Controlling genetic disorders in the french dairy cattle population

Andre A. Eggen, Amandine Duchesne, Pascal Laurent, Cécile Grohs, Catherine C. Denis, Mathieu M. Gautier, Didier Boichard, Alain Ducos

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– Development of Genetic Research and Animal Production –

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Japanese Society of Animal Breeding and Genetics

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Japanese Society of Breeding

Japan Livestock Technology Association
Livestock Improvement Association of Japan
Japan Racing Association

Japan National Tourist Organization

Contents

Time Table of the conference

Saturday September 11

9:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00	20:00
Registration (1F) Poster Mounting (2F)											
Executive Committee Meeting (310B)				Lunch at Liberty Tower (17F)	Workshops: (1) Pig Gene Mapping (309B) (2) Dog gene Mapping (309A) (3) Poultry Gene Mapping (309E) (4) Cattle Hereditary Diseases (309C) (5) Mouse Genome Informatics (309F)		Coffee Break	Workshops: (6-1) Cattle GeneMapping (309B) (7) Equine Genetics (309A) (3) Poultry Gene Mapping (309E) (8) Rabbit Gene Mapping (309C) (9) Gene Networks for Beef Marbling (309F)		Welcome Reception at Liberty Tower (23F)	
		Workshop: (R-1) Animal Forensics-1 (309F)									

Sunday September 12

9:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00	20:00
Registration (1F) Poster Exhibition (2F)											
Opening Ceremony (3F)	Plenary Session 1 Genetic Diversity of Livestock and its Utlization for Breeding (3F)			Lunch at Liberty Tower (17F)	Workshops: (6-2) Cattle Ggene Mapping (309B) (12) Fish and Shellfish Gene Mapping (309C) (10) Horse Gene Mapping (309A) (11) Dog Applied Genetics (309E)		Coffee Break	Workshops: (13) Comparative Mapping (309B) (15) ISAG/FAO Biodiversity (309C) (10) Horse Gene Mapping (309A) (14) Marker Assisted Selection (309E) (16) Applied Genetics in Sheep and Goats (309F)			
Special Exhibition “Native Domestic Animals of Eastern Asia” （9 F）											

Monday September 13

9:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00	20:00
Registration (1F) Poster Exhibition (2F)											
Plenary Session 2 Ultimate Chromosome Maps (3F)			Lunch at Liberty Tower (17F)		Poster Discussion: Odd numbers (2F)		Coffee Break	Oral Presentations of Selected Posters (3F)		Workshop: (17) Swine Genome Sequencing (309B)	
Special Exhibition “Native Domestic Animals of Eastern Asia” （9 F）											

Tuesday September 14

9:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00	20:00
Registration (1F) Poster Exhibition (2F)											
Plenary Session 3 Bright and Dark Sides of QTL Analysis (3F)			Lunch at Liberty Tower (17F)		Poster Discussion: Even numbers (2F)		Coffee Break	Oral Presentations of Selected Posters (3F)		Poster Removing	
Special Exhibition “Native Domestic Animals of Eastern Asia” （9 F）											

Wednesday September 15

9:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00	20:00
Regis.											
Plenary Session 4 Seeds for Future Genome Analysis (3F)	ISAG Business Meeting (3F)		Conference Tours with Lunch Box						Banquet at Tokyo Prince Hotel (～22:00)		

Thursesday September 16

9:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00	20:00
Registration											
Workshops: (18) Genome Sequencing (309B) (19) Cattle Molecular Markers (309A) (20) Avian Immunogenetics (309E) (R-2) Animal Forensics-2 (309F)				Executive Committee Meeting (310B)							

Plenary Sessions

1.Genetic Diversity of Livestock and its Utilization for Breeding

September 12 (Sunday) 10:00-13:00 at Auditorium (Academy Common 3F)

Chairs:Takao Namikawa (Nagoya University, Japan)
Johann-Nikolaus Meyer (Göttingen University, Germany)

1) **Several rivers from Babylon: The genetic legacy of Near Eastern cattle domestication** (P001)
Dan Bradley (Trinity College Dublin, Ireland)

2) **Genetic diversity evaluation and conservation strategy in pigs** (P002)
Louis Ollivier (INRA, Station de Génétique Quantitative et Appliquée, France)

3) **Genetic diversity of cattle in Southeast Asia** (P003)
Kazuaki Tanaka (School of Veterinary Medicine, Azabu University, Japan)

4) **Genetic diversity and phylogeny of pigs in Asia** (P004)
Yaetsu Kurosawa (Cattle Museum, Japan)

2. Ultimate Chromosome Map

September 13 (Monday) 9:00-12:00 at Auditorium (Academy Common 3F)

Chair: James E. Womack (Texas A&M University, USA)

1) **Creating the ultimate maps: Genomic sequencing of domesticated animals** (P005)
Lawrence B. Schook (Institute for Genomic Biology, University of Illinois, USA)

2) **Chicken genome sequence and the AvianNET Research Community** (P006)
David W. Burt (Roslin Institute, UK)

3) **From dynamic transcriptome analysis to future life science** (P007)
Yoshihide Hayashizaki (Jun Kawai in his behalf; Riken Genomic Sciences Center, Japan)

3. Bright and Dark Sides of QTL Analysis

September 14 (Tuesday) 9:00-12:00 at Auditorium (Academy Common 3F)

Chair: Denis Milan (INRA, France)

1) **Towards transgenic engineering of late-onset, male-specific double muscling** (P008)
Dimitri Pirottin, Luc Grobet, and Michel Georges (University of Liège, Belgium)

2) **Genome-wide scan of disease genes by association analysis using microsatellites** (P009)
Hidetoshi Inoko (Tokai University School of Medicine, Japan.)

3) **Gene-mapping strategy of human common metabolic disease of civilization in the post-genome era** (P010)
Ituro Inoue (Institute of Medical Science, University of Tokyo, Japan)

4. Seed for Future Genome Analysis

September 15 (Wednesday) 9:00-10:00 at Auditorium (Academy Common 3F)

Chair: Noelle E. Cockett (Utah State University, USA)

1) **Mouse models for human diseases – the German mouse clinic and the European mouse mutant archive-EMMA** (P011)
Martin Hrabé de Angelis (GSF National Research Center, Germany)

Workshops

1. **Pig Gene Mapping** (Chair: Gray Rohrer)
September 11 (Saturday) 13:30-16:30 at Room 309 B (Academy Common 9F)

2. **Dog Gene Mapping** (Chair: Melba S. Ketchum)
September 11 (Saturday) 13:30-15:30 at Room 309 A (Academy Common 9F)

3. **Poultry Gene Mapping** (Chair: Richard Crooijmans)
September 11 (Saturday) 13:30-15:30 and 16:00-18:00 at Room 309 E (Academy Common 9F)

Part 1: 13:30-15:30

Chicken genome

Chicken RH-map and alignment to the chicken linkage map – Alain Vignal (INRA)

Cytogentic map and alignment to the linkage map – .....

Chicken BAC libraries and alignment to the genetic map – Richard Crooijmans (Wageningen University)

The Chicken Genome Sequence – Dave Burt (Roslin Institute)

The chicken SNP-map – SNP-consortium

Evolution of tissue-specific genes – Nicholas J Dickens (University of Oxford)

Chicken MHC

Overview MHC-complex – Marcia Miller (Beckman Research Institute of the City of Hope)

Part 2: 16:00-18:00

Chicken QTLs

Overview QTLs integrated within Chickace (an Acedb database) – Annemieke Rattink (Nutreco Breeding Research Center)

QTL-detection guidelines – .....

QTL affecting egg production – Masaoki Tsudzuki (Hiroshima University)

Identification of a transferase in fatty livers – Takeshi Shibata (Kyushu Tokai University)

FADS1 candidate gene for egg yolk fatty ascid profiles – Xiquan Zhang (South China Agricultural Univeristy))

Quail genomics

Linkage map – Hideyuki Mannen (Kobe University)

Candidate gene analysis – Karl Schellander (University of Bonn)

QTLs – Francis Minvielle (INRA)

Ducks genomics

Linkage map – Ning Li (China Agricultural University)

Isolation of markers – Kimiaki Maruyama (Meiji University)

4. **Cattle Hereditary Diseases** (Chair: Tetsuo Kunieda)
September 11 (Saturday) 13:30-15:30 at Room 309 G (Academy Common 9F)

Renal tubular dysplasia, a disease caused by a deletion of the *Paracellin-1*/*Claudin-16* gene in Japanese Black cattle
Yasunori Ohba (Gifu University)

Controlling genetic disorders in the French dairy cattle population
André Eggen (INRA)

Mapping of the Gene Causing Disproportionate Dwarfism in Angus Cattle
James Reecy (Iowa State University)

Identification and control of a dwarfism gene in Japanese brown cattle
Haruko Takeda (University of Liège and Shirakawa Institute of Animal Genetics)

Mapping and identification of a the CVM-gene in cattle
Christian Bendixen (Danish Institute of Agricultural Sciences)

5. **Mouse Genome Informatics** (Chair: David R. Shaw)
September 11 (Saturday) 13:30-15:30 at Room 309 F (Academy Common 9F)

The Mouse Genome Informatics Database as a resource for mammalian phenotype and gene expression data
David R. Shaw (The Jackson Laboratory)

6. **Cattle Gene Mapping** (Chair: Eduardo Casas)
September 11 (Saturday) 17:00-18:00 at Room 309 B (Academy common 9F)
September 12 (Sunday) 15:00-17:00 at Room 309 B (Academy common 9F)

7. **Equine Genetics** (Chair: E. Gus Cothran)
September 11 (Saturday) 16:00-18:00 at Room 309 F (Academy common 9F)

Report on the 2003/2004 Equine Comparison Test
Alan Guthrie (University of Pretoria)

8. **Rabbit Gene Mapping** (Chair: Hein A. Van Lith)
September 11 (Saturday) 16:00-18:00 at Room 309 G (Academy common 9F)

9. **Gene Networks for Beef Marbling** (Chair: Kenji Oyama)
September 11 (Saturday) 16:00-18:00 at Room 309 F (Academy common 9F)

TG5 and other genes for beef marbling
William Barendse (CSIRO)

The effect of a leptin single nucleotide polymorphism on quality grade, yield grade and carcass weight of beef cattle
Leigh Marquess (Quantum Genetics Canada Inc.)

A short comment on leptin gene frequency of Japanese Black
Shinji Sasazaki (Kobe University)

Exploration of beef marbling genes based on gene expression profiling
Takahisa Yamada (Kyoto University)

Comparison between Japanese Black and Holstein cattle using gene expression profiling
Sigrid Lehnert (CSIRO)

Selective genotyping for beef marbling
Soichi Tsuji (Kobe University)

10. **Horse Gene Mapping** (Chair: Matthew Binns)
September 12 (Sunday) 15:00-19:30 at Room 309 A (Academy common 9F)

11. **Dog Applied Genetics** (Chair: Hein Van Haeringen)
September 12 (Sunday) 15:00-17:00 at Room 309 E (Academy common 9F)

12. **Fish and Shellfish Gene Mapping** (Chair: Nobuhiko Taniguchi and Nobuaki Okamoto)
September 12 (Sunday) 15:00-17:00 at Room 309 G (Academy common 9F)

How to break bilateral symmetry: learning from zebrafish and flounder
Tohru Suzuki (Tohoku University)

Mapping genes and QTL in tilapias
Gideon Hulata (Agricultural Research Organization)

EST analyses of pre-smolt Atlantic salmon *(Salmo salar)*
Bjorn Hoyheim (Norwegian School of Veterinary Science)

QTL analyses of viral disease resistance in fish
Nobuaki Okamoto (Tokyo University of Marine Science and Technology)

13. **Comparative Mapping** (Chair: André Eggen)
September 12 (Sunday) 17:30-20:30 at Room 309B (Academy common 9F)

Integrated maps and Oxford grids : maximising the power of comparative mapping.
Kyall Zenger (The University of Sidney)

Construction of a high resolution comparative gene map between human chromosome 14 and swine chromosomes using RH mapping.
Hiroshi Yasue (National Institute of Agrobiological Sciences)

A comprehensive radiation hybrid map of the bovine genome comprising 5757 loci
Akiko Takasuga (Shirakawa Institute of Animal Genetics)
A porcine physical map through comparative genomics
Lawrence Schook (University of Illinois Urbana-Champaign)
Comparisons of human, mouse and cattle chromosome maps reveal the reuse of evolutionary breakpoints.
Harris Lewin (University of Illinois Urbana-Champaign)
Comparative sequencing in the porcine MHC region.
Patrick Chardon (INRA-LREG)
The mouse Genome Informatics Database as a resource for mammalian phenotype and gene expression data.
David Shaw (The Jackson Laboratory)

14. **Marker Assisted Selection** (Chair: Jay Hetzel)
September 12 (Sunday) 17:30-19:30 at Room 309 E (Academy common 9F)

Opportunities for Marker Assisted Selection (MAS) in Livestock
John Gibson (University of New England)
Successful strategies for marker discovery
Bill Barendse (CSIRO Livestock Industries)
Commercial application of Marker Assisted Selection
The Japanese experience – Misao Kanemaki (Livestock Improvement Association)
Beef cattle – Gerhard Moser (Genetic Solutions)
Dairy cattle – Christa Kuehn (Research Institute for the Biology of Farm Animals)
Swine – Yuefu Liu (Canadian Centre for Swine Improvement)

15. **ISAG/FAO Biodiversity** (Chair: Henner Simianer)
September 12 (Sunday) 17:30-20:30 at Room 309 G (Academy common 9F)

Results of a Global Survey of Domestic Animal Diversity Studies
Roswitha Baumung (University of Natural Resources and Applied Life Sciences Vienna)
The further development of MoDAD in FAO's Global Strategy for the Management of Farm Animal Genetic Resources
Irene Hoffmann (FAO)
New MoDAD marker lists and future needs in global farm animal biodiversity studies
Henner Simianer (University of Goettingen)
Microsatellite DNA phylogeography and domestication and dispersion of domestic yak
Qi Xuebin, Han Jianlin, J.E.O. Rege and Olivier Hanotte (ILRI)
Domestication and genetic diversity of the Old World camelids: a molecular genetic perspective
Han Jianlin and Olivier Hanotte (ILRI)
Genetic Variability of Japanese Native Chickens Assessed by Means of Microsatellite DNA Polymorphisms
Masaoki Tsudzuki, Sayed A-M Osman, Masahide Nishibori and Yoshio Yamamoto (Hiroshima University)
Development of a Microsatellite Set for Diversity Studies in Ducks
Ning Li (China Agricultural University)

16. **Applied Genetics in Sheep and Goat** (Chair: Liliana Di Stasio)
September 12 (Sunday) 17:30-19:30 at Room 309 F (Academy common 9F)

17. **Swine Genome Sequencing** (The Swine Genome Sequencing Consortium)
September 13 (Monday) 18:30-21:00 at Room 309 B (Academy common 9F)

18. **Genome Sequencing** (Chair: Lawrence B. Schook)
September 16 (Tuesday) 9:00-12:00 at Room 309 B (Academy common 9F)

19. **Cattle Molecular Markers** (Chair: Maria Cecilia Penedo)
September 16 (Tuesday) 9:00-12:00 at Room 309 A (Academy common 9F)

20. **Avian Immunogenetics** (Chair: Marcia M. Miller)
September 16 (Tuesday) 9:00-12:00 at Room 309 E (Academy common 9F)

Genomic Comparisons of MHC Region in quail and chicken
Takashi Shiina (Tokai University School of Medicine)

Quail MHC class II beta gene diversity
Kazuyoshi Hosomuchi (Tokyo University of Agriculture)
Mapping of plumage color and blood protein genes on the microsatellite linkage map of the Japanese quail
Miho Inoue-Murayama and Mitsuru Miwa (Gifu University)
The c-type lectin-like genes in the chicken MHC *Y* region
Marcia M. Miller (Beckman Research Institute of the City of Hope)
Genotyping at the chicken MHC *B* region via microsatellite markers
Janet Fulton (Hy-Line International Dallas Center)
SSCP in *B* genotyping of indigenous chickens in Thailand
Kalaya Boonyanuwat (Ministry of Agriculture and Cooperative)
Genome-wide survey of genomic regions involved in genetic resistance of Rous sarcoma virus in the chicken
Hideji Yamashita (Kyushu Tokai University)
Evaluation of avian leukosis virus subgroup A and B receptor genes in recombinant congenic strains of chickens
Huanmin Zhang (USDA ARS Avian Disease and Oncology Laboratory)

21. **Genetics of Immune Response** (Chair: Herman Raadsma)
September 12 (Sunday) 15:00-17:00 at Room 309 F (Academy common 9F)

ROUND-TABLE MEETINGS

R-1. **Animal Forensics – 1** (Chair: Melba S. Ketchum)
September 11 (Saturday) 9:30-12:30 at Room 309 F (Academy Common 9F)

R-2. **Animal Forensics – 2** (Chair: Sreetharan Kanthaswamy)
September 16 (Tuesday) 9:00-12:00 at Room 309 F (Academy common 9F)

Introduction
Sree Kanthaswamy (Veterinary Genetics Laboratory- Forensics)
Domestic Animal Forensics
Joy Halverson (QuestGen)
QA in Animal Forensics
Bruce Budowle (US Federal Bureau of Investigations)
Wildlife Forensics
Holly Ernest (Veterinary Genetics Laboratory- Wildlife Unit) and Steve Fain (US Federal Wildlife Forensics Laboratory)

Oral Presentations of the Selected Posters

September 13 (Monday) at Auditorium (Academy Common 3F)

16:30 – 17:30 (Chair: Akiko Takasuga)

1. **Investigating the evolution of tissue-specific genes through comparisons of chicken gene expression information and human orthologues** (A017)
   NICHOLAS J DICKENS & CHRIS P PONTING
   University of Oxford, Dept. Human Anatomy & Genetics, England

2. **Identification of differentially expressed genes and transcriptome of four porcine tissues: Validating a 13,000 oligonucleotide microarray** (A010)
   SHU-HONG ZHAO [1], JUSTIN RECKNOR [2], DAN NETTLETON [2] & CHRISTOPHER K TUGGLE [1]
   [1] Department of Animal Science, and [2] Department of Statistics, Iowa State University, USA

3. **An integrated physical map of the porcine genome** (A004)
   DENIS MILAN [1], LAURENT SCHIBLER [2], CLAIRE ROGEL-GAILLARD [3], NATHALIE IANNUCCELLI [1], JULIE DEMARS [1] & PATRICK CHARDON [3]
   [1] Laboratoire de Génétique Cellulaire, INRA, [2] Laboratoire de Génétique Biochimique, INRA, and [3] Laboratoire de Radiobiologie et d'Etude du Génome, INRA-CEA, France

4. **cDNA Microarray and QRT-PCR Analysis of Host-Pathogen Interactions in a Porcine *in vitro* Model for Toxoplasmosis** (C030)
   MARGARET A. OKOMO-ADHIAMBO, CRAIG W. BEATTIE & ANETTE RINK
   Department of Animal Biotechnology, University of Nevada, USA

17:30 – 18:30 (Chair: Hiroshi Yasue)

5. **Loss of genetic variability through time in the American Standardbred horse** (E102)
   E. GUS COTHRAN [1] & RYTIS JURAS [1,2]
   [1] Department of Veterinary Science, University of Kentucky, USA, and [2] Siauliai University, Lithuania

6. **Mitochondrial DNA evidences for multiple maternal origins of domestic yak** (E029)
   QI XUEBIN [1,2], HAN JIANLIN [1,3], J.E.O. REGE [1] & OLIVIER HANOTTE [1]
   [1] International Livestock Research Institute (ILRI), Kenya, [2] State Key Laboratory of Arid Agro-Ecology, Lanzhou University, P.R. China, and [3] International Yak Information Center, Gansu Agricultural University, P.R. China

7. **Extent of linkage disequilibrium in Australian dairy cattle** (D020)
   M.S. KHATKAR [1], P.C. THOMSON [1], M.E. GODDARD [2] & H.W. RAADSMA [1]
   [1] Centre for Advanced Technologies in Animal Genetics and Reproduction (ReproGen), University of Sydney and CRC for Innovative Dairy Products, and [2] Victorian Institute of Animal Science, Australia

8. **Genome-wide linkage disequilibrium in Japanese Black cattle** (D021)
   MOTOI ODANI [1], TOSHIO WATANABE [2], KOU YOKOUCHI [2], YOSHIKAZU SUGIMOTO [2], TATSUO FUJITA [3] & YOSHIYUKI SASAKI [1]
   [1] Graduate School of Agriculture, Kyoto University, [2] Shirakawa Institute of Animal Genetics, and [3] Oita Prefectural Institute of Animal Industry, Japan

September 14 (Tuesday) at Auditorium (Academy Common 3F)

16:30 – 17:30 (Chair: Takahisa Yamada)

9. **Distribution of endogenous retroviruses in the Suidae family** (E067)
DENBIGH SIMOND [1], JAIME GONGORA [1], STEWART LOWDEN [2] & CHRIS MORAN [1]
[1] Faculty of Veterinary Science, University of Sydney, Australia, and [2] Royal School of Veterinary Studies, University of Edinburgh, United Kingdom

10. **Molecular evidence for hybridization of species in the genus *Gallus*** (E075)
MASAHIDE NISHIBORI [1], TAKESHI SHIMOGIRI [2], TAKESHI HAYASHI [3] & HIROSHI YASUE [3]
[1] Hiroshima University, [2] Kagoshima University, and [3] National Institute of Agrobiological Sciences, Japan

11. **A genetic linkage map of amago salmon (*Oncorhynchus masou ishikawae*) and mapping of two QTL associated with smoltification** (F095)
TAKUYA HARA [1], TAKASHI NAGASE1, TOMONORI KUWADA [2], TETSUYA TOKUHARA [2], AKIYUKI OZAKI [1], TAKASHI SAKAMOTO [1] & NOBUAKI OKAMOTO [1]
[1] Faculty of Marine Science, Tokyo University of Marine Science and Technology, and [2] Gifu Prefectural Fresh Water Fish Research Institute, Japan

12. **HEXA – A locus affecting predisposition for BSE in cattle?** (F004)
KATRIN JULING [1], SABINE WIEDEMANN [1], FEDERICA BELLA-GAMBA [1], JOHN WILLIAMS [2] & RUEDI FRIES [1]
[1] Chair of Animal Breeding, Technical University Munich, Germany, and [2] Division of Molecular Biology, Roslin Institute, United Kingdom

17:30 – 18:30 (Chair: Tetsuo Kunieda)

13. **Linkage and physiopathological analysis of spontaneous hereditary canine disorders segregating in French pedigrees** (C049)
LAURENT TIRET [1], MANUEL PELE [1], STEPHANE BLOT [2], JEAN-LOUIS KESSLER [1] & JEAN-JACQUES PANTHIER [1]
[1] UMR Molecular & Cellular Biology, and [2] Neurobiology Laboratory, Alfort National Veterinary School, France

14. **Identification of a major gene locus for marbling spanning 450 kb at the telomeric region of BTA 21 using Japanese Black cattle population** (F046)
YASUSHI MIZOGUCHI [1], EIJI IWAMOTO [2], KEN TATSUDA [2], TOSHIO WATANABE [1] & YOSHIKAZU SUGIMOTO [1]
[1] Shirakawa Institute of Animal Genetics, and [2] Hyogo Prefectural Institute of Agriculture, Forestry and Fisheries, Japan

15. ***SPP1* is a candidate gene for the QTL affecting milk protein concentration on BTA6 based on population-wide linkage disequilibrium, differential expression, and targeted inhibition** (F015)
MIRI COHEN [1], EYAL SEROUSSI [1], MARK R. BAND [2], HARRIS A. LEWIN [2], JAMES K. DRACKLEY [2], DENIS M. LARKIN [2], ANNELIE EVERTS-van der-WIND [2], JUN HEON LEE [2], JUAN J. LOOR [2], MOSHE SHANI [1], JOEL I. WELLER [1] & MICHA RON [1]
[1] Agricultural Research Organization, Israel, and [2] University of Illinois, USA

16. **FEZL affects somatic cell count through SEMA5A in Holstein-Friesian cattle** (F008)
MAYUMI SUGIMOTO [1], TSUYOSHI OHTAKE [1], AKIRA FUJIKAWA [2] & YOSHIKAZU SUGIMOTO [3]
[1] National Livestock Breeding Center, [2] Animal Research Center of Hokkaido, and [3] Shirakawa Institute of Animal Genetics, Japan

Best Posters

Recombineering: development of BAC-based experimental analysis (A013)

MEREDITH J. MAZUR, ANDREW HAUCK, LAWRENCE B. SCHOOK & MARGARITA B. ROGATCHEVA
Department of Animal Sciences, University of Illinois, Urbana, USA

Characteristics of eluted endogenous peptides define repertoires for two chicken MHC BF2 class I alleles (B018)

MARCIA M. MILLER [1], ROGER E. MOORE [1], RONALD M. GOTO [1], HENRY D HUNT [2] & TERRY D. LEE [1]
[1] Beckman Research Institute, City of Hope National Medical Center, Duarte, USA, and [2] USDA Agricultural Research Service, Avian Disease and Oncology Laboratory, East Lansing, USA

Identification of estrogen-responsive and oviduct-specific regulatory elements in chicken ovalbumin gene (C008)

TETSURO IWASAWA, OSAMU MUKAI, MASAKI SHIOTANI, HIDEO KAWAMOTO, SHINYA SAOTOME, YUKO FUNABASHI & KIMIAKI MARUYAMA
Department of Life Science, Faculty of Agriculture, Meiji University, Kawasaki, Japan

Developing a physical (BAC contig) map of the pig genome (D027)

SEAN HUMPHRAY [1], RICHARD CLARK [1], ROBERT PLUMB [1], CAROL SCOTT [1], JANE ROGERS [1] & ALAN L. ARCHIBALD [2]
[1] The Wellcome Trust Sanger Institute, Hinxton, CB10 1SA, U.K., and [2] Roslin Institute, Roslin, EH25 9PS, U.K.

Mitochondrial DNA evidences for multiple maternal origins of domestic yak (E029)

QI XUEBIN [1,2], HAN JIANLIN [1,3], J.E.O. REGE [1] & OLIVIER HANOTTE [1]
[1] International Livestock Research Institute (ILRI), Nairobi, Kenya, [2] State Key Laboratory of Arid Agro-Ecology, Lanzhou University, Lanzhou, P.R. China, and [3] International Yak Information Center, Gansu Agricultural University, Lanzhou, P.R. China

***SPP1* is a candidate gene for the QTL affecting milk protein concentration on BTA6 based on population-wide linkage disequilibrium, differential expression, and targeted inhibition (F015)**

MIRI COHEN [1], EYAL SEROUSSI [1], MARK R. BAND [2], HARRIS A. LEWIN [2], JAMES K. DRACKLEY [2], DENIS M. LARKIN [2], ANNELIE EVERTS-van der-WIND [2], JUN HEON LEE [2], JUAN J. LOOR [2], MOSHE SHANI [1], JOEL I. WELLER [1] & MICHA RON [1]
[1] Agricultural Research Organization, P.O.B. 6, Bet-Dagan 50-250, Israel, and [2] University of Illinois, 1201 W. Gregory Drive, 61801, Urbana, IL, USA

Other Program

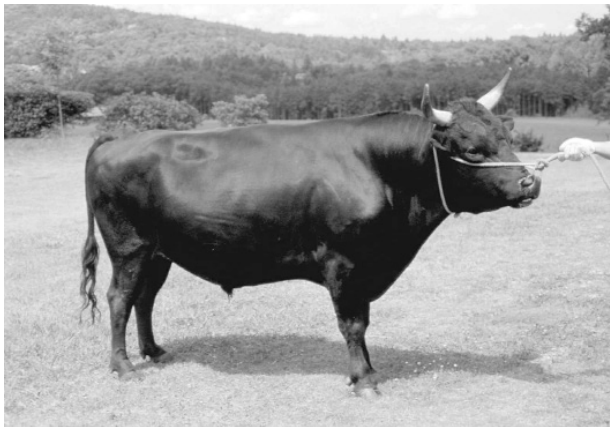
1. Special Exhibition

September 12-14, 9:00-18:30 at Academy Common 9F

Native Domestic Animals of Eastern Asia
Origin-Diversity-Culture

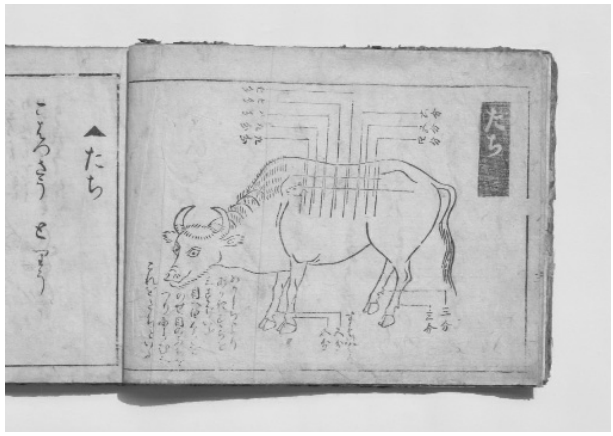
Presented by
Cattle Museum of Maesawa, Japan
The Society for Researches on Native Livestock, Japan
The Organizing Committee of ISAG2004/Tokyo

This exhibition introduce you a research history and the comprehensive results on domestic animals and their wild forms in eastern Asia, together with unique animal specimens, beautiful old paintings, Japanese food-culture materials, etc. One may not get any other opportunity to enjoy those exotic things except for this ISAG conference held in Asia.



Mishima Cattle (Bull)

The typical Japanese native cattle, which had been kept in a small island in Yamaguchi district. This cattle has hereditary capability to produce marbled beef.



Gyuuka-Satsuyo, 1720 (Edo period)

The veterinary guidebook of cattle which was published in mid-eighteenth century. The book describes conditions of patients and methods of treatments.

Guest Speakers

Dan Bradley studied genetics at Cambridge University (UK) from 1983-1986. His P.D. was awarded in 1991 by Trinity College Dublin (Ireland) for research in the mapping of a human disease gene for inherited blindness. He has been a lecturer in the Department of Genetics there since 1994. His current research interests include: ancient DNA in animals; Y chromosome diversity and Irish human origins; origins of livestock as discerned using genetic diversity; detection of recombination in mitochondrial genomes; population genetic detection of the signatures of past selection in the human and bovine genomes. For over 10 years he has been gazing hopefully at phyogenetic networks trying to understand the origins of cattle. (**P001**)

Louis Ollivier was trained as *ingénieur agronome* at the *Institut National Agronomique* of Paris, and received his MSc degree at the Iowa State University. He spent his entire research career at the Department of Animal Genetics of INRA (France), where he has been involved in quantitative genetics and applications to pig breeding. Since 1990, he has worked on gene mapping and genetic diversity evaluation in pigs, leading several international research projects funded by the European Union. He has also served as an expert for international organisations such as FAO and the European Association for Animal Production (EAAP). He chaired the EAAP Commission on Animal Genetics from 1982 to 1988, and was in charge of the EAAP working group on animal genetic resources from 1993 to 2002. (**P002**)

Kazuaki Tanaka studied animal genetics at Nagoya University (Japan) from 1992-1999. He received his Ph.D. in 1999. From 1999 to 2002 he was a research fellow of Japan Society of the Promotion of Science in Nagoya University. Since 2002 he is assistant professor of Azabu University (Japan). He is a young scientist of the society of researches on native livestock. His research interests are in the area of genetic diversity of native cattle in Asia. (**P003**)

Yaetsu Kurosawa studied the morphology and immunogenetics of wild and domestic pigs at Tokyo University of Agriculture's Institute of Animal Serology from 1976 to 1993. He obtained his Ph.D. in 1990. The following year, Kurosawa received a request from his hometown of Maesawa, Iwate Prefecture, to establish the Cattle Museum. In preparation for the museum's opening, Kurosawa studied museology for the next four years, and continues to work as curator of the museum. His primary research interests are the genetic diversity, phylogeny, and cultural history of domesticated animals in Asia. (**P004**)

Lawrence B. Schook is Professor of Comparative Genomics and Theme Leader in the Institute for Genomic Biology and Director of the Agricultural Genomics and Public Policy Training Program at the University of Illinois. He received his Ph.D. from the Wayne State School of Medicine and was a Fellow at the Institute of Clinical Immunology, Berne, Switzerland and the University of Michigan. He was Director, Food Animal Biotechnology Center and Associate Dean of Research at the University of Minnesota from 1993-2000. He was selected as a University Scholar, University of Illinois and has received the Funk Award in Agriculture and the Pfizer Award for Animal Health. His work has focused on the mechanisms of innate immunity, improving animal health, and using the pig as a biomedical model. (**P005**)

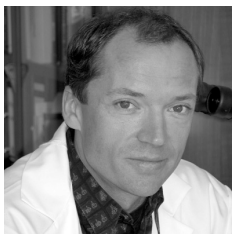
David W. Burt studied molecular genetics at Leicester University (UK) from 1977-1980 and received his Ph.D. in 1980. He was a research associate at the ICI (now AstraZeneca)/University of Leicester Joint Laboratory (UK) from 1980-1985, Harvard Medical School (USA) from 1985-1987, and Medical Research Council (UK) from 1987-1988, involved mainly in structural and gene expression studies. In 1988, he was appointed to a principal investigator at Roslin Institute (UK) to work on the control of muscle gene expression in poultry and now involve poultry genomics. His research interests consist of two mutually dependent paths: genome analysis of the chicken genome and characterization of genetic traits, mostly quantitative in nature. (**P006**)

Yoshihide Hayashizaki received his M.D. and Ph.D. from Osaka University Medical School in 1982 and 1986, respectively, and worked at Professor K. Matsubara and Professor K. Miyai's lab as a fellow until 1988. From 1988 to 1992, he worked as a research scientist at National Cardiovascular Center Research Institute (Department of Bioscience), Osaka and developed a new technology known as the Restriction Landmark Genome Scanning (RLGS) System. In 1992, he joined the Institute of Physical and Chemical (RIKEN), and was appointed Project Director for the RIKEN Genome Project in 1995. Since then he has been taking part and aiming to establish a Mouse Genome Encyclopedia. His present position is Project Director of Genome Exploration Research Group, Genomic Sciences Center, RIKEN, Yokohama and he aims to analyse the gene transcriptional network using the Mouse Genome Encyclopedia, for the development of new drugs, based on functional genomics in the post-genome era. (**P007**)

Michel Georges is presently Professor in Genetics and Genomics at the University of Liège, Belgium. He obtained a DVM degree from the University of Liège in 1983, a Masters degree in Molecular Biology at the Free University of Brussels in 1985, and became Agrégé de I'Enseignement Supérieur in 1991. From 1989 to 1993, he worked as senior scientists then director of research at Genmark in Salt Lake City, Utah. He has spent his scientific career working with his collaborators on the positional cloning of genes underlying complex traits. His team has primarily focused on economically important traits in livestock, including *double-muscling* in cattle, *callipyge* in sheep, QTL for carcass traits in pigs, and QTL for dairying traits in cattle. (**P008**)

Hidetoshi Inoko is Dean of Tokai University Graduate School of Medicine, and Chairman and Professor of Department of Molecular Life Science, Tokai University School of Medicine. He is a professor of Subassembly of Medical Sciences, Mongolian Academy of Sciences. He is a visiting professor of Harping University School of Medicine. He is President and CEO of GenoDive Pharma Inc. His interest is molecular genetics for identification of common diesease genes and for comparative genomics using genome information as post-genome sequence projects. (**P009**)

Ituro Inoue studied medical biochemistry at Kagoshima University from 1984-1988 and he received his Ph.D. in 1988. Between 1989 and 1998, he stayed in Department of Biochemistry and Department of Human Genetics at University of Utah, USA, studied biochemical and genetic aspects of human essential hypertension. In 2000, he was appointed to Associate Professor at Institute of Medical Science, University of Tokyo. His research interests are in the area of genetics of common metabolic disease of civilization especially on hypertension, asthma, intracranial aneurysm, and ossification of the posterior longitudinal ligament of the spine. (**P010**)



Martin Hrabé de Angelis studied biology at the Philipps University in Marburg and received his PhD in 1994. He worked as postdoctoral fellow from 94- 97 at the Jackson Laboratory in Bar Harbor/USA studying the Delta/Notch pathway and mouse mutant lines with impaired somitogenesis. He started his own research group in 97 at the GSF National research centre in Munich. In 2000 he was recruited as director of the Institute of Experimental Genetics at the GSF. Hrabe de Angelis is full professor at the Technical University in Munich and holds the chair of Experimental Genetics. At the same time he serves as director of the European Mouse Mutant Archive in Monterotondo/Rome Italy. His research focus is on large-scale mutagenesis, functional genomics using mouse models for the understanding of inherited diseases in man, the Delta/Notch pathway and bone related diseases. In 2002 he founded the German Mouse Clinic (GMC) for comprehensive phenotyping of mouse models. (**P011**)

Plenary Sessions

P 001

Several rivers from Babylon: The genetic legacy of Near Eastern cattle domestication

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Haplotypes are information-rich groups of markers which segregate together because they are situated closely together on a chromosome or on a special non-recombining unit (mitochondrial DNA or the Y chromosome). Mitochondrial DNA sequence has highlighted the Near Eastern affinity of European cattle. It also suggests a degree of separation of both African cattle from the original *Bos taurus* domesticates of the Near East and lends some support for an African domestication of the wild ox. African Y chromosome haplotypes are are also distinctive, but with both *Bos indicus* and Near Eastern influences. Interestingly, some divergence of Y chromosomes between regions in Europe suggests legacies of different migratory origins for northern and southern herds. A pattern of lack of diversity in Northern haplotypes mirrors that from other genetic systems but with the interesting exception of milk protein genes. The diversity of cattle in Britain is enigmatically high and suggests an input from both the southern and northern European agricultural streams.

P 002

Genetic diversity evaluation and conservation strategy in pigs

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Conservation of plant and animal variation is necessary to meet future agricultural challenges as well as to preserve the rich cultural heritage of the various regions of the world. A careful evaluation of each species genetic diversity is therefore required before setting the most appropriate conservation strategy. The data collected on a sample of 68 European breeds or lines of domestic pigs are used here as an example. The investigation (PigBioDiv1) was designed along recommendations made in the MoDAD report of FAO. The genetic markers chosen, microsatellites (50 loci) and AFLP (148 loci), allowed a fairly precise evaluation of the European pig genetic diversity, which could be used for defining conservation priorities. A new study (PigBioDiv2) is also under way to extend the work to Asian pig breeds.

Between-breed diversity is classically considered as a major criterion to be taken into account when setting priorities for conservation of domestic animal breeds. When diversity is evaluated on the basis of genetic markers, use can be made of measures of gene diversity or of pair-wise genetic distances between breeds in order to derive individual breed contributions to diversity. Such an approach, which may be termed conservationist, is somewhat different from the standard population geneticist approach, where the emphasis is put on time since divergence and phylogeny. The models underlying the classical genetic distances do not indeed strictly apply in the context of farm animals. This is shown in particular for the simplest model based on genetic drift underlying the Reynolds distance. On the other hand, a large distance between two breeds may reflect either large differences in allelic frequencies, i. e. a structural diversity, or the presence of alleles unique to one of the breeds considered, i. e. an allelic diversity. Many additional criteria are needed for establishing conservation priorities, among which the within-breed diversity, as measured for instance by the breed heterozygosities for the markers. The European data showed that more than half of the total European between-breed microsatellite diversity could be assigned to 29 endangered local breeds, while the average internal diversity of the latter was about 3 % below the overall mean. Individual breed contributions to between-breed diversity showed large differences whereas contributions to within-breed diversity varied very little between breeds. AFLP diversity, though overall markedly below microsatellites, showed very similar breakdown patterns.

Several methodological issues arise in the analysis and interpretation of genetic marker information. First, caution is needed in making inferences on the evolutionary forces explaining diversity for these markers. The central question is to ascertain the kind of diversity actually measured, which depends on the concordance between molecular and quantitative trait diversity. This issue is still a matter of debate. Though the DNA markers used are known to be neutral, there are pieces of evidence, such as estimated divergence times or heterogeneity of locus diversity, showing that genetic drift may not be the major evolutionary force explaining the markers diversity. Apart from well-established breed admixture effects in farm animals, we know, more generally, that neutral markers may be affected by the selection acting on genes located in the vicinity of markers. The rather intense selection pressures applied recently in pigs could thus explain part of the marker diversity observed.

Another important question is how the between-breed and the within-breed components of diversity should be combined in an "aggregate" diversity. For instance, one could weight the between-breed and the within-breed marginal contributions of each individual breed by F_{ST} and $1\text{-}F_{ST}$, respectively, since the fixation index F_{ST} of Wright represents the proportion of the total gene variation which is due to differences in allelic frequencies between populations. Such an approach applies in a situation of selection within a so-called "meta-population". However, in a more general context of animal breeding, when heterosis and complementarity between breeds have also to be considered, as well as adaptation to specific environments, more weight should be given to the between-breed variation.

The need to take into account the risk of extinction, or degree of endangerment, of the existing breeds is another difficulty in establishing conservation strategies. Long-term prediction of genetic diversity requires a precise evaluation of endangerment, known to be dependent on many factors, among which effective size of the populations, breed demography and organisation, geographical distribution and economic competitiveness. Genetic distances among breeds and breed internal diversities are also expected to change over time, in a way which can only be predicted under a model of genetic drift based on known effective sizes. Because of the difficulties of such predictions, a continuous monitoring of farm animal genetic resources is needed. Economic, demographic and cultural aspects should also be taken into consideration. Combining genetic and non-genetic data indeed remains the challenging goal of any conservation policy.

Acknowledgements

This work has received support from programmes of the European Union (contracts BI02-CT94-3044, RESGEN-CT95-012, BIO4-CT98-0188 and QLK5-CT2002-01059), which are gratefully acknowledged. Thanks are also due to the numerous providers of biological samples and marker genotypes, and to several participants in those projects for helpful discussions. Support of the European Association for Animal Production is gratefully acknowledged by the author.

P 003

Genetic diversity of cattle in Southeast Asia

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The presence of wild species of Bos is the feature of the genetic resources of cattle in Asia. The wild species of *Bos* consist of banteng (*B. banteng*), gaur (*B. gaurus*), yak (*B. mutus*), and kouprey (*B. sauveli*). The banteng is distributed over the Indochinese Peninsula, the Malay Peninsula, Java, and Borneo from the west of Arakan Yoma. The gaur is distributed over South and Southeast Asia from the forest area of the central part of India to the Indochinese Peninsula. However, it is not distributed over the islands. Yak is distributed over the high altitude area of the large range to Mongol from the Himalayas through Plateau of Tibet. The kouprey distributed over Thailand, Laos, and Vietnam centering on the northern part forest area in Cambodia, is now very rare and little is known of its present status. There are some domesticated forms of these wild species, for example, domestic gaur (gayal or mithan) in hills of separate India, Bhutan, Myanmar and Bangladesh, domestic yak in Nepal to highland China, and domestic banteng (Bali cattle) in Indonesia. It is possible that domestic cattle in tropical and subtropical Asia have acquired some genes from such wild species, because they can interbreed with domestic cattle and, though mostly male offspring are sterility, female hybrid has breeding capability. However, it has been thought that the genetic influence from these wildness kinds is limitation-like, because the domestic forms of wild *Bos* species are distributed only over the area restricted extremely now. The native cattle in South and Southeast Asia was generally regarded as humped cattle (*Zebu, indicus*) or crossbred between humpless (*taurus*) and humped cattle in various rates.

This time, I give a lecture about phylogenetic relationships of wild species of *Bos*, genetic composition of the domestic cattle in Southeast Asia, and some evidence of inflow of genes from the wild species to domestic cattle.

The 1140 bp cytochrome b gene of a mitochondrial DNA was sequenced for banteng, gaur, yak, Bali cattle, mithan, and American bison. And then, phylogenetic tree of these *Bos* species was constructed using the neighbor-joining method, in which Asian buffalo (*Bubalus bubalis*) and African buffalo (*Synceros caffer*) sequences were placed as outgroup. In the dendrogram, the genus of *Bos* (including the American bison) were divided into three clusters, one is cluster of *indicus* and *taurus* cattle, another is of banteng, gaur, Bali cattle and mithan, and the other is of yak and American bison. Using molecular clocks, the coalescence time for the present *Bos* species was estimated as approximately 5.5 million years. As the divergence time within the domestic cattle is less than 1 million years, the wild *Bos* species can be used as very diverged genetic resources for the domestic cattle.

Domestic cattle in the world were generally classified into three types, that is, *taurus* (humpless or European-type), *indicus* (humped or Zebu), and intermediate-type. The intermediate-type cattle were considered to have a hybrid origin between the *taurus* and the *indicus* cattle in various ratios. The native cattle of Indo-China area called Yellow Cattle was considered as this intermediate-type. Studies of the polymorphisms of Y-linked genetic marker can be used as a powerful tool to investigate the introgression or gene flow between the *taurus* and the *indicus* cattle through the male. The gene for *SRY* is one of well studied genes that can distinguish *taurus* and *indicus* origin. A total of 273 DNA samples of male cattle were collected in Vietnam (51), Laos (69), and Myanmar (153). Of all the 273 male cattle examined, 260 cattle had *indicus*-type SRY gene and the rest 13 cattle had *taurus*-type. Thus, most of the paternal background of these cattle is *indicus* and the gene flow from the *taurus* is very limited.

The hemoglobin-_ chain (Hbb) polymorphism is one of the most intensively investigated genetic characters in cattle. In Southeast Asia, most of the cattle populations were polymorphic with three electrophoretic bands: Hbb-A, -B, and -X with the three alleles, A, B, X at *Hbb* locus. The Hbb-X exists in the cow of the Southeast Asia specifically, and is contained by 10 to 30% of gene frequency. When analyzed by using isoelectrofocusing (IEF) method, the Hbb-X band can be further divided into two polymorphic bands, we temporarily call them X_1 and X_2 from the higher PI. The X_1 band was confirmed to be identical with the Hbb of mithan, on the other hand, the X_2 band showed no difference in the pI from the previous X (X^{Bali}) band of Bali cattle. In the continent part of Southeast Asia, the X_1 is far common compared with X_2. The Hbb-X appeared at highest frequency (27.4%) in the northern area of Vietnam where we can not see any domestic gaur and banteng. We believe that existence of Hbb-X_1 suggests that gaur played some important role as a source of genes for the cattle of this area.

P 004

Genetic Diversity and Phylogeny of Pigs in Asia

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The majority of domestic animals were first tamed and kept in Asia. Domestic pigs are thought to have originated separately in many areas because their ancestor, the wild pig (*Sus scrofa*), is widely distributed in Asia. Recently wild species other than *Sus scrofa*, such as the Warty pig (*Sus verrucosus*), have been added to the list of wild ancestors of the domestic pig. Of the pigs in Asia, the Chinese pigs are well known around the world. As a result of a long breeding history many highly domesticated breeds can be found there. And in South and Southeast Asia, most of the typical purebred native pigs remaining are limited to remote regions, such as the mountainous and island areas. Therefore the study of pigs in these areas is, at present, still limited. These local pigs are characterized by straight snouts and small ears, and are referred to as the "Short-eared pig" or "Small-eared pig".

The author and his coworkers had many opportunities to study the morphological and genetic variations of the Short-eared pig and some wild species in several countries of Asia in order to clarify their genetic diversity and phylogeny.

First, it is important to examine the breeding situation of the Short-eared pig in South and Southeast Asia. This pig is kept mainly by minority ethnic groups in remote regions. The pig's range also extends from mountainous areas of South China to the Tibetan plateau. This pig is generally kept in a loose housing arrangement, but varied traditional breeding methods can be found in some regions. For example, there is an interesting case of pig breeding by nomadic, non-Islamic people living in Muslim countries such as Bangladesh. It seems that the diversity of pig-breeding culture reflects the heterogeneous origins of pig domestication in Asia. The typical Short-eared pig is relatively small in body size, with some isolated mountain or island populations averaging only 30 or 40 kg. Domesticated pigs have coat coloring and a highly developed crest like wild pigs. So, it is difficult to distinguish between wild and domestic pigs at first glance. Pigs with these features are more frequently observed in South Asia rather than in Southeast Asia. The development of the crest is regarded as a typical feature of the Indian wild pig (*Sus scrofa cristatus*) rather than the other wild pigs (Lydekker, 1915). Whereas the variation of teat number in the Chinese pigs varied from 6 to 8 pairs, the Short-eared pigs range from 4 to 6 pairs and have 5 pairs at the highest frequency. Their teats were spaced evenly and symmetrically. These features are the same as the Asian wild pigs, and suggest that the morphogenetic similarity between the Short-eared and wild pigs is very high, and that the degree of domestication of this local pig is low. More specifically, it may be considered that gene flow is occurring from the wild pig into the populations of the Short-eared pig kept in remote regions. Indeed, there is even a case of the domestic population interbreeding with a wild species other than *Sus scrofa*: the Short-eared pig kept by a hill tribe on Mindoro island in the Philippines had variants of blood groups and blood proteins which originated from the Warty pig (*Sus verrucosus philippinsis*) (Kurosawa *et al*., 1989).

The results for blood group and biochemical polymorphism studied in the domestic and wild pigs of Asia were found to be very different from those of Europe. East-west, a geographical cline in the frequencies of several blood group antigens was clearly observed in parallel with the distribution of subspecies of the wild pig. And of the 22 genetic loci of the blood proteins examined by electrophoresis, the Tf and Am-1 loci were regarded as particularly useful genetic markers for phylogenetic relationship studies between domestic and wild pigs. That is, the Tf^B and $Am\text{-}1^B$ alleles are commonly found in the domestic and wild pigs from Asia and Europe; however, the domestic and wild pigs of Asia not only have those alleles, but also the Tf^C , $Am\text{-}1^A$ and $Am\text{-}1^C$ alleles at high frequency. It is probable that these alleles are variants which originated in Asian wild stock. Some rare variants such as Tf^X, $Tf^{D'}$,and $Am\text{-}1^Y$ are also present in the domestic and wild pigs of Asia. There are significant regional differences in the gene frequencies of both these loci between the populations of Short-eared pigs. Comparisons of gene frequency show that Tf^B and $Am\text{-}1^C$ tend to be more common in the populations of South Asia: $Am\text{-}1^C$ (0.611) was the highest in the Bangladeshi pig and was recognized in the Indian wild pigs (0.666~1.000). While the genes Tf^C (0.400~0.833) and $Am\text{-}1^A$ (0.333~0.919) show greater frequencies in the island populations of Indonesia than in South Asia and northern China.

The differences in these gene frequencies support the hypothesis that Asian native pigs were independently domesticated in locations different from those of European pigs. That is, in the process of domestication, the Short-eared pig, which has geographically diverse origins and has continued to have genetic connection with several species of its wild ancestors, should be considered to possess broad genetic diversity. However, the number of purebred Short-eared is drastically decreasing as modernization leads to crossbreeding with Chinese and Western pigs. In the future, preservation of the short-eared pig will need to focus not simply on its genetic resources, but also to take into account the traditional culture and social structures of the ethnic groups that are keeping these pigs.

P 005

Creating the Ultimate Maps: Genomic Sequencing of Domesticated Animals

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Over the past decade, tremendous progress has been made mapping and characterizing the swine genome. Currently, moderate to high-resolution genetic linkage maps containing highly polymorphic loci have been produced using independent mapping populations. Additionally, physical mapping methods such as somatic cell hybrid analysis, *in situ* hybridization and ZOO-FISH have been employed to enrich the gene map and to perform comparative analysis with map-rich species such as the human and mouse. To date, over 5,000 mapped loci have been assigned to the pig genome. Whole-genome radiation hybrid panels (7,500 and 12,500 rad) have been generated for swine resulting in yet another rapid increase in the number of comparative mapped loci. More recently, the swine genomics community has acquired access to resources such as bacterial artificial chromosome (BAC) libraries providing approximately 35X coverage of the swine genome. These BAC resources have facilitated the production of high-resolution physical maps in specific chromosomal regions that support construction of sequence-ready mapping resources for the porcine genome.

The porcine genome is now uniquely positioned for sequencing as a result of the development of these necessary tools and reagents. We have contributed to the development of a porcine BAC map with 20X coverage using fingerprinting and BAC end-sequencing. These resources have permitted selection of a minimum tilling path of BAC clones to be sequenced and complement a whole-genome shotgun sequencing approach. We have recently demonstrated our ability to "piggy BAC" human sequences to construct a high-resolution (approximately 1 Mb) comparative map of SSC13 that represents a fusion of HSA3 and HSA21. Thus, our approach links the sequence to the BAC clone map to support subsequent targeted closure of genomic regions of particular interest.

In current model systems (*Drosophila*, yeast, *C. elegans*, and mice), functional genomics is supported by the ability to develop congeneic inbred lines, cloning and creating mutants either by deletion or substitution of specific genes. In pigs, there are neither inbred lines nor embryonic stem lines to create gain-of-function (knock-in) or loss-of-function (knock-out) animals. In addition, the relatively long gestation time and costs of creating large breeding herds to map multigenic traits devoid of various background genes is not cost effective with respect to validating QTL or disease through breeding. Thus, there is an important need to develop models for transcription profiling similar to that developed for worms, fly and mice that will also allow the capture information from comparative genomes (creating models to validate hypotheses with respect to gene-gene interactions associated with multigenic traits).

Comparative genome maps provides the basis for evaluating phenotypes between rodents, man and pigs. We are using gene-trapping in porcine fetal fibroblasts to permit development of *comparative phenomics* between mouse, rat, dog, man and the pig. Because the sequence of the vector is known, it can be used to identify the insertion site, thereby creating a panel of thousands of gene disruptions in a system amenable to generating transgenic pigs. This resource is fast replacing the traditional transgenic mouse knockouts allowing rapid single or large-scale analysis of genes within a certain family, pathway, etc. A similar resource for the swine community provides an untold opportunity to study the genetics of swine development, growth and resistance to disease. Our approach permits the development of gene-targeted, clonable fibroblast lines that can then be used to develop appropriate animal models. Our long-term goal is to develop an approach for identifying genes and their related pathways that control growth and development and disease resistance in the pig. The ability to use phenotypic-driven screens and to use directed mutagenesis of characterized loci is now made possible for the pig due to the establishment of DNA sequence databases and appropriate target cells lines.

This porcine model system has broad implications for manipulating the maternal environment or *in vitro* culturing of fetal fibroblasts and embryos. Thus, we are creating a *comparative phenomics technology platform* that has broad applications in agricultural, life science and biomedical investigations. To date, we have been able to demonstrate the clonability of our porcine fibroblasts that were derived from the same animal that will serve as the genome-sequencing template (CHORI-242). Hence, we have a "syngenic" model that we have used to develop porcine phenotypic models where the mouse equivalent of the human diseases is not an appropriate phenocopy. This includes a genetically tractable cancer model and models for resolving complex genetic multifaceted inherited diseases such as ataxia-telangiectasia and neurofibromatosis Type 1.

P 006

Chicken Genome Sequence and the AvianNET Research Community

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In this paper the current status of chicken genomics is reviewed. This is timely given the current intense activity centred on sequencing the complete genome of this model species. The genome project is based on a decade of map building by genetic linkage and cytogenetic methods, now being replaced by high-resolution radiation hybrid and BAC contig maps. Markers for map building have generally depended on labour intensive screening procedures, but in recent years this has changed with the availability of almost 500,000 chicken EST sequences. These resources and tools have been critical in the final stages of the chicken genome sequence assembly (e.g. cross-checked with other maps) and its annotation (e.g. gene structures based on ESTs). The future for chicken genome and biological research is an exciting one, through the integration of these resources, for example through the proposed chicken Ensemble database, it will be possible to solve challenging scientific questions exploiting the power of a chicken model. One area is the study of developmental mechanisms and the discovery of regulatory networks throughout the genome. Another is the study of the molecular nature of quantitative genetic variation. No other animal species have been phenotyped and selected so intensively as our agricultural animals, and thus there is much to be learned in basic and medical biology from this research.

P 007

From Dynamic Transcriptome Analysis to Future Life Science

YOSHIHIDE HAYASHIZAKI (JUN KAWAI in his behalf)
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Since 1995, we have been establishing the comprehensive mouse full-length cDNA collection and sequence database to cover as many genes as we can, named Riken mouse genome encyclopedia. Using a series of our original technologies which we have developed, around 2,000,000 clones prepared from 267 tissues were end-sequenced to classify into 171,144 clusters and 60,770 representative clones were fully sequenced. As a conclusion, these sequences contained 33,409 unique sequences with more than 20,487 clear protein-encoding genes. Non-coding RNA is much more significant than we expected in the mouse transcriptome. In this analysis it was turned out that the significant population of non-coding RNA as well as protein, functions directly by various mechanisms. The geographical analogy of a second 'RNA continent' separate from the 'continent' of expressed proteins, aids the visualization of this concept. The basic strategies, which are used to encode the additional genetic information required for the regulation of complex life, differ between higher animals and plants. In higher animals, the additional genetic information is encoded using unexpectedly high levels of variation which is created through alternative splicing (more than 41% all genes, 80% of alternative splicing alter the amino acid sequence), 5'-end variation and 3'- end variation. Plants, in contrast, use a larger number of TUs rather than transcriptional variants to encode the additional information (only 13% of rice genes are alternatively spliced).

The current large-scale research in life science is to analyze the structure of biomolecules, such as genome, transcriptome, proteome, SNP and HapMap. However it is not enough to understand the biological phenomena at a system of biomolecules. Genome function database is essential, which explains the function of each biomolecule. Targets listed under "genome function database" could include analysis of expression regulatory regions, expression profiles, interactomes, intracellular dynamic kinesis of protein and RNA. Thus, these are essential platforms for genome network analysis, defined as the collection of molecular pathways which connect gene to phenotype, such as a disease gene to the disease symptom, a drug target to the drug effect, etc. The genome network analysis must be the ultimate goal for life science.

As the first trial for genome function database, we tried to establish a large-scale system to identify CAP sites and promoter region, named CAGE, Cap Analysis Gene Expression, which is based on preparation and sequencing of concatamers of DNA tags deriving from the initial 20 nt from 5' end mRNAs. CAGE provides us the enormous information of high-throughout gene expression and profiling of transcriptional starting site (TSP) including promoter usage analysis.

One of the big problems to be solved in genome network analysis is the delivery of full-length cDNA clone distribution. "DNA Book" proposed here is the powerful tool to distribute, in a timely and cost-effective manner, the full length cDNA clones essential for genome network research. In this technology, DNA clones or PCR products are printed directly onto the pages of books, and delivered to users along with relevant scientific information. The full-length cDNA clones can be collected by the PCR amplification or clone transformation into *E.coli*. DNA Book technology enable us to keep the whole full-length cDNA bank covering whole transcriptome on book shelf, not requiring deep freezer and scientist can start experiments after two hours PCR reaction.

The RIKEN Genome Exploration Research Group PhaseII team and the FANTOM Consortium *Nature* 409: 685-690
The FANTOM Consortium and the RIKEN Genome Exploration Research Group Phase I *Nature* 420: 563-573
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Kikuchi S.*et.al., Science* 301: 376-379

P 008

Towards transgenic engineering of late-onset, male-specific double muscling

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Intensive breeding programs implemented over the last fifty years have created cattle breeds that are highly specialized in either milk production (e.g. Holstein-Friesian, Jersey, ⋯) or meat production (e.g. Angus, Hereford, Charolais, Piedmontese, Belgian Blue, ⋯). Physiological antagonisms have indeed precluded the combination of superior abilities for both milk and meat production in the same animal. Despite its effectiveness, the resulting production system can be considered sub-optimal due to poor carcass and milk yield of respectively dairy and beef cattle.

A more efficient alternative can be envisaged based on a specialization by sex within the same population: a breed in which cows would be of dairy type while bulls would be of beef type. To achieve this goal while overcoming the physiological antagonisms between lactation and muscle development, we propose to use genetic engineering to target *trans*-inhibitor of myostatin (*MSTN*) on the Y chromosome. By doing so males are predicted to exhibit a muscular hypertrophy akin to "double-muscling" which in cattle results from naturally occurring *MSTN* loss-of-function mutations, while females will be non-transgenic and fully express their dairy potential. To avoid calving problems associated with increased birth weight we propose to inactivate *MSTN* after birth in order to induce a late-onset muscular hypertrophy.

To prove the feasibility of this concept we have (i) generated transgenic mice harbouring a floxed *MSTN* allele and shown that post-natal invalidation of the *MSTN* gene using an *MCK* promoter driven *cre* transgene causes a muscular hypertrophy of the same magnitude as the one obtained with constitutive *MSTN* knock-out mice, and (ii) produced transgenic mice in which only the males express a *MSTN trans*-inhibitor in skeletal muscle and show a 5 to 10 % increase in skeletal muscle mass. Latest result will be presented and discussed.

P 009

Genome-wide scan of disease genes by association analysis using microsatellites

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The "human genome diversity project" is conducted by disease gene mapping and association analyses using polymorphic genetic markers. For this purpose, SNP (single nucleotide polymorphism) markers are now being extensively and world-widely collected, and applied to disease mapping in a lot of laboratories. However, SNP is generally bi-allelic, and so polymorphism is not considered to be extensive enough to localize disease genes on the human genome by genome-wide mapping. Instead, we propose to use microsatellite which displays a high degree of polymorphism in repeat number of repetitious unit and so is expected to serve as a more useful genetic marker for genome-wide mapping.

To test this hypothesis, we have focused on mapping of diseases in the HLA region on chromosome 6p21.3, which is characterized by the presence of susceptible loci for more than 100 diseases. In this respect, the HLA region provides an excellent mini-genome model region for "human genome diversity project". By investigation of genetic polymorphisms in 78 microsatellite repeats in the HLA region, we could successfully reduce the critical regions for Behcet's disease (associated with B51) to a 100 kb segment around the HLA-B gene and for psoriasis vulgaris (associated with Cw6) to a 50 kb segment telomeric of the HLA-C gene. From these segments, strong candidate genes for these three diseases have been identified. Thus, refined microsatellites provide useful and valuable genetic markers for precise disease mapping. The length of linkage disequilibrium with disease locus observed for microsatellite is around 100 kb. This suggests that one microsatellite per 100 kb is enough for genome-wide mapping.

Collectively, the efficient method of genome-wide mapping is to first use microsatellites markers which enable to narrow down the critical region to 100 kb and thereafter to employ SNP markers within thus determined critical region for fine mapping to identify a causative gene. Based on this strategy, we collected 34,000 polymorphic microsatellite markers (one microsatellite per 100 kb) throughout the human genome and started genome-wide mapping of several complex diseases using them. As to rheumatoid arthritis, we have found 47 candidate regions for susceptible loci using 30.000 microsatellites in the genome-wide association study. By SNP association analysis of 7 candidate regions spanning approximately 100 kb among them, 7 susceptible loci for rheumatoid arthritis have been so far identified.

P 010

Gene-mapping strategy of human common metabolic disease of civilization in the post-genome era

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As the Human Genome Project accomplishes the completed human DNA sequences, research interests have rapidly shifted from common nucleotide sequences to nucleotide diversities in human populations. Human genome contains about 30 thousands of genes and probably about 5 millions of nucleotide variations, mainly single nucleotide polymorphisms (SNPs). Although much attentions were focused on SNPs that constitute key markers or causalities of diseases especially of common metabolic diseases of civilization such as hypertension, diabetes, and asthma, it is not realistic to analyze all the known SNPs to map disease gene despite that advent of modern technology enables us large scale typing with low cost. Therefore, genome-wide linkage analysis is still considered to be the legitimate and cost-effective strategy for narrowing the loci. In the common metabolic diseases, owing to undertermined mode of inheritance, low penetrance, delayed onset, and difficulty in recruiting families of multi-generations, classical parametric linkage analysis could not be applied, instead, non-parametric linkage analysis with affected sib-pairs is the most frequently performed to detect the loci. It is well recognized that non-parametric linkage analysis is not powerful to detect the loci and the linkage region spans 10-20 cM in general, so that still daunting efforts are required to identify disease gene. In linkage region, allelic association study with case-control comparison is performed to detect the susceptibility. Therefore, the characterizing the nucleotide diversity of single nucleotide polymorphisms (SNPs), their linkage disequilibrium (LD), and haplotype structures in the human genome have been rapidly prepared to pave the way for gene-mapping of common disease. Recently, a common pattern has emerged from several studies that the empirical distribution of LD in a number of different human chromosomal regions. LD appears to be organized in blocklike structure, in which a contiguous group of SNPs that constitute a block show high levels of pairwise LD between SNPs in different blocks. Accordingly, it is important to determine the LD block pattern of the linkage region to perform efficient association study.

Example: Ossification of the posterior longitudinal ligament of the spine as example of the genetic study from genome to gene.

For identification of disease gene, I would like to present our recent success of the ossification of the posterior longitudinal ligament of the spine (OPLL), which is commonly observed in Asian (prevalence: 2-4% of the general population >30 years of age in Japan) showing ectopic bone formation in the spinal ligament. We enrolled 142 affected sib-pairs and performed genome-wide linkage study with 420 microsatellite markers. In multipoint linkage analysis using GENEHUNTER-PLUS, evidence of linkage to OPLL was detected on chromosomes 1p, 6p, 11q, 14q, 16q, and 21q. The best evidence of linkage was detected near D21S1903 on chromosome 21q22.3 (maximum Z_{lr} = 3.97); therefore, the linkage region was extensively investigated for linkage disequilibrium with SNPs covering 20 Mb. In the linkage region, 600 SNPs of 150 genes were genotyped and LD mapping was performed. Several SNPs of collagen 6A1 gene (*COL6A1*) were strongly associated with OPLL and the best evidence of association, p = 0.000003, was observed with a SNP in intron 32. The haplotype analysis with three SNPs of *COL6A1* confers high significance (p = 0.0000007). These results strongly indicate that *COL6A1* constitutes the genetic susceptibility of OPLL, and more importantly our strategy performing genomewide linkage and LD mapping of the linkage region could successfully pin-point the disease gene.

In the lecture, I will mainly focus on genetic mapping of human common metabolic disease of civilization especially how we can and could identify the susceptible gene with genome-to-gene screening. In addition, I would like to discuss on quantitative trait loci (QTL) mapping for human diseases, which is directly relevant to domestic-animal genomics.

P 011

Mouse models for human diseases – the German mouse clinic and the European mouse mutant archive-EMMA.

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Here, we report of one of the largest ENU mutagenesis programs world-wide, the Munich Mouse Mutagenesis Project. To date, more than 550 mutant lines have been isolated. Novel dominant or recessive phenotypes have been identified with specific abnormalities comprising congenital malformations, biochemical alterations, immunological defects and complex traits such as behaviour or predispositions to allergies.

In addition to the large scale mutagenesis screen the "German Mouse Clinic" (GMC) has been established. The GMC, located at the GSF in Munich, offers a large scale standardized phenotype analysis of mouse mutants from various sources (transgenes, knockout mice, mutants from mutagenesis screens like ENU) to ensure an efficient, reliable and comprehensive analysis of the growing number of mouse mutants. Specialists from various fields of mouse phenotyping and from different institutions in Germany work side by side at one place.

In the field of hearing research our mutant lines contributed to most recent advances. With Beethoven (Bth) a semidominant mouse model for progressive hearing loss was found and mapped to mouse chromosome 19 to a 4.4-cM region. A point mutation in the Tmc1 gene, the mouse ortholog of the human deafness gene TMC1, is responsible for the phenotype. The latest result of an ALS model – the third most common neurodegenerative disease in man – will be presented. The European efforts in mouse genetics and genomics are very strong and mouse models are centrally archived and distributed in a well concerted European consortium – The European Mouse Mutant Archive – EMMA. An update of the structure and achievements will be discussed.

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Workshops

W 001

Renal tubular dysplasia, a disease caused by a deletion of the *Paracellin-1/Claudin-16* gene in Japanese Black cattle

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Renal tubular dysplasia (RTD) is an autosomal recessive hereditary disease of Japanese Black cattle characterized by renal failure, growth retardation and excessive growth of hooves. RTD has been observed since 1990 and the incidence rate was 3.51 % among newborn animals in 1995. The primary lesion is a dysplasia of epithelial cells at the renal proximal tubules in younger calves, followed by interstitial fibrosis with a reduction in the numbers of glomeruli and tubules in older cattle. In order to determine genetic factors of RTD, pedigree and medical records, which were related to affected animals, were collected and analyzed. It was suggested that an intensive inbreeding introduced to accelerate the fixation of the high marbling meet was the cause of the high incidence of RTD. We mapped the locus responsible for the disease (*rtd*) on bovine chromosome 1 (BTA1) by linkage analysis using microsatellite markers, and families of the affected calves. We found that a genomic segment of BTA1 was deleted in the affected animals. Construction of a physical map covering this region with BAC clones revealed that a 37-kb region was deleted in the affected animals. The deleted region included 1 to 4 of 5 exons of the bovine *Paracellin-1/Claudin-16 (PCLN-1)* gene, encoding a tight junction protein of renal epithelial cells. Cardinal symptom of RTD was different from that of a human disease caused by mutations of *PCLN-1* gene, familial hypomagnesemia with hypercalciuria and nephrocalcinosis. We also found that a stretch of highly GC-rich sequence was located at the boundary of the deletion, which can trigger the deletion of the *PCLN-1* gene.

W 002

Controlling genetic disorders in the french dairy cattle population

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Due to artificial insemination (AI) and to the particular structure of the dairy bovine population (heavy use of a few very popular AI sires in dairy populations like the Holstein breed resulting in increased inbreeding), dairy breeds present a large palette of different disorders some of which at a relatively high frequency causing not only economical losses but having a large impact on selection image. In France, a so-called "bovine genetic disease observatory" programme (BGDO) aiming at early detection of such defects has been developed since two years. This programme, coordinated by the animal genetics department of INRA, includes all the representative professional organizations involved in cattle production and breeding (including veterinarians) as partners. Among others, two disorders have been the subject of molecular studies in the French dairy populations :

1. During fall 1999, achondroplasia cases were observed in Western France in the progeny of a very popular Holstein bull called Igale Masc, underlining the genetic basis of the disease. We will present the study of the genetic determinism of this disorder and its mapping on BTA5 in the region containing a strong candidate gene.
2. Bovine syndactyly, also referred to as "mulefoot", has been previously shown to segregate as an autosomal recessive trait with incomplete penetrance and to map to BTA15 by an identity-by-descent mapping approach. We constructed a BAC contig spanning a large portion of the region of interest in order to start positional cloning of the underlying gene(s) and we identified several new genetic markers. Several "Mulefoot" affected embryos are currently being produced from an affected cow and homozygous or heterozygous sires and collected at different gestation stages. These biological samples will allow us to undertake sequence comparison and expression studies for several candidate genes in the chromosomal region.

Identification of the causal mutation for genetic disorders will facilitate eradication of these disorders in the dairy cattle population.

W 003

Mapping of the Gene Causing Disproportionate Dwarfism in Angus Cattle

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Disproportionate dwarfism has been reported in many cattle breeds including Dexter, Holstein, Aberdeen Angus, Hereford, Japanese brown and Shorthorn breeds. Recently, several Angus calves were diagnosed as dwarfs by gross and histo-pathological examination. Pedigree analysis indicated that all affected calves were inbred. Both affected and unrelated normal animals were genotyped for the presence of mutations known to cause bovine dwarfism. Only wild-type limbin and Dexter alleles were observed in all animals analyzed. Furthermore, genotyping of markers closely linked to these mutations indicate no loss of heterozygosity. Thus, mutations known to be associated with dwarfism are not present in these calves. To identify the causative mutation(s) for dwarfism in Angus, a directed genome scan was performed with a pedigree consisting of 26 individuals. Haplotype analysis indicated that the mutation is located on chromosome 6. Therefore, we cannot preclude the possibility that novel mutations exist in known dwarfism genes. Currently, we are fine mapping the critical region and have begun candidate gene analysis.

W 004

Identification and control of a dwarfism gene in Japanese brown cattle

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Bovine chondrodysplastic dwarfism (BCD) in Japanese brown cattle is an autosomal recessive disorder with the phenotype of short limbs, joint abnormality and ateliosis. By positional cloning, we identified a novel gene, *LIMBIN (LBN)*, that possessed two disease specific mutations in the affected calves. DNA tests based on the two mutations estimated the heterozygote frequencies at ~10%. Execution of these DNA tests has allowed breeders to eliminate dwarfism without losing genetic potential and biological diversity in Japanese brown cattle. Recently the human orthologue of *LBN, EVC2*, was identified as a gene responsible for Ellis-van Creveld syndrome (EVC). EVC is characterized by short limbs, short ribs, postaxial polydactyly, and dysplastic nails and teeth. Targeted disruption of *LBN* in mouse revealed that LBN plays critical roles in skeletogenesis and toothgenesis in the mouse as well. Future studies of LBN, both *in vivo* and *in vitro*, and further pathological examination of BCD will provide new insight into the complex biologic processes of mammalian skeletal development.

W 005

Mapping and identification of a the CVM-gene in cattle

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A new heritable disease called Complex Vertebral Malformation (CVM) was in 2000 observed with high frequency in the Holstein-Friesian breed. Pedigree analysis revealed that all diagnosed cases of CVM were genetically related to the US bull Carlin-M Ivanhoe Bell, which has been used extensively world wide, CVM thus presents a significant problem in Holstein-Friesian breeds all over the world. A genome-scan of families of affected animals using a panel of microsatellite markers was used to map the disease to a narrow region on bovine chromosome 3 (BTA3). A bovine BAC contig covering the region was established and candidate genes predicted from homologies to the human genome were evaluated by sequencing both healthy and diseased animals. The region covers approximately 3 million base pairs and contains about 25 known and predicted genes. Sequencing analysis revealed a mutation in a candidate gene encoding a nucleotide-sugar transporter. Functional analysis confirmed that the mutation disrupts the molecular function of the protein, demonstrating that the detected mutation is the direct cause of CVM.

W 006

The Mouse Genome Informatics Database as a resource for mammalian phenotype and gene expression data

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The Mouse Genome Informatics Database (MGI, http://www.informatics.jax.org/) provides free access to extensive information on the genetics, genomics and biology of the laboratory mouse. This hands-on workshop is designed to introduce Internet resources such as BLAST, comparative mapping data, phenotypic alleles and gene expression data. MGI curates comparative mapping data for mouse and 18 other mammalian species, including human and agriculturally important mammals. Over 11,000 phenotypic alleles, including spontaneous, targeted, transgene, conditional mutants and QTLs, are described. Researchers can also query gene expression data detailing when and where a gene is expressed and what genes are expressed in specific tissues and developmental stages. There will be ample time for personalized queries.

W 007

How to break bilateral symmetry: learning from zebrafish and flounder

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With respect to L-R asymmetry, flounder are quite unique in that in addition to the asymmetry of heart and gut found in most vertebrates, they are externally asymmetric, with both eyes located on a single lateral side. In contrast to the asymmetric morphogenesis of heart and gut, which initiates in embryos, eyes develop symmetrically in embryos, and only later, during metamorphosis, one of eyes migrates over the median plane to the other side of the animal. Recently, we obtained two important results on the molecular system that controls L-R asymmetry of flounder. The first is the discovery of the mechanism that breaks bilateral symmetry in embryos leading to asymmetrical morphogenesis of internal organs. The second is that the signals that function in embryonic L-R axial formation also participate in determining the direction of eye migration.

Using the zebrafish embryo, we show that symmetry is initially broken at Kupffer's vesicle, an organ that temporarily forms in tail bud by means of interaction between the gene *nodal* and its antagonist, *charon*. The *nodal* signal switched on on the left side of Kupffer's vesicle is relayed to left side of the gut, heart and brain. Knockdown of charon by morpholino randomizes the laterality of *nodal* expression, resulting in L-R abnormality of internal organs.

In embryos of flounder mutant, *reversed*, the laterality of expression of left-specific signals, including *nodal*, is randomized. This phenotype mimics that shown by *charon* knockdown. In *reversed* larvae, laterality of gut looping and eye migration independently becomes randomized. These results raise two hypotheses for the mechanism that establishes L-R asymmetry in flounder. First, a left-specific signal from the brain or heart primordium in embryonic stage gives the cue for eye migration. Alternatively, left specific signals may be switched on again in an asymmetrical manner in metamorphosing larvae. Current experiments are aimed towards distinguishing these hypotheses.

W 008

Mapping genes and QTL in tilapias

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The ability of tilapiine fishes to create viable interspecific hybrids makes them an ideal organism for genetic studies, using backcrosses or F_2 intercrosses as a segregating population. Several linkage maps of DNA markers were constructed for tilapias in recent years. The recent development of hundreds of microsatellite DNA markers enable coverage of the tilapia genome at 2.4 cM intervals on average, thus providing the infrastructure for systematic genome scans for detection of QTL. Markers associated with genes with deleterious alleles and distorted sex ratios were found in an inbred gynogenetic line of *Oreochromis aureus (Oa)*. Variation in a microsatellite was found to be associated with expression of the prolactin gene and growth in salinity-challenged fish from the F_2 of an *O. mossambicus (Om)* × *O. niloticus (On)* cross. A locus causing lack of melanin in a red tilapia was localized to LG3 through analysis of color segregation in two F_2 hybrid populations (*Oa* × *Red On* and *Red Om* × *On*). *Trp1*, a gene essential to melanin synthesis, was mapped to the same region, but is not the causative gene for this mutation. *MHC-1* and *TF*, related to the immune system, were mapped in the tilapia genome. Markers *UNH881* and *UNH208* are part of coding genes of the IgM light chain and Attractin, respectively. Chromosomal regions strongly associated with sex determination were localized to LG1 (Y haplotype) in *On*, and both LG1 (Y) and LG3 (W) in Oa. Analysis of epistatic interactions among the loci suggests the action of a dominant male repressor (the W haplotype on LG 3) and a dominant male determiner (the Y haplotype on LG1). A locus associated with sex determination in *Tilapia zillii* was also localized to LG1. A chromosomal region on LG23 with putative QTL for cold tolerance and body weight was detected in an F_2 hybrid population involving two species of tilapia, *Oa* and *Om*, and further supported by the results of another genome scan performed on a four-way cross between a (*On* × *S. galilaeus*) male and a (*Om* × *Oa*) female. Genome scan using 42 DNA markers, covering ~80% of the tilapia genome, done on another family of the *Om* × *Oa* F_2 hybrid revealed markers association with stress response, body weight and sex determination in four linkage groups: LG 1, 3, 4 and 23, confirming the location of QTL reported earlier.

W 009

EST analyses of pre-smolt Atlantic salmon (*Salmo salar*)

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Atlantic salmon is an important aquaculture species in Norway. As part of the Norwegian Salmon Genome Project we have developed cDNA libraries from pre-smolt Atlantic salmon. We focused on 15 different tissues and constructed single tissue libraries in a directional way using the pBluescript II XR cDNA library kit from Stratagene. Approx. 146000 non-amplified clones from the 15 libraries have been picked and gridded and so far approx. 40000 ESTs have been derived from the 5' end. In addition the remaining part of the libraries has been amplified and stored in the freezer for future use.

A preprocessing of the sequence data was performed using Phred to validate the sequence information and to eliminate vector and bad sequences. After clustering using a Phred/PHRAP pipeline we ended up with 14401 unique sequences. All clusters, singletons and singlets have been BLASTed against all relevant GenBank Databases and a Gene Ontology annotation of the data has been performed.

SNPs are currently being developed from the sequences and the first set has been mapped genetically.

W 010

QTL analyses of viral disease resistance in fish

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Gene marker technologies can be used for genetic improvement through selection for favorable traits, such as disease resistance. These traits are generally modeled as being controlled by many genes of small additive effects, which are known as quantitative trait loci (QTL).

Construction of a genetic linkage map, based on DNA markers at a large number of sites in the fish genome is necessary to identify quantitative trait loci (QTL) controlling traits of disease resistance. Linkage maps have been published for a large number of economically important fish species, such as rainbow trout, tilapia, catfish and Japanese flounder. Among these, the genetic linkage map of the rainbow trout and of Japanese flounder have permitted the identification of the QTL for infectious pancreatic necrosis (IPN) and infectious hematopoietic necrosis (IHN) resistance in rainbow trout, as well as lymphocystis disease (LD) resistance in Japanese flounder.

By identifying markers of high performance QTL in different strains or species, it may also be possible to successfully improve the performance of such traits in other strains through introgression of the desired QTL. One of the goals of selective breeding programs is to integrate genetic marker information from pedigreed brood stock fish into the successful management and culture. Such an approach, termed marker-assisted selection (MAS) and/or marker-assited gene introgression (MAI), is expected to increase genetic response by affecting intensity and accuracy of selection.

SECTION A

Bioinformatics
Statistical Genetics
Genome Technology

A 001

The Sino-Danish Porcine Genome Project: Genomic Survey and Gene Discovery

CHRISTIAN BENDIXEN [1], FRANK PANITZ [1], BO THOMSEN [1], HENRIK HORNSHØJ JENSEN [1], MERETE FREDHOLM [2], JAN GORODKIN [2], CLAUS B. JØRGENSEN [2], SUSANNA CIRERA [2], BIN LIU [3], JUN WANG [3], JIAN WANG [3], HUANMING YANG [3] & THE SINO-DANISH PIG GENOME CONSORTIUM

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In 2001, a collaboration, jointly funded by the Danish Slaughterhouses (DS), the National Committee of Pig Production (LUS) and the Chinese Academy of Sciences (CAS) was initiated between Danish and Chinese scientists with the purpose of performing an initial sequence analysis of the pig genome. Presently, the consortium has obtaine d more than 4.5 million genomic sequence reads and we have provided valuable knowledge about basic structural elements in the porcine genome, like sequence repeats, nucleotide composition, and extent of sequence conservation relative to other species.
The genome project also included a massive effort in gene identification with the sequencing of approx. 1 mill. EST sequences from 100 cDNA libraries. We will present the initial analysis of the EST and genomic sequences.

A 002

Improved Genotyping Through the Use of Real-Time PCR DNA Quantification

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The accurate genotyping of DNA samples requires a quantification technique that is both sensitive and discriminating. Given the wide origin of sample types in animal DNA testing, a precise quantification technique is required for determining template quality and quantity. This is especially true for multiplexed short tandem repeat (STR) assays which work optimally within a narrow range of template DNA concentrations. Quantification concerns are further compounded in veterinary forensics where sample mixtures between species can occur and therefore species-specific quantification is required. Furthermore, the quantification technique must be efficient when testing trace forensic samples that are often degraded or contaminated. In an attempt to address these issues, we turned to real-time PCR using an Applied Biosystems 7300 system. We have evaluated two real-time PCR-based assays to quantitate the amount of canine DNA in a variety of samples. The first method utilizes SYBR® Green I and canine-specific Short Interspersed Nuclear Elements (SINEs). The second method employs a 5'nuclease assay (the Taqman® assay) that uses a fluorescently-labeled probe to measure the progress of the PCR reaction. Real-Time PCR meets our criteria for both species specificity and the ability to quantify minute (< 5 pg) amounts of template DNA. In addition to being extremely sensitive, real-time PCR is a valuable quantification technique because it is in effect quantifying the PCR quality of the sample.

A 003

Allele specific real-time PCR for examination of X inactivation in *Bos taurus*

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Recent studies in the development and pathology of animals derived from somatic cell nuclear transfer indicate that the abnormalities may be due to improper epigenetic reprogramming including expression of X-linked genes. The recipient oocyte receives one active and one inactive X chromosome from the donor nucleus whereas normally in mammals, the female embryo acquires two active X chromosomes from both parents, of which one is randomly inactivated at the early blastocyst stage. In this study, we have developed an allele-specific TaqMan® real-time PCR system for detection of skewing of the X chromosome inactivation. This method is based on the quantification of transcripts from the paternal and maternal X chromosome of female cattle using an SNP located on the X inactivated gene MAOA. Non-equal expression of one allele would indicate skewing of X chromosome inactivation. This method was applied to samples from randomly selected cattle and two cloned females derived from fetal fibroblasts. The animals were genotyped for the SNP from genomic DNA. RNA was extracted from tissue samples from slaughterhouse cows and from fibroblast cell cultures derived from 3 skin biopsies of the cloned cattle. Reverse transcription and subsequent real-time PCR were performed and preliminary results indicate no significant skewing. These preliminary results suggest that cloning may not necessarily be associated with perturbation of X-inactivation.

A 004

An integrated physical map of the porcine genome

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To establish an integrated map of the porcine genome: 1) we produced a fingerprint for 7.10^4 BAC of INRA library by digesting BACs with HindIII-EcoRI-HaeIII and after migration of products on acrylamide gels. 2) we screened the BAC library by PCR with markers already mapped on genetic and RH maps and by hybridization with PCR fragments specific of known genes. Data were analyzed with FPC using a first Sulston score threshold of 10^{-12} and a tolerance of 0.5 base. After integration of information about markers found in BACs and iterative comparison of fingerprints of BAC mapped at the end of each contig, 7260 contigs were identified (11 contigs contain more than 50 clones, 320 contigs contain between 25 and 50 BACs, 1680 contigs contain between 25 and 10 BACs). 35000 BACs were then selected for BAC end sequencing at Génoscope Evry. This analysis is allowing us to anchor the BAC contigs on human genome. It provides both new high resolution comparative mapping information and a quality control of contig building. The INRA map is presently integrated with the international one by fingerprinting at UIUC and Sanger Centre of the 35000 BAC for which BES have been produced in France.
Finally this physical map of the pig genome is presently integrated with the pig-human compared map containing up to 4800 genes/markers mapped both on human sequence and porcine IMpRH map (in the frame of international collaborations).

A 005

Full-length bovine cDNA libraries: A development of library construction strategies

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Due to a shortage of ESTs, especially full-length sequences, for cattle, we have undertaken a project aimed at the production of numerous cDNA libraries from bovine tissues that are suitable for full-length cDNA sequencing. The full length sequences produced will not only serve as representative clones for DNA array experiments, but are indispensable in both assigning function to transcripts and as anchor points for the ensuing genome mapping and sequencing efforts. The aim of the study described here was to apply and refine the available methodologies in order to develop a system for producing high quality, universal libraries for full-length sequencing in our laboratory. In view of this, a standard cDNA library was produced from Peyer's patch tissue using available Cap-Trapper methodology. The library was cloned into p-Bluescript vector and evaluated. Analysis estimated that 74% of the clones produced were full-length. Average clone length was approximately 1138 bp. Strategies are examined to improve first strand synthesis and to universalize the above methodology through the incorporation of site-specific recombination approaches.

A 006

Construction of the full-length enriched cDNA libraries using Korean native pigs as a tool for gene discovery and cSNP development

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The development of functional genomic resources is essential to understand and utilize information generated from genome sequencing projects. Central to the development of this technology is the creation of high quality cDNA resources. It has been proven that full-length cDNA libraries are more informative than conventional cDNA libraries for defining genes as well as obtaining complete cDNAs for functional analysis of genes. In this context we constructed full-length cDNA libraries from Korean native pig tissues using SMART™ technology (CLONTECHniques), which is simpler in methods for full-length selection compared to other methods. As expected, the percentage of full-length cDNA with complete 5' ends was higher than conventional cDNA libraries. The size distribution of cDNA inserts was similar to that of a cDNA library constructed using conventional methods. Although the libraries constructed using SMART™ technology is enriched in full-length cDNAs than a conventional library, comparison of results using other technology such as cap trapper are needed to choose the better strategy for large scale full-length cDNA project of the pig genome. The libraries could serve as a useful resource for pig gene discovery and cSNP development.

A 007

Development of a bovine chromosome 5 SNP Chip

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Gene and QTL (quantitative trait locus) mapping and subsequent selection on genetic markers (termed marker-assisted selection) offers the potential to greatly increase the rate of genetic improvement of cattle. However, time and cost of genotyping have limited the application of marker-assisted selection. A SNP (single nucleotide polymorphism) DNA chip that interrogates thousands of SNPs (for a comprehensive, genome-wide chip) would reduce the time required for initial mapping studies from months to days when used in conjunction with selective, pooled DNA sampling. The aim of this pilot study is development of a DNA chip for genotyping approximately 300 SNPs on bovine chromosome 5 (BTA5) and software for genotype scoring, allele frequency estimation and gene or QTL mapping. Specific objectives supporting this aim are (1) validation of polymorphism in putative BTA5 SNPs, (2) estimation of within-breed allele frequencies for true SNPs, (3) creation of a SNP chip with validated SNPs, and 4) development of software for genotype scoring, allele frequency estimation and gene or QTL mapping. Putative SNPs for preliminary evaluation (n=600) have been obtained from the Interactive Bovine In Silico SNP (IBISS) database. SNP validation and allele frequency estimation will analyzed using pooled DNA samples from Holstein, Jersey, Angus, Hereford, Simmental and Limousin breeds. Preliminary data on SNP validation and allele frequency will be presented.

A 008

Validation of a bovine SNP panel for parentage and identity

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Single-nucleotide polymorphisms (SNP) are likely to become the standard marker for parentage verification and identity because of the ease of scoring, low cost assay development and high-throughput capability. Approximately 50 SNP markers at intermediate allele frequencies will provide 99% exclusion probabilities for parentage identification and in excess of 1×10^{-8} probability match rate for identity. To identify a robust SNP panel informative across several bovine breeds, a test group of 384 markers were selected for study. The test markers were selected from the MMI Genomics proprietary bovine SNP map of over 700,000 putative SNP markers using the following criteria: markers were GA alleles, all markers were unlinked, and the contig containing the described SNP was comparatively mapped to ENSEMBL human sequence. Markers were multiplexed in groups of twelve and each marker was evaluated in 7 breeds of cattle: Brahman, Angus, Hereford, Limousin, Simmental, Charolais, and Gelbvieh with 26 to 29 animals per breed for a total of 190 individuals. In addition, markers were tested for Mendelian inheritance using trios of 20 animals. Fifty-two SNP markers passed the criterion of heterozygosity in the majority of the breeds with minor allele frequency ranging from 0.20 to 0.49. These markers form the basis of a panel that can be used for a variety of applications in cattle populations.

A 009

A comparison of the utility of SNPs discovered by different methods

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One of the obstacles to generating a high density SNP map of the porcine genome is cost associated with SNP discovery and validation. We compared the efficiency of discovery, validation and mapping of SNPs by three methods. Potential porcine SNPs were identified by 1) clustering of 185,000 EST sequences (eSNP), 2) clustering of genomic sequences from a reduced representation library (rrSNP), and 3) PCR re-sequencing of genomic sequences, using template from 12 unrelated animals of the same line (rsSNP). Potential SNP sequences were masked for repetitive elements and primer extension assays were attempted for 76 eSNP, 78 rrSNP and 178 rsSNP. All SNPs with working assays were genotyped on a "diversity" panel of 184 samples representing eight lines from four breeds (Yorkshire, Landrace, Duroc & Pietrain). The assay conversion efficiency (no. working assays/no. attempted assays) for eSNPs was 0.78, vs. 0.55 & 0.65 for rrSNP and rsSNP, respectively. A larger percentage of eSNPs showed no allelic variation (38%) vs. rrSNP (17%) and rsSNP (6%) on the diversity panel. In addition, new SNPs have limited value without information on chromosomal location and relative order of markers. The MARC porcine reference panel was also genotyped to assess the efficiency with which SNPs could be mapped. Of the working assays that had >1% minor allele frequency on the diversity panel, the percentage of eSNP, rrSNP and rsSNP that could be assigned to linkage groups was 47, 77 & 76%, respectively. Low mapping efficiency for eSNPs was because a much larger proportion had no informative meioses. Nonetheless, the poor success of mapping eSNP is offset by much lower discovery costs, compared to rrSNP and rsSNP.

A 010

Identification of differentially expressed genes and transcriptome of four porcine tissues: Validating a 13,000 oligonucleotide microarray

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Establishing relationships between tissue transcriptomes (genes expressed in a tissue) under specific physiological conditions/genetic background can elucidate interesting genes contributing to phenotypes. A new oligonucleotide set representing 13,000 porcine genes has been designed by Qiagen-Operon. These 70-mer oligonucleotides were designed to be specific to the gene of interest, thus both utility and specificity of these elements need to be tested. Validation of glass slide microarrays containing these oligonucleotides was conducted by hybridizing target made from adult liver, lung, muscle, or small intestine, collected from three cross-bred pigs. A loop design was employed, and 24 measurements (with dye swap) were obtained from 12 slides of Cy3 or Cy5 labeled total RNA. To determine the tissue transcriptome, the signal intensity of each gene spot was divided by the median signal intensity of negative control spots for each combination of slide and dye. The geometric mean of these ratios was computed for each combination of gene and tissue. In any one tissue, ≥56% of the genes exhibited mean ratios >3 and ≥9% of the genes exhibited mean ratios >20. These results show significant utility of the microarray for measuring the transcriptome of these tissues. In total, 386 genes were found to be differentially expressed (Holm's adjusted p value < 0.05) across tissues by mixed model analysis with slide and animal as random factors and tissue as a fixed factor. There were 2,479 genes that showed differential expression between at least two tissues at 0.1% false discovery rate. Expression patterns for selected genes showed agreement with orthologous human/mouse expression data.

A 011

Gene expression profiling of different group of Japanese flounder (*Paralichthys olivaceus*) by using DNA microarray analysis

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Microarray is a powerful tool that allows characterization of number of gene expression profiling in a rapid and efficient fashion. The ability to survey the expression pattern of hundreds of thousands of genes at once has major implications to modern aquaculture species improvement. Microarray analysis can be combined with quantitative trait loci analysis to provide not only valuable molecular markers link to the trait, but also the ability to understand the molecular basis of the trait by finding genes whose expression are closely associated with the phenotype under investigation. Recently, we developed the cDNA microarray which was spotted with approximately 900 different cDNAs including unknown sequences from our EST studies of Japanese flounder. In this study, we examined the immune responses at the molecular level of groups of Japanese flounder groups moderately resistant and sensitive to *Edwardsierlla tarda* infection using cDNA microarray. Four different groups of Japanese flounder (groups A, B, C and D) were artifactually infected with *E tarda*. Groups A and C were killed by *E. tarda*, whereas about 50% of fishes in groups B and D survived. Microarray analyses showed that expression of immune relate genes in low sensitivity groups (group B and D) were induced earlier than the high sensitivity groups (group A and C) after challenge with *E. tarda*, suggesting that early immune response might be important for resistance to infectious disease. DNA microarray technology will be a useful tool for selective breeding.

A 012

A repertoire of cDNA probes for gene expression profiling in muscles of beef-producing cattle

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Array technology has been increasingly used to monitor global gene expression patterns in various tissues. However, applications to muscle development as well as meat production in livestock species have been hampered by the lack of appropriate cDNA collections. To overcome this problem, a directed cDNA library was constructed starting from 23 muscles of meat-producing bovines to derive a first collection of 3,573 clones. A preliminary sequence characterization of this collection indicated that the most abundant transcripts correspond to genes encoding proteins involved in energy metabolism (COX and NADH dehydrogenase subunits) and belonging to the contractile apparatus (myosin chains and troponin isoforms). Most of the clones represent known genes but interestingly, 20% have unknown function. In addition, our work identified new bovine genes as compared with other bovine libraries constructed from pooled tissues. From this first cDNA collection, we selected a set of 435 clones representing 340 different genes, of which 24 were novel. This collection was subsequently enriched with 75 specific cDNA probes for genes of interest in Meat Science. The bovine "muscle cDNA repertoire" thus designed was spotted onto a nylon membrane in order to test its relevance to further investigate the transcriptome of bovine muscles in relation with meat quality traits. Hybridizations with muscle samples confirmed the quality of the arrays, since at least 40% of the muscle cDNA probes gave valid hybridization signals.

A 013

Recombineering: development of BAC-based experimental analysis

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Bacterial artificial chromosomes (BACs) provide valuable resources for the analysis and manipulation of large genomic fragments. Using recombineering to make directed genetic modifications of genomic DNA cloned into BACs, we are developing approaches to assist in the resolution of complex human diseases with the pig biomedical model. To overcome difficulties associated with the introduction of BACs with large inserts into mammalian cells, we carried out experiments to determine the effects of various parameters regarding the efficiency of DNA transfection into porcine fibroblast cells. The BAC clone 403O9 from the RPCI-44 library was recombineered by introduction of a cassette encoding enhanced green fluorescent protein (EGFP) and used to monitor the effectiveness of BAC DNA uptake into the fibroblast cells. Since the rate of successful BAC transfection depends on factors including the purity of BAC DNA, the transfecting agent, and the target cell line, we compared the effects of varying these parameters on transfection efficiency. The efficiencies were determined by flow cytometry analysis of transient EGFP expression in the transfected cells. We determined that the time course of EGFP expression varied depending on the method of transfection (non-liposomal lipid reagent with DNA-condensing enhancer, lipid-based transfection reagent, or electroporation), observed the transfected cells expressed EGFP for up to two weeks, and optimized the conditions for transfection. Co-transfecting an EGFP BAC with another BAC of interest may provide a rapid method of selecting successful transfectants. (Supported by USDA grant AG2002-35205-12712).

A 014

Transfection of EGFP gene into chick embryo directly by sonoporation

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The resent progress is that transgenic technology can be applied for poultry breeding, to produce medical products in eggs and to study gene functions that underlie avian embryo genesis. Several methods to generate transgenic chickens have been improved during these 20 years. Among them retrovirus or adenovirus techniques could generate transgenic chickens efficiently. However this method requires virus vector construction or viral preparation, including packaging. It is not desirable from the point of safety that transgenic chicken generated by virus vector are eaten.

Findings of recent studies have shown that ultrasound can induce or increase cell membrane permeability of various agents, including genes. This phenomenon can be enhanced with using echo-contrast miclobubbles. This new gene transduction technique named sonoporation has been used in studies of gene therapy in adult organs. In this study we tried to transfect EGFP gene to chicken embryos by sonoporation. Manipulated embryos were incubated until hatching. Before hatching some embryos died, and then they were dissected and GFP gene expression was observed under fluorescent microscope. EGFP gene expression was detected in various chick embryonic tissues in them. Some embryos hatched will be test mated when they reach their sexual maturity. Sonoporation was found not to damage embryonic tissues or cells extremely. It was suggested that there is a possibility of using sonoporation as a useful method to generate transgenic chickens in future.

A 015

Sex manipulation using artificially induced gynogenesis and marker-centromere mapping in Barfin flounder (*Verasper moseri*)

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Barfin flounder *Verasper moseri* is a large flatfish, inhabiting cold sea basins around the east of Hokkaido. This species is suitable for aquaculture in northern Japan due to its commercial value and high growth rate at low temperatures. All-female production is desirable in this species because of the females exhibit higher growth rate than males. Artificial gynogenesis is a useful tool to produce all-female population in the male heterogametic (XX female-XY male) species, but optimal conditions of chromosome manipulation have not been determined in this species.

The optimum UV irradiation for genetic inactivation of sperm was the dose higher than 10 mJ/cm^2 irradiation. Insemination of UV irradiated sperm triggered haploid gynogenetic development. Gynogenetic diploids were obtained by inhibiting the second polar body release using cold shock (-1.5℃, 60-90 min) at 3-9 min after fertilization or pressure shock (650 kg/cm^2, 6min) at 7 min after fertilization. Although female rates varied among gynogenetic families, a family exhibited 95% females, suggesting the presence of XX-XY sex determination system in this species. The occurrence of 5-47% gynogenetic males can be explained by temperature dependent sex determination.

To verify all-female inheritance in gynogenetic diploids, we isolated 181 $(GT)_n$ microsatellite arrays from random genomic library and developed PCR primer sets from a part of these arrays. Currently, we are mapping microsatellite-loci in relation to the centromere of chromosomes using some gynogenetic diploid families.

A 017

Investigating the evolution of tissue-specific genes through comparisons of chicken gene expression information and human orthologues

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Functional and morphological specialization of tissues occurs as a result of differences in gene expression. To identify tissue-specific expression of genes in the chicken we have examined all of the ESTs that are contained in the NCBI Genbank [1], EBI EMBL [2] and a chicken tissue specific library held at UMIST [3] databases. These were mapped to the chicken genome sequence using BLAT, and a 95% threshold.

EST sequences were mapped to the chicken assembly and to predicted genes. Chicken genes were thus assigned tissue expression information based on their representation amongst EST libraries. Those chicken genes that had single orthologues in the human genome (1:1s) were then used to investigate sequence conservation between chicken and human genes. A percentage amino acid sequence identity value for each of the 1:1 genes was calculated.

The genes were classified in 10 tissue categories (brain, fat & skin, bone & connective, heart, kidney & adrenal, immune, liver, female reproduction, alimentary, and testis) and partitioned according to the number of these tissues in which they were expressed. Partition A: 1-3 tissues, partition B: 4-6 tissues and partition C: ≥7 tissues. The distributions of percentage amino acid identities for partitions A and C were significantly different (p=0.0006, Kolmogorov-Smirnov test). In addition to this, sorting the tissue categories by median percentage identity demonstrates that brain-expressed genes are most highly conserved, whereas testis-expressed genes are the least.

1. http://www.ncbi.nlm.nih.gov/Genbank/index.html
2. http://srs.ebi.ac.uk/
3. http://www.chick.umist.ac.uk/

This work was carried out in collaboration with the International Chicken Genome Sequencing Consortium. With special thanks to the EBI and EMBL.

A 018

Cloning, annotation and mRNA expression analysis of novel brain cDNAs related to highly reproductive performance in Taiwan Country Chickens

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To identify novel genes or expression sequence tags (ESTs) that are expressed specially or preferentially in the chicken hypothalamus and pituitary gland related to highly reproductive performance in Taiwan Country chickens, we constructed two reciprocal cDNA libraries using a suppression subtractive hybridization technique. Two different strains of Taiwan Country chicken, L2 (n=12) and B (n=12), which were originated from one single stain and further subjected to 40-wk egg production and body weight selection, respectively, for 19 years, were used in our study. A total of 532 and 435 clones were identified from L2-subtract-B and B-subtract-L2, hypothalamus/pituitary cDNA libraries. After performing sequencing and annotation, approximately 38% of the clones show no similarity to any known genes with only matches to identified ESTs from a broad range of organisms, including plants under specific conditions. Among known genes, prolactin (*PRL*), pituitary glycoprotein hormone alpha subunit (*CGA*), selenoprotein P (*SEPP1*), mitochondrial cytochrome oxidase Ⅱ and Ⅲ (*MTCO2* & *MTCO3*) were highly represented in the L2-subtract-B brain cDNA library. Some these genes have been validated at RNA expression level from another three commercial chicken populations including red- and black-feather Taiwan Country chickens and Leghorn layers by quantitative reverse transcription PCR.

A 019

Suppression subtraction hybridization analysis on the high temperature resistance in the guppy Poecilia reticulata

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The resistance to high temperature is one of the most important traits for aquaculture because of its large influence to the survival, growth, and maturation in the fishes. The resistance to high temperature was examined as the survival rate and/or time to death in an examined temperature, and the genetic influence to the individual, and strain differences were estimated from the pedigree analysis and strain comparison. Furthermore, some genes were estimated from QTL analysis in the rainbow trout and sex-linked inheritance of major gene was also suggested from cross experiment in the guppy. In each case, small number of gene was estimated, however, the type, products and act of gene was unknown still now. On the other hand, suppression subtraction hybridization analysis was developed for the identification of the gene, which expressing at specific condition from the comparison between different conditions. In this study, the genes were compared between the individuals in normal condition and survived individuals in 35˚C 24h treatment as high temperature resistance by suppression subtraction hybridization analysis in the guppy *Poecilia reticulata*. The obtained up-regulate and down - regulate fragment of genes were compared of their sequences with the sequence which registered on the database of DDBJ, and the functions on the high temperature condition were estimated.

A 020

Identification of betaine homocysteine S-methyltransferase in fatty livers of chicken by peptide mass fingerprinting

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Thyroidectomized chickens produce remarkable fatty livers. We carried out proteome analysis for this fatty liver. Then, we found out some proteins that were highly expressed in them. The amino acid sequence of molecular mass 45-kDa protein in these protein showed a high degree of homology with the Betaine-homocysteine S-methyltransferase (BHMT, EC 2.1.1.5) of some mammalian species with the basic local alignment search tool (BLAST). We identified this protein as chicken BHMT because there were similarities that were also observed between this 45-kDa protein and mammalian BHMT with respect to molecular mass and isoelectric behavior. Fatty livers in chicken are also observed in livers of laying hen and embryonic livers on hatch. We carried out peptide mass fingerprinting (PMF) using a high performance liquid chromatography (HPLC) to identify protein that is also highly expressed in those fatty livers. Mouse BHMT which was determined all amino acid sequence was analyzed together to compare with PMF of chicken BHMT. The result of PMF between chicken and mouse BHMT digested with lysyl endopeptidase showed a good agreement. But those patterns digested with tripsin were different a little. Main peaks in these two kinds of 45-kDa protein of PMF which were digested with lysyl endopeptidase and tripsin in laying hen liver and embryonic liver on hatching were very similar to those of chicken BHMT. As this result, it was expected that BHMT would be highly expressed in these fatty livers. The reason of why BHMT gene has been expressed in fatty liver is not clear, because this enzyme works in the amino acid, homocysteine metabolic pathway.

A 021

The Mouse Genome Informatics Database as a resource for mammalian phenotype and gene expression data

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The Mouse Genome Informatics Database (MGI, http://www.informatics.jax.org/) provides free access to extensive information on the genetics, genomics and biology of the laboratory mouse. Core data include gene and mutant descriptions, DNA and protein sequences, and mapping data. Integrated with this core information are phenotypic and expression data and comparative data on orthologous genes and human disease models. Over 11,000 phenotypic alleles, including spontaneous, targeted, transgene, conditional mutants and QTLs, are described. Researchers can also query gene expression data detailing when and where a gene is expressed and what genes are expressed in specific tissues and developmental stages. To facilitate these searches, structured vocabularies for phenotypes and mouse anatomy are provided. Comparative mapping data are curated for orthologous genes between mouse and 18 other mammalian species, including human and agriculturally important mammals, and are linked to the appropriate species-specific databases. MGI is updated with new data nightly and continually adds new datasets and enhances the user interface. MGI's dedicated User Support group is available at:
mgi-help@informatics.jax.org.

A 022

Database directed full-length cDNA generation using EST data from normalized bovine cDNA libraries

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Full-length cDNA including the 5' untranslated region (UTR), entire coding sequence, and 3' UTR are vitally important in producing accurate gene models and resolving ambiguities in gene models built solely upon computational prediction, genomic and expressed sequence tag (EST) data. Annotation of the impending bovine genome sequence assembly will benefit greatly from full-length cDNA sequence data. Therefore, we developed a database for directed full-length bovine cDNA prediction, sequencing, analysis, and annotation. To facilitate the economical production of bovine full-length cDNA, EST data produced from normalized cDNA libraries were computationally screened for potential full-length clone candidates via similarity to transcripts in the NCBI RefSeq database. Using primer walking, the entire length of the candidate clone inserts were sequenced and assembled in the database pipeline. We routinely sequenced and contiged 384 different candidate clones per plate. The pipeline is tolerant to sequencing failures so that sequencing of different clones with different walk steps may occur simultaneously on the same plate in any given sequencing run. Over 1,000 full-length cDNA have been processed through this analysis and annotation pipeline prior to submission to the NCBI FLIC (Full-Length Insert Clone) database.

A 023

HorseMap Viewer: A web based tool for visualizing and exploring the Horse Genome map

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We present the first version of HorseMap Viewer, a visualization tool designed for graphical presentation of horse gene mapping data. The tool, developed in conjunction with a separate database system, provides the user with a relatively easy way to access and compare mapping data and results. Comparison between data sets is presented graphically with colored lines and discrepancies are highlighted for visual inspection. HorseMap Viewer presents data from several resources and integrates existing databases all within a single graphical access point.
http://www.vgl.ucdavis.edu/equine/caballus/
Four classes of data are presented for each chromosome; cytogenetic (FISH), radiation hybrid, linkage and comparative. Additional data classes, map updates, and analyses can be readily integrated. Additionally, comparisons can be performed across data classes and versions. The front or cover page enables the user to directly access data for each chromosome. It also provides the option to search for specific markers. The user is also provided with the option to view and or compare all classes simultaneously as well as a "zoom" option that allows the user to change map resolution.

A 024

GenomeMapper is a software to generate and compare graphical marker maps which can be linked to online databases

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GenomeMapper is written in Java™ and therefore can be used on common operating systems such as Windows, Mac, Linux or UNIX. It is organized in three major input modes, the data, the design and the idiogram mode. In the data mode the user enters or imports data e.g. from CRI-MAP or FASTLINK. The loci are listed in a chart where the user can add a primary key for accessing data in a database. Further, the locus type, e.g. microsatellite or gene, and the mode of support for an order are required. After the data are entered, GenomeMapper automatically creates a map which can be viewed in the design mode. In this mode the user can modify the display of the map, e.g. adding zoom, a title, a legend, a centromer, and changing colors, fonts, sizes, positions, the map gradation and other features. In the idiogram mode the user can connect loci to their physical position in relation to an idiogram which has to be imported. GenomeMapper also calculates the coordinates so that the physical positions of loci do not superimpose. The generated map can be implemented in the users web application as a html page or as an applet. In a html page the map is implemented as an image where hyperlinks point to the users database. The image can have formats such as jpeg, tiff, png or Scalable Vector Graphics. If an applet is to present the map a property file is required where additional information are stored such as e.g. the appearance of the applet and optionally any desired parameters. GenomeMapper not only generates maps but it can also compare maps. It is implemented in the genome database DogMap (http://www.dogmap.ch).

A 025

Online genetic databases at the Vetsuisse Faculty, University of Berne, Switzerland

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DogMap and *DogBAC* are genetic databases internationally accessible at *http://www.dogmap.ch.* The user interfaces have been developed with Uniface 7.206 and Java. The RDBMS-System is Sybase 12.5 running on a Tru64-UNIX-System (AlphaServer 1200, 2 CPU's). In the main menu of *DogMap* you can choose 'Loci List', 'Get a printable summary on a locus', 'Chromosome maps', 'Primer List' or 'Contact'. Selecting 'Loci List' you can search for a locus, get information about the homology, polymorphism and the bibliography. Looking at the details of a locus you see the effects, category and mapping mode, get the EMBL number or by clicking on the direct link to *DogBAC Library* you can search for a clone. Choosing 'Get a printable summary on a locus' you can search for a locus and you will get a summary with reference families, primers, EMBL and bibliography for print out. Choosing 'Chromosome Maps' you get the full map and the idiogram. Clicking on a locus you get the details. In chromosome maps you can select the map (DogMap, Dalmatians, the comparison between DogMap and Dalmatians and vice versa) and a specific chromosome. Selecting 'Primer List' you see the locus symbol, locus name, the database, a link to the bibliography and the primer. You can search for a locus symbol, locus name or within a database (DogMap, Dalmatians) and get details of the locus symbol or the bibliography. In 'Contact' you can search for a member of the international collaboration.

A 026

DNA diagnosis and national program for controlling cattle genetic disorders in Japan

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Since 1990 when BLAD testing was initiated, the program to control genetic disorders (GD) in cattle has been established and is making rapid progress in Japan. The gene-testing laboratory in the Livestock Improvement Association of Japan is currently performing 7 kinds of DNA testing for cattle GDs. Japanese researchers have developed technologies, other than BLAD and CVM in Holsteins, for testing Japanese Black Cattle, the most important beef cattle in Japan. The National Guidelines to control cattle GDs have been outlined by the "Committee for Controlling Cattle GDs". This Committee is responsible for announcing the presence of new GDs, designating some as strictly controlled GDs, publishing the prevalence of the mutant genes among the population, disclosing the carrier sires, and recommending the monitoring of the frequencies of carriers among the population. To fulfill this system, many institutions involved in the animal husbandry industry are cooperating with each other. Preliminary information from local veterinarians is to be accumulated by committee members of the Japanese Society of Veterinary Science and the Society for Veterinary Clinical Genetics. Intensive research on clinical, pathological, and etiological studies are performed in the veterinary laboratories. The cattle genome project team works to identify the gene responsible for the disorders, to specify the mutation, and to develop the technology of DNA diagnosis. Cattle improvement organizations including AI societies and registration societies are to follow these guidelines. In this manner, the incidence of GDs in cattle is effectively controlled in Japan.

A 027

Italian Horse Racing Population and forensic certified DNA database: high throughput DNA isolation and STR analysis using robotic sample processors

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In Italy in 2002 legislation became effective to establish "Horse Competition Court Prosecutors" involved in investigations about "crimes" committed in the "Racing Horse World" like frauds about bets (substitution of standardbred with thoroughbred during competitions and reproduction, doping, ill-treatment, etc.), and the necessity occurred to set up a centralized DNA database with an automated procedure to analyse all the samples of interest for genetic identification, paternity tests, kinship tests and future comparison. For the first time, forensic procedures were applied on animals to the entire process, from sample collection to diagnosis in order to certify all the steps for possible use in the "Horse Competition Court".
Two automatic workstations were set up with different protocols, able to isolate DNA from several kind of sample like hair roots, blood, saliva, urine, trace and finally to set up PCR and electrophoresis mix. FTA Classic Cards were chosen for DNA storage and a magnetic beads based procedure was used for DNA extraction in order to obtain high quality purified nucleic acid already quantified for PCR. A new 17 loci five colours kit was the only possible way for genotyping using a certified kit patented for horse identification and forensic use. Analysis and database management software were also selected between the forensic patented ones. Through these procedures is possible to process with just one complete robotic station easily 576 samples each day that means more than 200.000 samples per year with only two operators. Complete routine diagnosis is possible from 3 to 24 hours depending on sample and question requested (simple profile, paternity test, kinship test, forensic analysis). Because of the potential productivity of this kind of system, it has been set up for all the available animal forensic patented kits like cattle, dogs and for human identification of course.

A 028

The impact of canine inbreeding on likelihood calculations for the significance of DNA matches in forensic investigations

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Canine microsatellite loci, successfully amplified from dog hairs and other sample types, have contributed to over 20 criminal investigations since 1996. Evidence from these investigations has been admitted in nine criminal trials in the United States and Great Britain. Analysis was performed with a commercial PCR multiplex kit consisting of 1 tri- and 9 tetranucleotide repeat loci. An additional multiplex of 7 tetranucleotide loci was used in many cases. A database of 558 dogs has been examined for both Hardy Weinberg (HW) equilibrium and linkage equilibrium at the 17 loci. Analysis showed significant HW disequilibrium (non-random allele associations within loci) but no evidence of linkage disequilibrium (associations between loci). Inbreeding in canine populations is ten-fold higher than human populations; average θ values ranging from 0.08-0.15 in dogs are in marked contrast to the θ value of 0.01 found in humans. Thus, the high θ value in dogs plays an important role in DNA match calculations. In 1996 the US National Reseat Council recommended the use of a likelihood ratio to report the significance of a DNA match. In the likelihood ratio the numerator is the prosecution's explanation of the events; that the evidence sample(s) matches the reference sample because they came from the same dog (and is, thus, equal to 1). The denominator of the ratio is the defense's explanation of the events and is generally that the match has occurred by random chance (though there may be other explanations). Thus the denominator is generally the match probability based on the product of genotypic frequencies for each locus as determined by the allele frequencies in the database. The θ value is incorporated into the calculation of the genotype frequencies and corrects the denominator for the inbreeding found in canine populations. The use of a conservative θ value guarantees the defense a fair likelihood calculation. The canine microsatellites described, however, are sufficiently informative that a DNA match at six loci or more gives very strong support to the prosecution's case.

A 029

Bias in allele frequency estimates for RAPD loci at different levels of inbreeding

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Two "phenotypes" of a RAPD marker locus are characterized by the presence or absence of specific DNA sequences and the heterozygous genotype in a locus is not distinguished from the dominant homozygous. Therefore, assuming HWE in order to estimate allelic frequencies for such markers is required. In practice this requirement is rarely met. It is known that inbreeding would increase the number of homozygous genotypes and decrease the number of heterozygous ones, hence, introducing departure from HWE. This study quantifies this departure from HWE. Simulation was used to produce populations with finite size. The bias in frequency estimates of marker alleles was quantified by comparing true allelic frequencies with those obtained as square roots of the frequency of recessive homozygous. Results of this study indicate that allelic frequency estimates could be heavily biased when true frequency of the recessive allele is low and sampled individuals have moderate to high levels of inbreeding. When the true frequency of the recessive allele is equal to 0.05, an inbreeding coefficient of 0.20 would double the frequency estimate while an inbreeding coefficient of 0.40 would triple the estimate. The same levels of inbreeding would only cause a mild increase in estimates for the recessive allele if the actual frequency is equal to 0.95. Results suggest the need for correcting such bias in estimates for RAPD loci when information is obtained from individuals not in HWE. Making such correction is very important when true frequency of the null allele is low. Methods of correcting such bias is discussed.

A 031

The BovMAS Consortium: An approximate interval mapping procedure for selective DNA pooling

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Selective DNA pooling is an efficient method for QTL detection. Applied to a Daughter Design, milk samples of offspring with extreme phenotypic values for a trait of interest are assigned to high and low pools, respectively, and within each pool the pooled DNA is subsequently used for genotyping. A single marker test for linkage between marker and QTL is based on the estimation of marker allele frequency differences between the high and low pools. Given the test statistics at marker positions for which a sire was heterozygous, an approximate multiple marker method was developed to predict test statistics for markers for which a sire was homozygous or at any other location on the chromosome, to extract maximum information on linkage. A simple selection index analogy was used to make multipoint predictions, taking into account that the prediction of a test statistic at location j, given an observed test statistic on location i, is only a function of the genetic distance and hence the recombination rate. This method was applied to single sire and across sire analysis for data from a selective DNA pooling experiment. Results indicate that the method may increase power of detection and map resolution.

A 032

Use of across-family QTL mapping analysis to reduce the false discovery rate associated with large numbers of analyses

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QTL mapping often involves a high number of analyses, resulting in the detection of numerous QTL including a number of false positives. As the subsequent cost of following a false-positive is high, approaches which reduce the false discovery rate (FDR) are extremely valuable. Our experience with a QTL mapping experiment for production traits in sheep, involving interval mapping over 5 chromosomal regions for ~45 traits in 15 half-sib families, is that the FDR is significantly reduced by using across family analysis compared to within family analysis. Under across family analysis the number of QTL significant at the 5% and 1% chromosome wide empirical levels were, respectively, 25 and 8 compared to expected numbers of 11 and 2 (i.e. a FDR of 0.44 and 0.25). In comparison, for within family analysis the number of QTL significant at the 5% and 1% levels were, respectively, 159 and 36 compared to expected numbers of 158 and 32 (i.e. a FDR of 0.99 and 0.89). The across family analysis was generally significant when the within family analysis showed a significant or near significant QTL for multiple families, although in one case the across family analysis was significant when the within family analysis showed a significant but large (α=1.6 σ_P) QTL in one family only. It was interesting that of the 15 families analysed, the maximal number of families segregating at the 5% level for any particular QTL was 4, despite the number of QTL detected per family ranging from 5 to 16.

A 033

Pedigree transmission disequilibrium test for QTL fine mapping

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Although linkage analysis has been proved a successful method for QTL mapping in farm animals, it usually maps QTL on a region of 10-20cM, this resolution is too poor to be used to identify QTL. Therefore, exploring new method on QTL fine mapping is important and a precondition for QTL positional cloning. In human genetics, the transmission disequilibrium test (TDT) has received great attention recently in locating human disease genes due to its simplicity and powerfulness. But TDT only deals with data from independent nuclear families and information will be lost for extended pedigree which incorporates information not only from parents and siblings but also from other relatives. In this study, pedigree transmission disequilibrium test (PTDT) is proposed, which can be used to analyze the extended pedigree for threshold traits in farm animals. And three transforming methods of within mixed-family selection, within full-sib family selection and EBV (Evaluation Breeding Value) selection are presented, too. Using these three transforming methods, quantitative traits can be converted into threshold traits and analyzed by PTDT. The power and type I error of PTDT for quantitative traits are investigated by Monte Carlo simulation. It is shown that PTDT is a robust and valid approach for mapping QTL of quantitative traits, and among the three transforming methods, within mixed-family selection is most effective under different parameter combinations. These results indicate that PTDT can be a new approach for QTL fine mapping based on the existing coarse mapping information.

A 034

Testing population-wide versus within-family linkage disequilibria using large half-sib families

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Large half-sib families are widely used for testing linkage disequilibrium within families. This is, the association of marker alleles with alleles at the QTL is tested using the performance of the offspring inheriting alternative marker alleles. This approach has proven to be efficient to map QTL in cattle and sheep. However, determining which candidate gene segregating in the chromosomal fragment is the causal mutation has been proven to be a difficult task because strong linkage disequilibrium within families requires a very large number of meiosis to discriminate between linked candidate genes in that chromosomal area. In this study, we propose the use maximum likelihood methods to estimate within-family and population-wide linkage disequilibria using offspring performance and the allele inherited from sire and dam. It allows testing population-wide linkage disequilibrium of candidate genes in a chromosomal interval where QTL have been previously detected. A likelihood ratio statistics is proposed to test if both disequilibria are statistically different. For a two alleles model (e.g. SNP at he candidate gene) allele frequencies at the dam population must be very different of 0.5 in order to discriminate between both disequibria. In that scenario it is proposed the use of flanking markers to determine paternal or maternal inheritance of alleles at the candidate gene for heterozygous offspring.

A 035

A simplified method for estimation of QTL effects through marker-trait association in swine populations

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To incorporate genomic information into the genetic evaluation system of Canadian Swine Improvement Program, a statistical procedure was developed for estimating QTL effects from marker-trait associations using important linked-marker information. The proposed procedure includes methodological improvements using the conventionally used gametic model as a starting point. The proposed procedure has several advantages. First, it incorporates the QTL effects into animal effects in order to reduce the number of linear model equations and save computing time. The numerator relationship matrices for QTLs are converted from gametic relationship matrices. Second, the procedure includes a reasonable approximation for constructing gametic relationship matrix due to QTL effects based on a flanking marker approach. Third, the procedure offers a simplified approach for estimating non-additive effects. Initially, during the computing phase, only additive effects are included in the mixed model equations. In the second phase, the solutions of the additive effects and the relationship matrices between dominance effects of QTLs can be used to estimate dominance effects. For polytocous animals with a large number of fullsibs such as swine, it is important to be able to estimate dominance effects. This allows removing the non-additive effects from breeding values. It also provides a way to achieve more precise QTL mapping from estimating non-additive QTL effects.

A 036

Accounting for heterogeneity of variances to improve the precision of QTL mapping in dairy cattle

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A maximum likelihood approach of QTL mapping for heterogeneous half-sib data has been developed and compared with a homogeneous maximum likelihood approach using dairy production data of 433 sons from 6 sire families. Sires and sons were genotyped for 69 microsatellite markers. The likelihood ratio statistic of heterogeneous approach is larger than that of the homogeneous approach on most of the detected putative QTL positions. Though likelihood ratios may sometimes be smaller in heterogeneous approach, the maximum log-likelihood of the heterogeneous approach is always bigger than that of the homogeneous approach, both in the full model and the reduced model. This means that the heterogeneous model always has a better fit to the data and provide more reliable conclusions in the likelihood-based hypothesis test of QTL positions. The average residual variances of the heterogeneous approach are consistently smaller than the estimated residual variances from the homogeneous approach. This is another indication of a better fit of the heterogeneous model to the data. The method developed in this study is useful for the joint analysis of the data from different experimenters, for QTL mapping of multiple crossbred populations, or for the analysis of heterogeneous purebred data.

A 037

A hierarchical model to identify gene networks underlying a quantitative trait using expression profile and molecular marker data

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Large scale mRNA studies on segregating populations have made it possible to map determinants affecting gene expression (eQTLs). Although this strategy is a more promising approach to reconstructing pathways compared with the methods using expression data only, a further problem of interest is to connect the eQTLs via networks with classical quantitative traits. The purpose of this study is to describe a model for inference of gene networks associated with a quantitative trait. We consider a crossed population derived from inbred lines, and for simplicity, assume that QTLs and eQTLs exhibit only additive effects. The observations for individuals can be described by macro - environmental, genetic and residual effects. For the second tier, the vector of the genetic effect can be modeled as $\mathbf{u} = \mathbf{WHDv} + \varepsilon$, where $\mathbf{v}$ is the vector including gene expression and QTLs effects, ε is the vector of residual genetic effects, $\mathbf{W}$ is the matrix to indicate genotypes of markers and expressed genes, $\mathbf{H}$ is the matrix containing identity matrix and eQTLs effects within networks, and $\mathbf{D}$ is the diagonal matrix whose elements are indicator variables denoting whether the gene products and/or the QTLs affect the trait or not. This model can be further extended to refine expression data by taking account of residual genetic effect for each expression. Although the method for selecting expressed genes related to the trait before eQTL analysis has been developed, in such an approach some important genes might be missed. A Bayesian hierarchical model presented here can implement the simultaneous QTL and eQTL analysis without pre-selection of genes and inference of gene networks underlying the trait.

A 038

A deterministic method for calculating the identity-by-descent matrix and its inverse based on the chromosome segment model when marker phases are unknown

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Linkage analysis of quantitative trait loci (QTLs) and marker-assisted prediction of breeding values based on a mixed linear model require the inverse of identity-by-descent (IBD) matrix. Previously, assuming that marker phases are known, we developed a recursive method to calculate the inverted IBD matrix for the chromosome segment model (CSM) considering transmission of the marked segment rather than a single marked QTL. In this study, we extend this method to allow unknown phases and investigate the properties of the resulting method. For the single QTL model (SQM), a deterministic method (DET) for constructing the IBD matrix has been available in the case of unknown phases, in which a recursive method ignoring markers not fully informative and a pair-wise fashion for sibs are combined. Then we employ the similar scheme to extend the previous method for CSM. Because DET is partly recursive, the inverted IBD matrix cannot be obtained directly, even if SQM is used. Thus, a reasonable approach to obtaining the inverse is: 1) the vector of ancestral contributions is derived by using the elements of IBD matrix themselves, and then 2) the recursive formula is used to calculate the inverse. This strategy is similar to that for handling with the total relationship matrix and our previous method for CSM, hence the substantial cost of building the inverse does not increase. By using closed forms for expected IBD proportions between sibs, the computational burden could further be reduced.

A 039

Fast computation of the identity-by-descent probabilities for a marked quantitative trait locus

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Probabilities of identity-by-descent (IBD) for marked QTL alleles are necessary for QTL analyses. Calculating IBD probabilities is, however, a time-consuming task in large populations. Implementation of a direct method in which a small subset of IBD matrix is required to compute its diagonal for a QTL linked to a marker has already been described. In this study, an indirect method in which each paternal (or maternal) group is evaluated simultaneously was applied to compute IBD within each animal at a QTL flanked by two markers. Using simulated data, a comparison was conducted between the indirect and the direct methods. Markers genotypes were assumed to be known for all animals. Probabilities that a QTL allele in an offspring descended from its sire or its dam (PDQ) were computed conditional on markers information. When the linkage phase (LP) between two flanking markers was not certain, probabilities of LPs were obtained using information of progeny with certain LP and probabilities of LPs of parents. With the simulated data, the indirect algorithm turned out to be approximately 2.3 times faster, and needs substantially less memory, than the direct algorithm, when 30 generations evaluated. The computation time of the indirect algorithm depends highly on the number of sires or dams (whichever is less), therefore relative results might be different according to the population structure. Since the number of markers linked to a QTL is involved in the calculation of PDQs, the current indirect algorithm would be applicable to any configuration of markers and a QTL assuming known PDQs.

A 040

Effects of inaccurate variance estimates on marker-assisted empirical BLUP of breeding values using single QTL and marker-haplotype models

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The objective of this study was, using a stochastic simulation, to examine effects of using inaccurate values of variance components on the accuracies of the marker-assisted empirical best linear unbiased prediction of breeding values employing information on upstream as well as flanking markers. Using an additive finite loci model, some true genetic settings in which a single QTL was bracketed with two franking and upstream markers were simulated. Heritability of a quantitative trait was .25, and the fraction of the additive genetic variance due to the marked QTL to the total genetic variance 30%. Five non-overlapping generations with random mating were generated. Three mixed linear models, or the so-called animal models, employed were: the conventional polygene model using no marker information (CM), the single QTL model (SM), and the marker-haplotype model (HM). Relative to CM, accuracies of the marked QTL effects predicted with SM and HM were approximately 20 to 45% high. For comparison between SM and HM, accuracies of total breeding values predicted with HM were consistently lower than those with SM, resulting in the values almost equal to those with SM in some cases. For example, when overestimated marked QTL variances with the true variance for the remaining polygenes were used, the accuracies with SM were totally higher than those with HM for all the settings of maker interval, the number of marker alleles and the QTL position.

A 041

Segregation analysis with mixed inheritance models for binary traits in livestock: A comparison between Bayesian and maximum likelihood methods

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Many disorders in animals are expressed in a categorical way and generally they do not show a simple Mendelian mode of inheritance. To find the presence of a single major locus segregating in a population and to clarify its mode of inheritance is one of the important tasks of animal genetics. Knowing the mode of inheritance of genetic disorders is a prerequisite for linkage analysis. In this study we developed a Bayesian approach to analyze the segregation of binary traits based on the mixed inheritance model with Gibbs sampling. Our method was compared with the maximum likelihood (ML) approach applied in the PAP program by using simulated half-sib populations. Power of detection of the major locus and precisions of the estimated parameters were investigated (allele frequency, dominance effect, displacement, heritability of polygenes). The power was better with the ML method but the precisions of the estimates were rather lower than with our Bayesian method. The latter method showed no false positive detection of major locus. Further it could handle complex pedigrees more easily. In conclusion, the ML method cannot be replaced by the proposed Bayesian method; both methods have their own merits. The Bayesian method, however, can compensate the insufficiencies of the ML method for segregation analysis for binary traits in livestock populations.

A 042

Evaluation of degree and amount of heterogeneous variance for carcass weight in Japanese Black cattle

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Heterogeneous variances among contemporary groups (CG) in population data are a potential source of bias in livestock genetic evaluation. The degree and amount of heterogeneous phenotypic variance for carcass weight in the Japanese Black cattle population were evaluated and a simple method was developed to account for the heterogeneity. The impact of this method on the genetic evaluation was investigated too. Carcass weight records were collected from steers slaughtered between 1993 and 2002. In total 54,058 observations were grouped in 1,580 farm-market-year-sex CGs. The heterogeneous phenotypic variance and the mean within CG were estimated by an empirical Bayesian method simultaneously. The connections between CGs were obtained through common prior distributions. These estimators were used to adapt the heterogeneity through a pre-adjustment of records to a standardized baseline variance. In the uncorrected data, the absolute predicted breeding values were significantly biased among the three classes of phenotypic variances. These three classes were formed according to the level of the phenotypic variance (large, medium and small). By the adjustment, however, the bias was reduced, and the index of inequality was reduced by 49%. Spearman's correlation coefficients of elite sires and dams (top 1%) between before and after adjustments were 0.93 and 0.83, respectively. These results suggest that selection of dams becomes more accurate by the procedure described here.

A 044

Research on selection and breeding for two high production SM duck lines in Vietnam

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To have the high egg/ meat production duck lines from exotic Super Meat type ducks (SM) to reduce the cost for duck production, the research was starting from T_1 (male line) and T_4 (female line) by individual selection method. The aim focused into high-meat production trait for T_5 (from T_1) and high-egg production trait for T_6 (from T_4) lines through 4 generations. The results achieved that: T_5 showed 60 - 115 g weight gain per bird, higher than T_1 ($h^2 = 0.75$; $p < 0.01$) at 7 weeks of age; egg production at 68 weeks of age was 223 - 232 eggs / female ($h^2 = 0.46$; $p < 0.05$). T_6 showed egg production higher than T_4 at 68 weeks of age (235.6 - 249.3 eggs / female; $h^2 = 0.55$; $p < 0.01$); h^2 of body weight gain was 0.57 ($p < 0.01$); h^2 of hatchability was 0.21 ($p > 0.05$). Selection response Re = 1.71 - 10.32 egg per female; crossed ducks from $T_5 \times T_6$ have super dominance with heterosis is 10.2%; body weight at 7 weeks of age is 3154.2 g per bird; feed conversion is 2.35 kg per kg weight gain. T_5, T_6 and crossed ducks from $T_5 \times T_6$ is divised into households and appreciated by farmers for more benefits.

SECTION B

MHC Immunogenetics

B 001

Construction and Validation of the Expression Array in MHC Region in Pig

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In different studies, the region surrounding the major histocompatibility complex (MHC) region contains QTL influencing many traits such as growth, back fat thickness, carcass composition and immune response. To help identification of responsible genes, a transcriptomic strategy that integrates comparative mapping, bioinformatics, and expression arrays were developed. Here, we report the construction of an expression array specific to the extended MHC region in pig. 655 genes located on the 35 Mb chromosome7q region harboring and flanking SLA region were selected to be included in the chips. cDNA clones coming from 1PIG and 2PIG MARC libraries (Scott C. 2002) and INRA cDNA libraries were chosen by BLAST against the available sequence from pig and human. Primers were designed according to the sequence coming from its human homologues genes and to amplify and subcloned from pig genomic DNA to develop specific probe represent the genes absent in the cDNA libraries. Arrays were printed on nylon membranes and hybridizd by the 33P labeled complex cDNAs. RNA samples isolated from liver, adrenal, muscles and fat tissue from Meishan and Large White pig breeds were employed to perform hybridization. Our result has validate the construction of this array, further analysis demonstrate the power of this strategy for identifying candidate genes encoding QTLs for important economic traits. This array will be also be helpful for study the immune response in pigs as well.

B 002

Porcine EST database constructed with full-length cDNA libraries and its utilization for the SLA research

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Collection of cDNA clones containing full-length CDS is beneficial for analyses using protein products translated from the genes, such as the preparation of antibodies against porcine molecules. We constructed full-length cDNA libraries with 10 porcine tissues, and performed large-scale EST analysis from 5'-ends. The 120,603 reads, obtained to date, were clustered and assembled into 7,204 contigs and 42,359 singlets, and the assemblies were subjected to BLAST search to the nucleotide sequences of mammalian genes and the human genome. The assembled sequences and their BLAST search results were stored into a database, equipped with web interfaces for various kinds of searches (http://pede.dna.affrc.go.jp/). The database, Pig EST Data Explorer (PEDE), enables to utilize porcine cDNA clones, estimated to include full-length of 6,000 different genes at least. We are also constructing an expressed SLA database in PEDE, based on the SLA cDNA sequences extracted from the cDNA clones. The expressed SLA database also provides an interface to discriminate from and identify with the known SLA alleles with the sequence in the library and those of users. The PEDE database contributes for exploring genes that may be responsible for traits like disease susceptibility. (This work was supported by MAFF, Japan and JRA.)

B 003

Genomic structure analysis of a 238 kb segment between the *TRIM15* and *UBD* genes in the *SLA* class I region

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Genomic sequences of the 2.37 Mb entire swine leukocyte antigen (SLA) region have been recently determined. In this study, we analyzed the genomic structure of a 237,633 bp long segment spanning the *TRIM15* to *UBD* genes located in the telomeric side of the classical *SLA* class I gene cluster. Fourteen genes including ring finger-related family genes and olfactory receptor genes were identified in this segment. Genomic sequences obtained in this 238 kb segment and a 342 kb segment containing the classical *SLA* class I gene cluster were compared to those of the corresponding 1,030 kb human *MHC* gene segment. A human segment spanning about 350 kb from the *HLA-J* to *HLA-F* genes was absent from the 238 kb swine segment spanning the *TRIM15* to *UBD* genes. Furthermore, the gene order of a swine segment carrying the classical *SLA* class I gene cluster was markedly different from that of a human segment around the *HLA-92* gene. In contrast, the gene order of the *MHC* non-class I genes was highly conserved between the human and swine. Therefore, taken together, the remarkable comparative differences between the gene organization of the swine and human *MHC* class I regions further highlight the power and diversity of evolutionary forces acting selectively on different genomic regions of the *MHC*.

B 004

Boundary of insertion of the genomic region in porcine MHC extended-class II is identical with that in the murine genome

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A major QTL for backfat thickness had been detected at the q-arm of SSC7 in our swine resource family. The region of this QTL was restricted to the portion including the outer region of the major histocompatibility complex (MHC) class Ⅱ region, designated "extended-class Ⅱ" and those in rodents includes MHC class I loci such as H-2K and its pseudogene in mice, whereas no class I loci has been observed in other mammals like primates. We constructed a BAC contig that encompassed this QTL, and completely determined the nucleotide sequence of three BAC clones in the contig, which included the distal side of the extended class Ⅱ region in the porcine genome. The nucleotide sequence elucidated precise genomic structure of the two non-MHC genes that were located in the porcine extended-class Ⅱ, BPAG1 and COL21A1, the counterparts of which are located in the region apart from the MHC and its "extended" region in humans and mice. Surprisingly, the distal boundary of the insertion of a genomic region in the porcine extended-class Ⅱ was exactly identical with that in the murine genome. This suggests the genomic instability at the specific site in the MHC-encoding region, and contributes the research of the evolution of MHC. (This work was supported by the Grant-in-Aid from the Japan Racing Association, and the Animal Genome Research Project by MAFF, Japan.)

B 006

Nucleotide sequences and molecular evolution of the bovine major histocompatibility complex (BoLA) class I genes

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To date, more than 28 cDNA clones coding for bovine major histocompatibility complex (MHC) (BoLA) class I antigen have been isolated, whereas that of Japanese black is not yet obtained. Therefore, in this study, two full-length cDNAs for BoLA class I antigen were isolated from the cDNA library constructed from the bovine lymphoid cell line BLSC KU-1 by PCR and characterized. Both alleles, with 1092-bp insert, encoded a translated product of 363 amino acids, which included signal peptide (SP), alpha1, alpha2, alpha3, transmembrane (TM) and cytoplasmic tail (CY) domain. The conserved cysteine residues that form the intramolecular disulfide bridges in the alpha2 and alpha3, as well as the conserved site for potential N-linked glycosylation can all be found. Comparison of the sequences and construction of a phylogenetic tree among our clones and 28 distinct BoLA class I genes previously isolated revealed that both clones were different from those of all previously characterized BoLA class I alleles. Moreover, the extent of identity between bovine protein and putative primate, rodent, artiodactyls and perissodactyl proteins is rather high, ranging from 77% to 89%. In addition, this analysis showed that cattle and sheep were the most closely related. On the other hand, we determined sequences of TM, CY, 3'-untranslated region and compared with those of other BoLA class I genes. It appeared that these sequences were classified in 3 major groups and our clones belonged to same group.

B 007

Cloning of cDNAs and molecular evolution of *DO* and *TAP* genes mapped at class IIb region of bovine major histocompatibility complex (BoLA)

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The bovine major histocompatibility complex (MHC) (*BoLA*) class II genes are located within two distinct regions of chromosome 23: class IIa which contains the classical class II genes, *DR* and *DQ*, and class IIb which contains the non-classical class II genes, *DO, DY* and *DM*. Moreover, the class IIb contains bovine *TAP* and *LMP* genes. However, the expression of *DO, LMP* and *TAP* genes is not yet determined. In this study, cDNA clones encoding BoLA-DO alpha- and beta-chains, and TAP1 and TAP2 were isolated from mRNA of cattle peripheral blood lymphocytes or a cDNA library in pCDM8 that had been derived from bovine lymphoid cell line. The *BoLA-DOA* cDNA clone encoded a primary translated product of 250 amino acids (aa), which included a signal peptide of 24aa and a mature polypeptide of 225aa. The *DOB* cDNA clones encoded a primary translated product of 271aa, with a signal peptide of 26aa and a mature polypeptide of 247aa. Comparison of sequences among several species and construction of phylogenetic tree revealed that cattle and sheep were the most closely related. Next, we obtain bovine *TAP1* and *TAP2* cDNA clones. Both of two genes were conserved 6 transmembrane, 2 peptide binding domain, Walker A and B, and ABC domain. Bovine *TAP1* and *TAP2* exhibited 86% and 81% identities to human *TAP1* and *TAP2*, respectively. It appeared that bovine *TAP2* has polymorphism in its peptide binding domain, suggesting that *TAP2* allele may affect to antigen presentation and regulated cellular immunoresponse via class I-peptide-TCR association.

B 008

Polymorphism of Bovine Lymphocyte Antigen DRB3.2 Alleles in Iranian Holstein Cattle.

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Breeding goals for dairy cattle have focused mainly on increasing productivity and have ignored disease resistance. Molecular techniques have been developed for identification of bovine genes responsible for production traits and host immunity. In addition, these techniques have been used to clarify the potential association of bovine major histocompatibility (MHC) or bovine leukocyte antigen (BoLA) alleles with disease resistance and production. In this study polymorphism of the second exon of BoLA-DRB3 gene of 96 Iranian cattle was investigated. Genomic DNA extracted from whole blood samples and two-step polymerase chain reaction (PCR) was carried out in order to amplify a 284 bp fragment of target gene. Nested-PCR products were digested with three restriction endonucleases RsaI, BstYI and HaeIII. Digested fragments were analyzed by Polyacrylamid gel electrophoresis. Twenty-two BoLA-DRB3 alleles were distinguished with frequencies ranging from 0.5 to 19.3%. Identified alleles include: BoLA-DRB3.2*3, *6, *7, *8, *9, *10, *11, *12, *13, *14, *15, *16, *20, *21, *22, *23, *24, *25, *27, *28, *32 and *51. Their frequencies found to be 2.6, 2.6, 1.0, 13.5, 0.5, .05, 13.0, 1.6, 1.6, 0.5, 2.6, 14.1, 0.5, 1.6, 7.3, 5.2, 19.3, 2.1, 2.1, 1.6, 0.5 and 5.7% respectively. The most frequent alleles (BoLA-DRB3*8, *11, *16, *22 and *24) accounted for 67.2% of the observed alleles. Results of this study indicate that BoLA-DRB3 locus is highly polymorphic among tested animals.

B 009

Identification of SBT (Sequence Based Typing) alleles for BoLA DRB3 exon 2 in Hanwoo (Korean Cattle)

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The bovine leukocyte antigen (BoLA) system is the major histocompatibility complex (MHC) of cattle. The genes located in the MHC class II region encode glycoproteins that are composed of alpha- and beta-chains and are expressed on the surface of antigen presenting cells. Within the class II region, the DRB3 gene displays a high degree of polymorphism, with at least 90 different previously identified alleles and most of the polymorphisms are located in the second exon, which encodes peptide-binding residues. Previous researches indicated that the DRB3 gene had correlations with various disease traits as well as some production traits in cattle. As a first step to find new DRB3 alleles in Hanwoo and improve their disease resistance status based on DRB3 typing, 281 bp of partial DRB3 exon 2 from 20 unrelated Hanwoo was direct-sequenced and their allele types were identified. Preliminary results show that 30 polymorphic nucleotide sites were identified including previously known PCR-RFLP sites. Further researches about these DRB3 exon 2 alleles can give general strategy for improving the disease resistance in Hanwoo.

B 010

Linkage analysis between the upstream regulatory region and the second exon of bovine *DRB3* gene

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The *BoLA-DRB3* gene extended polymorphism are located mainly in the second exon that encode the antigen binding site (ABS) of the molecule. Additional variation have also been reported in the promoter and in the third exon. It can be hypothesized that the exon 2 and the promoter (URR) are under different types of natural selection. In order to underline the coevolution of these sequences that are tightly linked and probably suffer different types of selection, we analyzed the polymorphism within each, URR and exon 2 of the gene. The URR and the exon 2 of DRB3 gene were genotyped by PCR-SSCP and -RFLP respectively, in 112 DNA samples from 12 cattle breeds. All URR variants and some DRB3.2 alleles were cloned and sequenced. We associate the seven URR variants detected in the sample studied with the exon 2 genotypes. This study may evidence the following: (i) lack of a clear pattern of linkage association between the URR and exon 2; (ii) the most abundant URR variants were spreading along the entire neighbour-joining tree of DRB3 exon 2 peptide sequences, while rare alleles were restricted to a specific cluster; (iii) each URR variant corresponded to more than one DRB3.2 allele; and (iv) any DRB3 type could be associated with more than one URR variant. A plausible explanation for these evidences was that the URR variation are stable, and old polymorphism was purified by positive selection, while the exon 2 alleles were originated by both point mutation and gene conversion and maintained by overdominace selection.

B 011

Allelic frequency of PCR-RFLP type of BoLA DRB3 in the Japanese Holstein herds and the relation to mastitis

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Holstein Cows (n=703) from 26 Dairy Herds in Tama Area, Tokyo were examined for polymorphisms of the second exon of the BoLA-DRB3 gene, using the polymerase chain reaction (PCR), followed by digestion of the amplified fragments with three restriction endonucleases. 35 alleles were observed in the cows and allelic frequencies ranged from 13.7 to < 1%. DRB3.2*16 had the highest allelic frequency (13.7%), followed by DRB3.2*23 (10.2%), DRB3.2*13 (9.6%) and DRB3.2*8 (8.8%). 12 alleles (16, 23, 13, 8, 15, 10, 2, 9, 22, 24, 12 and 11 in order) had frequencies >2%. 86.9% of the whole was occupied by these 12 alleles. Somatic cells were used to classify mastitis cows that had one test of SCC > 500,000 or two consecutive tests of SCC > 300,000 in their milking records. 212 of 703 cows were mastitis (30.2%). Allelic number of DRB 3.2*16 had the highest rate to the total number of alleles of mastitis cows (15.6%). However, the individual cows that have a one copy at least for every allelic type were collected and calculated the rate of the mastitis cow in each allelic type, DRB 3.2*12 had the highest rate (40.0%), followed by DRB 3.2*24(39.7%) and DRB 3.2*16 (34.4%).

B 012

Characterization of bovine MHC (*BoLA*)-*DQA1* and *DQA2* of bovine leukemia virus-infected cattle by using newly established PCR-sequence based typing

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Bovine leukemia virus (BLV) is an exogenous retrovirus that is manifested as 3 progressive stage of disease, an aleukemic healthy stage, persistent lymphocytosis and development of B lymphoma. Previously we described that progression of BLV-induced lymphoma correlates with polymorphism of bovine MHC *(BoLA)-DRB3* gene. However, the association between alleles of *BoLA-DQA* functional genes being highly polymorphic and BLV-induced lymphoma is still unknown. In this study, we developed the technique of PCR-sequence based typing which can assign *BoLA-DQA1* and *DQA2* alleles of large numbers of animals in DNA sequence level and identified these alleles of BLV-infected cattle. We firstly determined the intron sequence around exon 2 of *DQA1* and *DQA2*, designed the specific primers to amplify two exon 2 and determined sequences of two amplicons by direct-sequencing. Next, we tested 29 BLV-infected but healthy cattle and 23 cattle with lymphoma by the established method in order to characterize BLV-induced leukemogenesis. Twelve distinct *DQA1* alleles and 7 distinct *DQA2* alleles were identified in these animals. The proportion of aleukemic animals positive for *DQA1*0101* (37%) was significant higher than that of animals with lymphoma (0%) (p=0.001185). This result suggests the *DQA1*0101* allele is associated with resistance to BLV-induced leukemogenesis.

B 013

Identification of cosmid clones spanning the sheep MHC region

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Previous research completed at Curtin and other places have demonstrated an association between genetic markers within the class I region of the sheep major histocompatibility complex (MHC) and resistance to parasitic nematodes. This project aims to extend the study of these associations by using single nucleotide polymorphisms (SNP's) as markers for haplotypic variation. A novel method has been employed to identify sheep cosmid clones containing genes from the MHC region. Mouse mRNA sequences, were aligned with human MHC genomic DNA sequences. Primers were identified on the basis of highly conserved regions within a single exon and sheep DNA amplified using PCR. Nine probes including CAT56 and UK genes in the class I region, RING3 and TAPBP genes in the class Ⅱ region and BAT3, BAT4, NTH, G6D, and G7c genes in the class Ⅲ region. Hybridisation of sheep cosmid library with TAPASINBP probe has identified cosmid clones containing the TAPBP gene. Subsequent sequencing has revealed that this cosmid spans the region from B3GALT4, BING4, HKE2, RGL2, TAPBP, and BING1 gene. Meanwhile, another cosmid clone containing CAT56 gene in the class I region has also been identified. Cosmids spanning the class Ⅲ region from C2 to TNXB gene have also previously been identified. Primers have been designed based on the end sequences of these known cosmid clones and will be used to generate probes to identify overlapping regions. More detailed sequencing of these sheep genes will then facilitate the detection of polymorphic genetic markers referred to as short tandem repeats (STR) and single nucleotide polymorphisms (SNPs).

B 014

Optimization of Mhc-DRB1 gene typing and association with Maedi-Visna virus infection in sheep

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Maedi-Visna is a slow, progressive and fatal lymphoproliferative disease of sheep associated with infection by a lentivirus of the Retroviridae family. It is distributed worldwide and neither antiviral treatment nor vaccination is available. In other retroviral infections, such as EBL in cattle or CAE in goats, polymorphism at the MHC has been related with disease susceptibility, so we started looking for associations in the MHC Class Ⅱ DRB1 gene of this genomic region. The genetic polymorphism in the second exon of this gene was analyzed in a sample of Basque Latxa breed sheep. The detection of some familial incompatibilities and the low heterozygosity led us to consider the presence of one or more non-amplifying alleles. Therefore, we changed the PCR primers and conditions. After SSCP analysis, 18 different alleles were detected in the analysed material, 8 of them never described before. The observed heterozygosity was 92 %. In order to detect any possible association between the susceptibility/resistance to infection and the DRB1 gene, we analysed the allele distribution in 103 infected and non-infected animals of at least 2 years old. The highest OR value (2.5) corresponds to the 1w allele, the Chi square being marginally significant at the 10% level. This means that individuals presenting this allele have a 2.5 times greater risk of being infected by the MV virus than non presenters. We are now analysing diseased animals and other MHC and non-MHC genes.

B 015

Characterization of MHC class I genes of the horse

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Recently, a contig of the horse Major Histocompatibility Complex (MHC) has been assembled by screening the CHORI-241 library for MHC class I, class Ⅱ, and framework non-immune system genes (Gustafson *et al*., 2003). This contig spans approximately 2.4 Mb and is continuous except for a break between the class Ⅱ and class Ⅲ region. Using MHC class I constant region probes, MHC class I genes have been localized to three regions of the contig: 1) between GNL1 and TRIM26 in the class I region, 2) between BAT1 of the class Ⅲ region and the MIC genes of the class I region, and 3) in the class Ⅱ region near BTNL2. Individual MHC class I genes from these regions are being sequenced using conserved primers from a recently published horse MHC class I genomic sequence (Carpenter *et al*., 2001) and from cDNA sequences of classical and non-classical MHC class I genes that were previously determined (Ellis *et al*., 1995). These studies are aided by the fact that the donor horse for the BAC library has an MHC haplotype that is very similar to one of the haplotypes studied by Ellis *et al.* (ELA-A3). This has facilitated the process of identifying the locations of known expressed MHC class I genes from the horse, and for identifying homologous alleles from different haplotypes (Barbis *et al*., 1994, Carpenter *et al*., 2001, Chung *et al*., 2003). As expected, the sequences obtained include pseudogenes, identified by stop codons or large deletions, and novel sequences, in addition to the previously described genes.

B 016

Analysis of *DLA-DRB1* exon 2 polymorphism in dogs by PCR-RFLP and PCR-SSCP

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The polymorphism of the canine histocompatibility complex (DLA) class Ⅱ *DRB1* gene was analyzed. Genomic DNA of complete blood samples was extracted from 60 Shar-pei dogs (many closely related), 10 Beagles and 10 Argentinean mastiff. The second exon of *DLA-DRB1* gene was analyzed by PCR-RFLP and PCR-SSCP methods. The fifty-four *DLA-DRB1* exon 2 previously reported sequences were analyzed for *Hae*Ⅲ, *Rsa*I and *Msp*I restriction sites. This theorical analysis showed: (1) one to four restriction sites per sequence resulting in nine restriction patterns for *Hae*Ⅲ; (2) zero to three restriction sites per sequence resulting in seven restriction patterns for *Rsa*I; (3) one to five restriction sites per sequence resulting in two to four restriction patterns for *Msp*I. Consequently, twenty eigth PCR-RFLP defined alleles could be detected by this method. In the screened samples, the PCR-RFLP analysis revealed two *Hae*Ⅲ patterns, four *Rsa*I patterns and five *Msp*I patterns. Additional patterns that did not account for known exon 2 DNA sequences were observed, suggesting the existence of novel *DLA-DRB1* alleles. PCR-SSCP analysis show up to now nine different band patterns in the sample studied. As expected both methods showed: i) few alleles were detected within each breed; ii) different alleles were observed among breeds. The improvement of this PCR-RFLP and PCR-SSCP methods should provide a simple and rapid technique for an accurate definition of *DLA-DRB1* typing in dogs and studies related to diseases.

B 017

Primary studies on the genomic DNA from the hybrids of female quail and male chicken

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The quail-chicken hybrids are important animals for molecular biological analysis of development, heat shock resistance and disease resistance of fowl and/or avian. Therefore, in the present studies, we have carried out 1) sex determination, 2) maternal inheritance for mitochondrial (mt) DNA, 3) polymorphisms of heat shock protein 70 (*HSP70*) gene, and 4) analysis of major histocompatibility complex (*Mhc*) gene, in order to clarify the basic knowledge about the characteristics of hybrid genes. 1) PCR products from W chromosome specific sequences (USP: 390bp and CHDw: 118bp) were observed in only one embryo (day 13) out of thirty hybrids. 2) The size and sequence of the PCR product for the partial *D-loop* region of mtDNA from the hybrids corresponded to those of their maternal quail (1,247bp), but disagreed with those of the paternal chicken (1,324bp). 3) RFLP pattern and base sequence of the PCR product for the peptide-binding region of *HSP70* (615bp) from the hybrids showed that the allele of *HSP70* from quail and chicken was impartially inherited to the hybrids. 4) In the Southern hybridization analysis, partial cDNA from chicken MHC class I (*BF*: 872bp) were used for probe. The hybrid genome, which was processed by restriction enzyme *Pst* I and *Pvu* Ⅱ that had two and three unique bands, respectively, were not observed in the parents. The PCR product (345bp) from an MHC class I locus (*MhcCojaB1*) of quail was observed with the quail and hybrids, but was not detected in the chicken. As mentioned above, the knowledge acquired by this study was considered to be very the useful to future molecular biological research of quail and chicken.

B 018

Characteristics of eluted endogenous peptides define repertoires for two chicken MHC *BF2* class I alleles

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Major histocompatibility complex haplotypes are known to strongly influence disease resistance in the chicken. For example, the *B21* haplotype confers resistance to the induction of tumors following infection with the highly oncogenic Marek's disease (MD) herpesvirus. It is suggested that the *B21* allele at the dominantly expressed MHC class I *BF2* locus may be responsible for the observed association between reduced MD tumor formation and *B21*. However, the basis for the association of resistance with *B21* remains undefined. To identify the peptide repertoire for BF2*21 a FLAG epitope-tagged *BF2*21* cDNA clone was expressed in RP9 cells using the RCASBP(A) vector. The FLAG_BF2*21 molecules were purified by affinity chromatography using FLAG specific monoclonal antibodies. Peptides were eluted and analyzed by LC/MS/MS. To validate our results we also analyzed peptides from BF2*13, for which a motif was previously defined. Twenty-eight peptides from BF2*13 showed strong preferences at three positions consistent with the earlier motif, but also showed strong secondary preferences at the remaining positions resulting in BF2*13 peptides all being relatively similar. In contrast, the 46 peptides from BF2*21 align at the c-terminus with leucine strongly dominating this position. Although additional strong and intermediate preferences are present, two positions were without preference. Thus BF2*21 has the capacity to bind a broad variety of peptides which may result in greater *B21* immune responsiveness.

B 019

Molecular genotype identification of the chicken major histocompatibility complex

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The chicken major histocompatibility complex (MHC) is commonly defined by serologic reactions between erythrocytes and antibodies specific to the MHC class I (BF) antigens and to the highly polymorphic MHC class IV (BG). The microsatellite marker LEI0258 is known to be physically located within the MHC, between the BG and BF regions. DNA from various serologically defined MHC haplotypes was PCR amplified with primers specific for this marker. Among twenty-six distinctive allele sizes identified, fourteen LEI0258 alleles had a unique association with a serologically defined MHC haplotype. The remaining 12 LEI0258 alleles were each found in 2 to 10 different MHC haplotypes. Some serologically well-defined MHC haplotypes shared a common LEI0258 allele size. The association between LEI0258 allele size and MHC haplotype were very consistent for the same haplotype from multiple sources. Sequence information for the region defined by LEI0258 was obtained for 14 different alleles. This marker encompasses two internal repeats whose lengths were 13bp and 12 bp, respectively. Alleles size variation ranges from 185 to 552 and is due to changes in the number of both the 12 and 13 bp repeats, plus the presence or absence of an 8 bp deletion in the unique sequence region between the repeat and the primer. This marker will be a useful tool to identify MHC haplotype in chickens particularly for the initial development of serological reagents.

B 020

Single-Strand Conformation Polymorphism (SSCP) Assays for Major Histocompatibility Complex *B* Genotyping in Thai Indigenous Chickens

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Blood samples of seventy-one Thai indigenous chickens kept at Kabinburi Livestock Research and Breeding Center, Department of Livestock Development (DLD), Ministry of Agriculture and Cooperative, Thailand, were individually withdrawn from the wing vein and precipitated for the DNA. Diluted DNA was amplified by primers from exon2 of BF and BLβ genes. The PCR products were automated sequenced and compared with MHC haplotypes in GenBank to identify MHC classI haplotypes and MHC classII haplotypes. PCR products of BF and BLβ genes were run on SSCP method to identify classI and classII haplotypes by SSCP paterns. The results of 13 classI haplotypes, 9 classII haplotypes, represented many different groups of SSCP patterns, difficultly interpreting. However, SSCP assays can be performed in laboratories having access to a PCR thermal cycler, equipment for PAGE and computers supporting commonly used graphics software. The SSCP patterns are detected by silver staining, eliminating the need to handle ^{32}P-labeled nucleic acids. This method can be used well in laboratories having nonradioactive label. It is necessary to develop this method to be appropriate for MHC haplotype typing.

B 021

Genomic diversity of quail *Mhc* (*Coja*) major expressed class IIB region elucidated by genomic sequencing

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The major histocompatibility complex (*Mhc*) contains two multigene families that encode the *Mhc* class I and class II molecules, being responsible for graft rejection and a variety of other T lymphocyte recognition phenomena. We already analyzed the nucleotide sequence of the 180-kb quail *Mhc* (*Coja* haplotype-1) and RT-PCR products of *Mhc* class IIB (*Coja IIB*), and suggested two major expressed *Coja IIB* loci (*Coja-DAB1* and *-DBB1*) of the haplotype-1 were located between *Tapasin* and *RING3* (*Tapasin-RING3*) same as the major expressed chicken *Mhc* class IIB locus (*BLB2*) (Shiina *et al*., 2004). We identified also five *Coja* haplotypes (-1 ~ -5) based on microsatellite polymorphisms, SNPs and major expressed *Coja IIB* sequences. In this study, in order to clarify the genomic diversity of *Tapasin-RING3* region among five Coja haplotypes, we isolated cosmid clones of Tapasin-RING3 positive from haplotype-2/3 and -4/5 heterozygote quails, and determined the nucleotide sequences and compared them. The haplotype-2 and -5 had only one major expressed *Coja IIB* locus, *CojaIIB-13* and *-14* respectively, in the *Tapasin-RING3* segment, though the haplotype-1 had two major expressed *Coja IIB* loci in the same region. The nucleotide sequence of *CojaIIB*-13 will be also to show that this locus is hybrid of *Coja-DAB1* and *-DBB1*. Taken together, these results suggest that genomic structure of the *Tapasin-RING3* segment from *Coja* haplotype-2 and -5 is similar to the chicken *Mhc*.

B 022

Comparative genomic analysis of two avian (quail and chicken) major histocompatibility complex regions

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We sequenced one of the quail haplotype for comparative genomic analysis with a previously sequenced haplotype of the chicken *Mhc*. The quail *Mhc* region spans 180 kb of genomic sequence, encoding a total of 41 genes compared to only 19 genes within the 92 kb chicken *Mhc*. Both species have the same basic set of gene family members that were previously described in the chicken 'minimal essential' *Mhc*. The two *Mhc* regions have a similar overall organization but differ markedly in that the quail has an expanded number of duplicated genes with 7 *class I*, 10 *class IIB*, 4 *natural killer* (*NK*), 6 *lectin* and 8 *B-G* genes. Comparisons between the quail and chicken *Mhc* class I and class II gene sequences by phylogenetic analysis showed that they were more closely related within species than between species, suggesting that the quail *Mhc* genes were duplicated after the separation of these two species from their common ancestor. The proteins encoded by the *NK* and *class I* genes are known to interact as ligands and receptors, but unlike in the quail and the chicken, the genes encoding these proteins in mammals are found on different chromosomes. The finding of NK-like genes in the quail *Mhc* strongly suggests an evolutionary connection between the NK c-type lectin-like superfamily and the *Mhc*, providing support for future studies on the NK, lectin, class I and class II interaction in birds.

B 023

Cloning and comparative analysis of swine pre-T-cell receptor α-chain gene

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In human and mouse, it has been demonstrated that the T-cell receptor (TCR) β-chain gene is rearranged and subsequently expressed prior to the rearrangement of TCR α-chain gene in immature T-cell. At this stage, TCR β-chain forms heterodimer with the pre-T-cell receptor α-chain (pTα) through disulfide bond between the two chains in association with CD3 complex. Recently, pigs have been candidate as a model animal for biomedical research. Therefore, in this study, to compare the features of swine pTα with those of human and mouse, we have attempted to obtain cDNA of swine pTα gene and its genomic segment. PCR primer pairs were designed based on the published pTα sequences of various animal species to obtain swine BAC clones containing pTα. The BAC clones thus obtained were subjected to sequencing to examine the genomic structure of swine pTα gene. Based on the putative exon sequences, primer pairs were designed to perform RT-PCR using thymus RNA as well as to determine terminal region of pTα transcripts. The swine pTα gene was shown to span about 7 kb consisting of five exons. The cDNA sequences demonstrated that alternative splicing occurred in the transcription of swine pTα gene as is the case of human and mouse. pTα polypeptide deduced from cDNA sequences were indicated to be quite different from those of human and mouse, especially in C-terminal region, suggesting that pTα chain might have different functional spectrum from those of human and mouse.

B 024

Genomic organization and generation of diversity of the porcine TCRα/δ chain gene

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T-cells express heterodimeric receptors (T-cell receptor; TCR) utilized to recognize antigens presented on the MHC and related molecules. To understand the molecular basis for generation of the large repertoire of the porcine TCRα and δ chain and difference of lineage in $\alpha\beta$ and $\gamma\delta$ T-cells in swine, we determined the germline nucleotide sequence, which was 646kb in length, encompassing all of the joining (J) and diversity (D) segments and constant (C) regions of the TCRα and δ, and most of the variable (V) segments utilized for the TCRδ chain gene. It included 36 Vα/Vδ and six Dδ segments. Among the demonstrated segments, 20 Vα/Vδ and all of the Dδ segments were confirmed to be expressed in swine by RT-PCR. In comparison with the human counterpart, apparent repetition was demonstrated around Vδ1 and its flanking sequences and Dδ segments in the porcine TCRδ germline sequence. The porcine Dδ segments were frequently used tandem in the recombination process, and several transcripts exploited at least three Dδ segments in each transcript. The structure of the porcine TCRα/δ chain gene revealed in this study demonstrates that the high diversity in the porcine TCRδ chain is caused by wide availability of V and D segments in rearrangement. (This work was supported by the Animal Genome Research Project by MAFF, Japan.)

B 025

The genomic structure of porcine T-cell receptor gamma locus: involvement of exon-shuffling in creating the Cγ3 gene

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Vertebrates have T and B cells that play essential roles in immune responses in an antigen-specific manner. T cells express heterodimeric T-cell receptors (TCR) on their cell surface and are divided into 2 major groups, $\alpha\ \beta$ T cells and $\gamma\ \delta$ T cells due to the TCRs expressed on their surface. The frequency and distribution of $\alpha\ \beta$ and $\gamma\ \delta$ T cells varies among different animals. In pigs, $\gamma\ \delta$ cells in blood comprise 10-60% of the total T-cell population, while those of mouse and human are reported to be 0.3-10%. The TCR γ chain polypeptides are encoded in the genome as variable segments (V), joining segments (J) and constant region genes (C) in mouse and human. The random assortment of the various V and J segments, as well as the junctional diversity that occurs during V J recombination, provides a repertoire for antigen recognition. Here, we determined the genomic sequences of 230 kb covering the porcine TCR Vγ , Jγ and Cγ region. We assigned the 7 V segments, 5 J segments and 4 C genes. These segments and genes span about 130 kb in this region, and the germ-line configuration is Vγ1-Vγ2-Vγ3-Vγ4-Jγ1.1-Jγ1.2-Cγ1-Vγ5-Jγ2-Cγ2-Vγ6-Jγ3-Cγ3-Vγ7-Jγ4-Cγ4. This configuration is similar to that of mouse with 4 V-J-C clusters, rather than that of human with V-J-C-J-C. The Cγ1 gene is composed of 3 exons, but the other Cγ genes, Cγ2, Cγ3 and Cγ4 contain 5 exons. The exon 1 of the Cγ3 gene shows 99% homology with the exon 1 of the Cγ4 gene, and the exons 2 to 4 show 96% homology with the exons 2 to 4 of the Cγ2 gene. We found the SINE (short interspersed nuclear element) sequences at the recombination junctions in the introns. The Cγ3 gene was deduced to have been created from the Cγ2 and Cγ4 genes by exon shuffling.

B 026

Porcine T cell receptor genes: the genomic structure and the expression in piglets

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The immune system recognizes a wide range of polymorphic antigens by creating a diverse set of antigen receptors; i.e., Immunoglobulin Heavy chains and Light chains, and T-cell receptor (TCR) α-, β-, γ- and δ- chains. TCR chains form either $\alpha\beta$ complex or $\gamma\delta$ complex that are expressed in two different classes of T-cells. Porcine TCR gene cDNA clones were isolated from thymi and peripheral blood monocytes of piglets including TCR α-, β-, γ- and δ- chains, and CD3γ and δ chains. We also analyzed the genomic sequences of these TCR chains and other genes involved in T-cell functions including RAG-1 (AB091392), RAG-2(AB091391), IL-2(AB041341) and Fas-ligand (AB069764: accession# DDBJ/NCBI/EMBL). In the genomic sequence of TCR β-chain (AB079894) from LW strain, we found 2 D- and 14 J- gene segments including 2 pseudo J-gene segments, Jβ1.5 and Jβ1.7 without cDNA expression. On the other hand, NIH miniature swine expressed Jβ1.5 gene segments indicating strain polymorphism of this J-gene segment. The polymorphisms in TCR genes may indicate the difference in the T cell reactivity among various strains towards pathogens. It is important to investigate whether these polymorphisms could be used as markers to select for the swine strains with stronger acquired immunity. (This work was supported by the Animal Genome Research Project by MAFF, Japan.)

B 027

Porcine Mx2 gene: cDNA isolation, genomic analysis and mRNA expression

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An interferon-inducible Mx protein was isolated in various animals. Two *Mx* genes have been found in some species, and much about the difference of their antiviral activity has been known. In the pig, two *Mx* genes, *Mx1* and *Mx2*, have also been detected. As for the porcine *Mx2*, only a partial sequence has been determined so far. In this study, we have isolated a full-length *Mx2* cDNA of the pig. The amino acid sequence deduced from porcine *Mx2* cDNA showed a high similarity to those of bovine Mx2 and human MxB proteins. The porcine *Mx2* as well as *Mx* genes of other animals expressed in cultured cells by IFN induction. On the other hand, it was expressed weakly in various tissues without IFN treatment. Genomic organization of porcine *Mx2* gene was determined using a BAC clone. As a result, it was revealed that the porcine *Mx2* consisted of 14 exons and existed tandem with porcine *Mx1* gene. We mapped the locus of porcine *Mx* genes to SSC13 using radiation hybrid panels. We previously reported a genetic variation in the porcine *Mx1* that anticipated the relationship to the antiviral activity. Single nucleotide polymorphisms of *Mx2* were detected in several exons. This gene might be one of candidate genes for disease resistance in farm animals. (This work was supported by JRA and MAFF, JAPAN.)

B 028

Molecular characterization of novel porcine CD1 genes and their genomic structure

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T lymphocytes recognize wide variety of antigens including peptides, glycolipids and phosphorylated metabolites presented by MHC or related molecules. It has been revealed that lipid antigens such as lipopeptides of mycobacteria and marine sponge-derived glycolipid (α-galactosylceramide) are recognized by human and murine TCRs in the context of CD1 molecules. CD1 genes are thought to have an origin identical with genes encoded in MHC, and have an exon-intron structure similar to those of MHC-class I genes. To date, five CD1 genes have identified (CD1A, CD1B, CD1C, CD1D and CD1E) on chromosome 1 in humans, whereas only two CD1D orthologs have been ascertained on chromosome 3 in mice. In swine, a complete nucleotide sequence of CD1.1, which shares high similarity to CD1A, has been reported so far. We had performed large-scale porcine 5' EST analysis based on full-length cDNA libraries, and constructed a database of assembled sequences of these ESTs. In this database, we found three types of CD1 sequences newly identified in porcine. They showed high similarity to those of human CD1B, CD1D and CD1E, respectively. For analysis of their function and polymorphisms, we determined complete nucleotide sequences of their cDNAs. We also determined genomic organization of the porcine CD1 genes using BAC clones. The analysis of genomic and cDNA sequences of porcine CD1 families presented here contribute to analysis of varied host options in immune response to diverse spectra of pathogens. (This work was supported by MAFF, Japan and JRA.)

B 029

New single nucleotide polymorphisms in the horse genes encoding interleukin-12 and its receptor molecules

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Interleukin-12 (IL-12) and its receptor (IL-12R) play an important role in immunity against infection. IL-12 consists of two heterodimeric subunits - p40 and p35, encoded each by a specific gene. Expression of IL-12, representing a link between innate and adaptive immunity, is essential for switching the immune response to Th-1 type, IL-12R is composed of two chains - β1 and β2, also encoded by two genes, expressed on the surface of T-cells. Searching for single nucleotide polymorphisms (SNPs), we designed multiple sets of horse primers amplifying regions likely to contain SNPs, based on analogy with other species. An intra-intronic (intron 3) and synonymous exonic (exon 5) SNP in the p35 sub-unit encoding gene and a synonymous exonic SNP (exon 6) in the p40 subunit gene were identified by selective re-sequencing. Each of them can be genotyped by PCR-RFLP. Out of the two IL-12R genes, the β2-chain was analyzed. Primers were designed based on GenBank human mRNA and DNA sequences. Complete horse mRNA sequence containing two SNPs was determined. Besides an intra-intronic SNP (intron 5), a non-synonymous exonic SNP (exon 6) was found. Effect of the SNPs identified on the level of mRNA expression in the corresponding cell populations is currently investigated.

B 031

Mammalian Population Genomics and Selection: Detection of Immunogenes with Functionally Significant Variation in the Bovine Genome

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Domestication involved changes resulting in new and severe selective pressures on cattle and other animals. The epidemiology of infectious diseases would have changed as a result of sharp and permanent increases in population densities, while the new proximity of humans and animal domesticates would have increased the probability of disease transfer between species. One of the most likely sources of detectable selective effects in genomic variation lies in the genes which influence susceptibility to infectious diseases. Thus the examination of the genome for selective effects has the potential to inform on those genes that are most important in these most critical of traits. In the absence of genomic sequence, Expressed Sequence Tags (ESTs) provide a novel source of sequence information that can be utilised in the gene discovery process. We have assembled, using the TGICL software, more than 332,000 *Bos taurus* and *Bos indicus* ESTs and mRNAs into approximately 32,000 clusters consisting of more than one sequence. Homology searching has led to the identification of 1896 contigs with significant BLAST hits to human genes annotated by Gene Ontology as having a role in immunity or defense. Immunogenes, including several Toll-like receptors, have been sequenced in diverse bovine populations and tested for deviations from neutrality. Additionally we are employing PAML software to examine for bovine-specific acceleration in the rate of nonsynonymous substitutions with respect to the human, mouse and pig lineages.

B 033

Immunomodulatory effects of fish and soyabean oils in sheep

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Fish and soyabean oils (0.2 gm/kg. body weight) were separately given *per os* by gavage daily to mature rams in 2 equal groups each of 5 (40-45 kg. Body weight) for 14 successive days. Two blood samples were collected from each ram at zero time, and at the end of the 1st, 3rd , 7th and 14th day post oil administration. The first blood sample was collected in presence of heparin (50 IU/ml) as an anticoagulant for separation of mononuclear leukocytes to study the effect of oils on cellular immune response. The second blood sample was collected in a centrifuge tube without anticoagulant for serum separation to assay immunoglobulins and study effects on lipogram.. Total lipids, total cholesterol, HDL-c, LDL-c and triglycerides were markedly lowered along course of the experiment. The cellular immune response represented by the lymphocyte stimulation index stimulated by the nonspecific mitogen (phytohaemagglutinin), chemotaxis index, phagocytosis% and killing% was markedly elevated along the entire period of the experiment. Whereas, the nitroblue tetrazolium reduction (NBR)% was slightly elevated along the whole period of the experiment. Serum total proteins and gamma-globulins were significantly increased at the end of the 3rd, 7th and 14th day post oils administration, while alpha-globulins were significantly elevated after end of the 3rd and 14th day post oils administration when compared with pretreatment values. The serum albumin and b-globulin were not affected. Serum immunoglobulins (IgG, IgM and IgA) were markedly elevated along the entire period of the experiment .It is concluded that fish and soyabean oils can be considered a non specific immuno-stimulant agents which might be of great value in cases of autoimmune disease.

SECTION C

Functional Genomics

C 002

Two growth hormone genes in common carp (*Cyprinus carpio L.*) and their differential expression during ontogenesis

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The cyprinid fish species common carp is one of a few vertebrates in which a genome duplication took place quite recently (20-50 Myr BP). Thus, it was proposed to have more than one growth hormone (GH) gene. Using published information on one genome sequence and two cDNAs coding different GH products we were able to show that common carp really possess a second GH gene. In one individual three sequences of the GH gene were found. Two of them were very similar to each other: only 11 substitutions and two deletions/insertions could be found. One of these deletions was 341 bp long and located in the third intron. Both sequences coded the same protein product and were therefore considered as allelic variants of the same gene *GH1*. The third sequence was very different from these two (245 and 397 substitutions, and 30 and 34 deletions/insertions compared to the short and long allele at *GH1*, respectively) and the coded protein product differed by six amino acids from that of *GH1*. Therefore, it was considered as a second gene *GH2*. The introns of the two genes showed differences in potential binding sites for transcription factors. The two common carp GH genes were also used for a phylogenetic comparison with literature data on the duplicated GH genes of the tetraploid goldfish and single GH genes from other diploid cyprinid species. This analysis showed that the duplicated GH genes of common carp have probably arisen through that recent genome duplication. In our studies we could also show that both genes have different expression rates during ontogenesis indicating a different biological significance.

C 003

Promoter analyses of two inducible genes derived from Japanese flounder (*Paralichthys olivaceus*)

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Transgenic fish is useful and powerful technique not only understanding functions of genes and their regulation mechanisms of expression but aiso to obtain commercially important traits. In this study, two inducible gene promoters derived from Japanese flounder, *Paralichthys olvaceus* were identified and examined their promoter activity. cDNA and genes of Japanese flounder complement ubiquitin-liken protein (JFUb) and apoptosis regulator NR13-like protein (JFNR13) were cloned and sequenced. The 5'-upstream regions of JFUb and JFNR13 linked to the green fluorescence protein (GFP) as the reporter gene were constructed. Following which each of the promoter regulatory constructs was introduced into HINAE cells derived from Japanese flounder embryo by lipofection method. Transformed HINAE cells were stimulated with phorbol myristate acetate (PMA) or ployinosine-ploycytidine acid (ploy I:C) and observed under fluorescence microscope for GFP expression. The number of GFP positive cells containing JFUb-EGFP and JFNR13-EGFP were increased by Poly I:C and PMA treatments, respectively. These results indicate that both JFUb and JFNR13 promoter might be useful tools for transgenic research to regulate foreign gene expression. To test the in vivo activity of the promoters, we introduce JFUb-EGFP and JFNR13-EGFP into fertilized zebrafish eggs by microinjection for establishment of transgenic zebrafish lines. Two separate transgenic zebrafish lines (F0) containing the JFUb-EGFP and JFNR13-EGFP have been obtained. The GFP positive embryos are presently being raised and mated with wild type zebrafish to select for germ line transmission.

C 004

Production of disease resistance transgenic zebrafish expressing chicken lysozyme gene

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Lysozyme is known as an antimicrobial enzyme that catalyzes the hydrolysis of the cell walls of most bacteria. It has important roles in non-specific innate immunity against bacterial infection. In this study, we generated transgenic zebrafish that expressed chicken lysozyme gene under the control of Japanese flounder keratin gene promoter, and investigated whether this transgenic strain could show resistance against bacterial pathogen infection. For the generation of the lysozyme transgenic construct, Japanese flounder keratin promoter linked with hen egg white (HEW) lyoszyme gene and green fluorescence protein (GFP) gene were used as a marker for selection of transgenic strains. This recombinant plasmid was introduced into fertilized zebrafish eggs by microinjection method. GFP positive embryos were raised and mated with wild type to select for germ line transmission. F2 transgenic zebrafish showed very strong GFP expression in the epithelial tissues, liver and gill from embryonic stage to adult fish. Western blot analysis showed that HEW lysozyme existed in the protein extracts from liver of F2 transgenic zebrafish. These indicate that both HEW lysozyme and GFP were expressed in the same tissues under the control of keratin promoter. The lytic activity of protein extracts from liver of both wild type and F2 transgenic zebrafish was assessed by lysoplate assay using *Micrococcus lysodeikticus* as substrate. The extracted protein from F2 transgenic zebrafish showed higher lytic activity than that that of wild type. F2 transgenic were challenged with *Flexibacter columnaris* and *Edwardsiella tarda*. Challenge studies showed that 100% of control fish were killed by both pathogens, while 40 ~ 45 % of F2 transgenic zebrafish were killed by both pathogens. These results indicated that the transgenic zebrafish carrying HEW lysozyme transgene had resistance to bacterial infection.

C 005

Fine analysis of regulatory region of the mouse Sry gene

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Sry (Sex determining region on Y chromosome) gene expression is restricted in undifferentiated gonads of mammals. There have been few studies on the regulatory elements of the *Sry* gene, mainly because no *Sry*-expressing cell lines have yet been established. We developed a useful tool for investigating the regulatory upstream region of *Sry* by means of the *in vitro* Cre/*loxP* system using primary cultured cells from CAG (cytomegalovirus enhancer and beta-actin promoter) /loxP/CAT/loxP/LacZ transgenic mice. We constructed a *Sry*/*Cre* fusion gene plasmid in which *Cre* expression is controlled by the 5' and 3' untranslated regions of mouse *Sry* that have been previously shown to induce testis development in XX transgenic mice. When *Sry*/*Cre* plasmids were transfected into the cells that had been prepared from the gonads, the brains and the livers of CAG/loxP/CAT/loxP/LacZ transgenic fetuses on 11.5 days post-coitus, only a small number of X-gal-stained cells were detected among the primary cultured cells from male and female gonads, while none were detected among the cells from the other tissues. The X-gal-positive cells were negative for alkaline phosphatase, indicating that these cells were somatic cells expressing *Sry*. Next, we constructed *Sry*/*Cre* plasmids with a differentially sized-upstream region of *Sry*. When *Sry*/*Cre* plasmid with 0.4kb upstream region of *Sry* was transfected into cells from various tissues, a large number of X-gal-positive cells were observed in the cells from tissues in which *Sry* is not expressed, indicating the loss of the stage- and tissue-specific expression of Sry. The *Sry*/*Cre* with a 0.5 kb upstream region maintained stage- and tissue-specific activity of *Sry*. The results suggested that the 0.4-0.5 kb upstream region of *Sry* is important for stage- and tissue- specific expression of *Sry*.

C 006

Comparative Analyses of Whey Acidic Protein (WAP): Function of Human WAP gene

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The whey acidic protein (WAP) has been identified in milk of various species of monotreme and marsupial. Our previous studies have demonstrated that mouse WAP inhibits the proliferation of mouse mammary epithelial cells. However, the presence of WAP was not reported for milk of ruminants and primates. We investigated computationally and biochemically human WAP. Comparative analyses of nucleotide and the predicted amino acid sequences showed that putative human WAP cDNA sequence has a homology of 64%, 66%, 74%, 73% and 97% with WAP cDNA from mouse, rat, rabbit, pig and chimpanzee, respectively. However, the human WAP gene sequence showed the loss of a translation start codon, a point-deletion at the exon-intron boundaries, and a point mutation substituted to the stop codon. This result accounts for the absence of WAP from human milk found by HPLC and Western blotting analysis. We repaired these three nucleotide mutations in the human WAP ORF. The transfection of expression vector of repaired human WAP has indicated that human WAP has inhibitory function on the proliferation of MCF-7 cells (human mammary tumor cell line) as seen in mouse WAP. Next, we constructed the fusion gene of the human WAP promoter region (2.6 kb) with the human growth hormone structure gene (hGH). The transfection of hWAP/hGH fusion gene showed that human WAP promoter was active.

C 007

Comparative gene expression analyses of canine villus and crypt small intestinal cells using laser capture microdissection

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The objective of this study was to compare the effects of diet and age on gene expression profiles of villus and crypt intestinal cell populations in the canine. Twelve geriatric (12 yr old) and 12 young adult (1 yr old) beagles were used. Six dogs from each age group were randomly assigned to one of two dietary treatments: 1) high quality, animal-protein based diet, or 2) plant-based diet. Intestinal tissues were collected after animals had been on experiment for one year. Laser capture microdissection (LCM) was used to isolate individual villus and crypt epithelial cells from ileal samples. RNA was isolated and amplified using the PicoPure™ RNA Isolation Kit and RiboAmp®OA RNA Amplification Kit (Arcturus), respectively. Gene expression profiles were generated by hybridizing amplified RNA to the Affymetrix GeneChip® Canine Genome Arrays. Preliminary results show that genes more highly expressed in the crypt epithelial cells include genes related to apoptosis, cell cycle, DNA replication, and energy metabolism. In contrast, genes more highly expressed in villus than crypt were associated with matrix or structural proteins. The use of LCM provides cell-specific gene expression profiles of distinct intestinal cell populations. While villus cells are composed of differentiated cell populations possessing specific functions, crypt regions are composed of undifferentiated cells. Research in this area may identify factors associated with cellular differentiation and lead to the development of therapies for intestinal disease and may be used as a screening tool for gene targets associated with growth promotion. (Supported by Pyxis Genomics, Inc. and the Critical Research Initiative (CRI) at the University of Illinois).

C 008

Identification of estrogen-responsive and oviduct-specific regulatory elements in chicken ovalbumin gene

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The expression of chicken ovalbumin is estrogen-responsive and tissue-specific in the oviduct. In order to direct the expression of a transgene in the oviduct, the regulatory element for ovalbumin gene is the choice. However, the size of the regulatory element-promoter complex is too large to be incorporated in the expression vector. In this 6666-base complex, four protein-binding regions have been identified by the DNase I hypersensitive (DH) assay. These fragments are NRE, SDRE, DH3, and DH4. The objective of this study was to identify the regions responsible for estrogen-dependent and oviduct-specific expression. For this purpose, we constructed a series of expression vectors containing a DH fragment or their combination, the constitutive promoter, and EGFP and transfected LMH cell line with them. The transfected cells were cultured in the presence of β-estradiol or corticosterone and the intensity of EGFP expression was measured to evaluate the transcriptional activation by regulatory fragments. When the vector contained a single fragment, there was no enhancement of EGFP expression. On the other hand, the vector containing NRE, SDRE, DH3, and DH4 fragments produced the higher level of EGFP expression independent of steroid hormones. The enhanced response exclusive to β-estradiol was observed with the trio of NRE, SDRE, and DH3, the pair of NRE and DH4, and the pair of DH3 and DH4. However, when SDRE was added to NRE and DH4, the β-estradiol-responsive enhancement was lost.

C 009

Nucleotide sequence of constitutive androstane receptor (CAR) mRNA of Japanese quail

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The constitutive androstane receptor (CAR) belongs to the subfamily I of the nuclear receptor class 1(NR1I3). The previous studies suggest the importance of the CAR gene for physiological mechanisms of the vertebrates, especially metabolism of steroid hormones (Min *et al.* 2002). The present study determines the nucleotide sequence of the full length mRNA of Japanese quail (*Coturnix japonica*) CAR gene by the rapid amplification of cDNA end (RACE) method. The mRNA consists of 1365bp, which is 25bp longer than the chicken CAR (Genbank accession No.AF276753). The A/B domain of the quail CAR gene was highly variable and the revealed sequence difference between mammals and birds. The fifteen amino acids deletion of the mammals CAR and six amino acids insertion of the chicken CAR were found in the D domain in comparison to the Japanese quail CAR. No insertion and deletion were found in the DNA binding domain (C domain) and the ligand binding domain (E domain) among Japanese quail and other animals, however the identity percentage of amino acids between Japanese quail and human is quite low, such as 64% in the C domain and 59% in the E domain. Expression of Japanese quail CAR was detected in duodenum, liver, and kidney using one-tube RT-PCR method. The expression pattern of the quail CAR is similar with the expression pattern of the mammals CAR.

C 010

Identification of complete cDNA for three heat shock protein (HSP) 70 homologues of quail

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The heat shock protein 70 (HSPA) is a major family for molecular chaperones and plays an important role as a highly elaborate quality control mechanism for many proteins. The HSPA is a useful biomarker for the study of environmental stress in poultry. The Japanese quail (*Coturnix japonica*) is an excellent bird to use for poultry and experiments because of their small size, fast growth rate, fecundity and environmental resistances. We identified complete cDNA sequence of three quail HSPA homologues: *HSP70, HSC70* and *GRP78*, by using the RACE method. The nucleotide sequences of the open reading frame for the quail *HSP70, HSC70* and *GRP78* had a close similarity to the chicken *HSP70*: 96.5%; *HSC70*: 96.5%; and *GRP78*: 96.1% respectively, and those translated amino acid sequences showed a higher sequence identity to several vertebrates HSAP2, 8 and 5 genes than other members of the HSPA gene family respectively. The amino acid sequences of these quail HSPA homologues contained three conserved HSP70 proteins family signatures, the ATP/GTP-binding site, the N-linked glycosylation site and the leucine zipper. The amino acid sequences of the quail *HSP70* and *HSC70* contained also the nuclear localized signal motif site and the C-terminal EEDV, though the amino acid sequence of the quail *GRP78* contained the bipartite nuclear targeting sequence and the ER-target. Furthermore, the quail *HSP70* and *HSC70* had *HSPA2* specific insertion and HSPA8 specific insertion respectively. We therefore, concluded that these cDNA sequences show quail HSPA2, 8 and 5 orthologue genes: *CjHSPA2*, *CjHSPA8* and *CjHSPA5*.

C 011

Cloning, sequencing and diversity analysis of 9 chicken functional genes correlated with growth traits

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Based on former reported sequences, 21 pairs of primers were designed and synthesized to amplify unknown sequences of chicken growth hormone receptor (GHR), growth hormone releasing hormone (GHRH), ghrelin, insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding factor-2 (IGFBP-2), insulin, pituitary transcriptional factor-1 (PTF-1 or PIT-1), somatostatin (SS) and thyroid stimulating hormone beta subunit (TSH-β) gene. With the use of genomic DNA from a White Leghorn layer, each PCR product amplified by above primers was cloned into T-vector and then sequenced. Later, all sequences were analyzed and their diversity was studied.

Results showed, 4 sequences of GHR, 1 of GHRH, 3 of ghrelin, 2 of IGF-1, 2 of IGFBP-2, 2 of insulin, 5 of PIT-1, 1 of SS, and 1 of TSH-β gene were obtained. These 21 sequences spanned 32 195 bps in length in which 5 428 bps were overlapped with reported regions. These 21 sequences were proved to be what we anticipated of each gene for their high consistency with reported sequences in overlapped regions and high homology to genome sequences (http://mgc.ucsc.edu/cgi-bin/hgBlat). These 21 sequences were all accepted by database in http://www.ncbi.nlm.nih.gov and could be helpful for other research on the above genes. Additionally, by comparing these 5 428 bp sequences in this study with other reported ones, dozens of SNPs and some other mutations were detected either in coding regions or in non-coding regions, and several SNPs led to the change of either translated precursors or mature proteins. The potential effect of these mutations remained unclear and still awaited further investigation.

C 012

Isolation and molecular characterization of the porcine stearoyl-CoA desaturase (*SCD*) gene

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Stearoyl-CoA desaturase (SCD) is a rate-limiting enzyme in the biosynthesis of unsaturated fatty acids. We isolated and characterized the full-length cDNA and the genomic DNA sequence of the porcine *SCD* gene. The 5134 bp cDNA contains a 1,080 bp open reading frame encoding a protein of 359 amino acids with a calculated molecular mass of 41.3 kDa and a theoretical isoelectric point of 9.4. The porcine SCD protein shares high identity (>80%) with the other mammalian SCD. The 20,985 bp of genomic DNA sequence is similar to the other mammalian orthologs, particularly in term of exon size and exon/intron boundaries. The porcine *SCD* gene spans a transcription unit of 16,186 bp, consisting of six exons with sizes ranging from 131 bp to 4,048 bp, and five introns varying in size from 518 bp to 4,784 bp. The gene reveals a 176-bp long 5' UTR and possesses an unusually long 3'UTR of 3,848 bp in the last exon. Comparison of different mammalian *SCD* promoters identified some regulatory domains required for the transcription regulation in the 5' flanking sequence of the porcine *SCD* gene. A total of 21 gene polymorphisms were revealed in the 21 kb DNA sequence, including 19 single nucleotide polymorphisms (SNPs), a 24-bp long fragment length polymorphism in the fourth intron and a triplet nucleotide insertion in the fifth intron. Reverse transcription (RT)-PCR result indicates that the *SCD* gene is expressed ubiquitously in pigs.

C 013

Characterization of novel splicing variants of PPAR-gamma in pig and human

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The peroxisome proliferator-activated receptor gamma (PPAR-γ) belong to the steroid / thyroid / retinoid receptor superfamily which are primarily expressed in the fat tissue. Recently, we have reported the presence of novel splicing variants derived from new exon A' in the franking region of the pig *PPAR-γ* gene (XXVIII ISAG meeting, Germany, 2002). To characterize the expression of the exon A' derived transcripts, the relative amount of expressions in the PPAR-γ transcripts was analysed in different adipose tissues. In addition, we attempted to isolate the exon A' derived transcript in human adipose tissue. The real time RT-PCR analysis showed that the exon A1 derived transcripts were not significantly different expressed in visceral fat (lamina subserosa) or subcutaneous fat (back fat, inner and outer layer). In contrast, exon A' derived transcripts were gradually higher expressed in the inner and outer layer of the subcutaneous fat than in visceral fat. The same expression pattern was also observed for PPAR-γ2. The exon A' derived transcript was also isolated from human adipose tissue, which was a novel human PPAR-γ splice variant (GenBank accession No. AB097931). The sequence homology between the human and the pig in exon A' was around 78%. We postulate the presence of two distinct promoters, which regulate the PPAR-γ1 depending on the localization of the fat tissue.

C 014

Characterization of bovine solute carrier family 27 member 1 (slc27a1)

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SLC27A1 is a transmembrane protein involved in translocation of long-chain fatty acids (LCFAs) across the plasma membranes. It may play a pivotal role in regulating available LCFAs substrates from exogenous sources in tissues undergoing high levels of beta-oxidation or triglicerides synthesis. SLC27A1 belongs to a family of transpoters caracterized by the presence of an signature sequence of 311 aminoacid that is highly conserved among FATP family members. It also belongs to the ATP-dependent AMP-binding enzyme family.
We used the human SLC27A1 gene sequence (AX015325) to desing specific primers and determine the entire coding sequence of the bovine gene. It shows an 88% similarity with human and a 83% with mouse genes. A C/T SNP at 212 position of the predicted protein sequence was isolated. Primers amplifying a 500 pb fragment corresponding to human genomic sequence were used to screen a bovine BAC library. Positive clones allowed us to assign SLC27A1 to BTA7q11-q12 by FISH. This localization is in agreement with the corresponding human localization in HSA19p13.12. In adittion, we have also mapped SLC27A1 in goat and sheep in CHI7q11-q12 and OAR5q11-q12 respectively. Expresion pattern of the SLC27A1 gene has been evaluated in different tissues (heart, lung, liver, spleen, brain, muscle, kidney, testitcle, and adipose tissues) by quantitative real time PCR.

C 015

Functional polymorphism within bovine prolactin and STAT5A genes

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Genes coding for prolactin (PRL) and signal transducer and activator of transcription 5A (STAT5A) are considered as candidates for genetic markers of productive traits in farm animals. Using PCR-SSCP, PCR-heteroduplex, PCR-RFLP and sequencing methods we identified several new polymorphisms within coding and regulatory sequences of bovine PRL, GH, and STAT5A genes. The A/G transition was found at position –488 in the promoter region of the STAT5A gene. With RT-PCR, Real-Time PCR and Western-blotting we showed that the STAT5A expression level in the liver was higher in cattle with AA than GG genotype. EMSA showed that A→G transition increased the STAT5A gene promoter binding capacity for liver nuclear proteins (possibly HNF-3). Another transition - T/C was found at position 12,743 in the exon 16 of STAT5A gene that changes amino acids sequence in the protein - V/A at position 686. Significant differences in DNA-binding capacity were observed between proteins extracted from cell nuclei of liver tissues of bulls with different genotypes; those from CC animals always showed less DNA protein complexes than TT. Effects of sequence polymorphism in the promoter region of bovine PRL gene (TG_2 deletion at position –877) were estimated. It was shown that the deletion totally abolished a GR binding site. PRL gene expression was significantly higher in pituitaries of bulls carrying the TG_2 deletion than in those without deletion.

C 016

cDNA cloning, expression and sequence analysis of bovine glycerol phosphate acyl transferase mitochondrial (GPAM) gene

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GPAM catalizes the first step of glycerolipid biosynthesis. It plays a key role in the regulation of cellular triacylglycerol and phospholipid levels. There are two isoforms of Glycerol-3- phosphate acyltransferase in mammals, a mitochondrial and a cytosolic form. The mitocondrial form (GPAM) prefers saturated fatty acyl-CoA as a substrate, whereas the cytosolic enzyme uses both saturated and unsatured fatty acyl-Co. We assigned GPAM to BTA 26q22 by FISH. This is in agreement with the corresponding human and mouse localizations on HSA 10q24-q26 and MMU 19 respectively. We used the mouse GPAM gene sequence to design primers in order to amplify the bovine gene. In this work, cDNA of bovine *GPAM* was cloned, characterized and compared to the human and rat orthologs. Comparative analysis reveals evolutionarily conserved exonic regions. RT-PCR and Northern have been used for expression studies from mRNA of kidney, spleen, heart, liver, testis, skeletal muscle, brain, lung, subcutaneous and mesenteric adipose. *GPAM* was expressed in all the tissues examined, with the highest expressions in adipose tissues. In man and nonruminant animals, lipogenesis occurs primarily in the liver whereas in ruminants, the main site of fatty acid synthesis is adipose tissue.

C 017

Genomic organization and promoter analysis of the bovine ADAM12 gene

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ADAM12 is a member of the ADAM (a disintegrin and metalloprotease) family possessing a putative role in a variety of biological processes such as modulation of proteolytic processing, cell adhesion, cell fusion, and signaling. Recently, it has been suggested that ADAM12 is involved in regulation of adipogenesis as well as myogenesis. In this study, we have determined the genomic structure of 5'- and 3'-regions in the bovine *ADAM12* gene. We observed a lower homology of its exon 2 with the human counterpart. The exon S19 encodes for the sequence specific to a shorter secreted form of ADAM12S in human. The bovine *ADAM12* gene had no canonical 3'-splice acceptor site before the putative exon S19. It suggested that the cattle could not produce an ADAM12S counterpart. The exon/intron organization of 5'- and 3'-regions were conserved between cattle and human, except for the exon S19. Sequence analysis of the proximal promoter of the bovine *ADAM12* gene revealed to be a potential C/EBP response element. When linked to a luciferase reporter and transiently transfected into mouse 3T3-L1 preadipocyte, the ADAM12 promoter was activated 1.8-, 3.0- and 4.6-fold by co-expression with C/EBPalpha, beta and delta, respectively, whereas co-expression with PPARgamma reduced the promoter activity. These data suggested that the expressional regulation of the bovine *ADAM12* gene during adipogenesis might be mediated by C/EBP family and PPARgamma.

C 018

Expression of agouti protein in cattle

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Agouti protein is secreted from the cells surrounding hair-bulb melanocytes, which binds to the MC1R preventing α-MSH binding, and thus shifts pigment synthesis from black eumelanin to red/yellow pheomelanin. Wild type agouti hair is characterized by a sub-terminal or terminal yellow band; the rest of hair shows black or brown eumelanin pigment. In mouse, dominant agouti alleles associated with yellow coat color also cause obesity, diabetes and development of tumors due to the ubiquitous expression of agouti protein. In wild type mice, agouti is expressed only in skin and in testes. In humans, agouti was shown to be expressed in adipose tissue, heart, ovary and testes, and at lower level in liver, kidney and foreskin. In cattle, there are no reports on polymorphisms found in the coding sequence of the agouti gene so far. Since polymorphisms affecting gene expression in the non-coding sequence of agouti in mouse have been described, the expression of agouti in cattle was investigated. Real time PCR was performed with cDNA reverse transcribed using RNA extracted from different tissues of four cattle breeds. Comparative C_T method was used to compare expression between samples (tissues and breeds). Agouti was expressed at high levels in the skin of Simmental, Red Holstein and Brown Swiss breeds, in testes of Simmental breed and in heart, liver, ovaries and testes of Red Holstein breed. Heart, liver, ovaries and testes of Brown Swiss and Holstein, heart, liver and ovaries of Simmental and liver of Red Holstein showed very low expression of agouti.

C 019

Structure, expression and regulation of the bovine calpastatin gene

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Calpastatin is a specific inhibitor of the ubiquitous calcium dependent proteinases μ- and m-calpain. The calpain-calpastatin system is involved in various physiological and pathological processes such as myoblast fusion, cell cycle or Alzeihmer's disease. It seems also to be the major system implied in meat ageing, particularly in the myofibrillar degradation during *post-mortem* tenderization. Up to now, calpastatin has been suggested to be the main regulator of the calpain-calpastatin system. In the present study, we determined the bovine gene and mRNA structures as well as the protein expression in various tissues and demonstrated that, at least, three promoters exist. The bovine calpastatin gene harbours 35 exons spanning 130 kb on genomic DNA. Its structure is similar to that of mouse and pig gene but with larger introns. As previously shown in mouse, we found that four different types of mRNAs (named I, II, III and IV), differing from their 5' ends, are transcribed from the bovine calpastatin gene. One seems to be specifically transcribed in testis (IV) whereas the others are ubiquitous. Based on reporter gene experiments, we found that three promoter regions are located upstream the first exons of type I, II and IV mRNAs. Multiple calpastatin protein isoforms ranging from 70 kDa to 150 kDa have been highlighted. The establishment of a correspondance between protein isoforms and transcripts is under process. The aim of this study is to establish a potential correlation between varations in meat ageing kinetics and differential expression of the bovine calpastatin gene.

C 020

Muscular fatty acid composition affected by genetic variants and mRNA expression of delta-5-fatty acid desaturase in pigs

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Fatty acid desaturases are critical enzymes involving in the biosynthesis of essential polyunsaturated fatty acids. The 418 bp of porcine Δ5-desaturase cDNA has been sequenced and registered in the NCBI GenBank as AY512560. This fraction of Δ5-desaturase cDNA had 92% identity with that in human. The gene expression of Δ5-desaturase in hepatocytes was detected by real-time PCR. The result showed that the gene expression of Δ5-desaturase was induced by additional docosahexaenoic acid (DHA) or palmitic acid (PA) in the medium. As compared with control group (n = 4), increments of 7.32 and 2.56 folds of gene expression of Δ5-desaturase were observed in the hepatocytes 1 hr after incubations with DHA and PA treatments, respectively. Therefore, the Δ5-desaturase gene is possibly an inducible functional gene. The corresponding genomic DNA of this fraction of Δ5-desaturase includes 2 introns and each intron has three genetic variants. The genetic polymorphism of Δ5-desaturase gene was examined by using PCR-RFLP. The variants GG, GC, CC of different breeds (LD, n = 37; YD, n = 6) was carried out in the same farm. The polyunsaturated fatty acid contents of the meat with GC genotype (n = 19) were significantly higher than those with GG genotype (n = 24) ($p < 0.01$). The results imply that genetic variants of fatty acid desaturases can be a possible indicator for improving muscular quality in pig breeding.

C 021

Expression of Stearoyl-CoA Desaturase mRNA in bovine preadipocyte during differentiation

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Stearoyl-CoA desaturase (SCD) is the enzyme that catalyzes the desaturation of stearic acid and palmitic acid into oleic acid and palmitoleic acid, respectively. Japanese Black cattle have a fatty acid profile that differs from that of other breeds in terms of unsaturated fatty acids. Japanese Black cattle have higher rate of unsaturated fatty acid than that of other breeds. Furthermore, it is reported that Japanese Black cattle have high level of SCD mRNA expression compared to Holstein cattle in adipose tissue. In this study, we isolated adipocytes from adipose tissue of castrated male in Japanese Black and Holstein cattle. Preadipocytes were obtained from adipocytes by the "ceiling culture" method. The preadipocytes were cultured and induced the differentiation into multilocular adipocytes that contained intracytoplasmic lipid droplets. Total RNA in several differentiation stages (0, 1, 2, 4 and 8 days) were extracted. We determined the expression level of SCD mRNA during differentiation by using quantitative real-time RT-PCR and compared the expression level between the breeds. In consequence, SCD mRNA expressions in both breeds were gradually increased during the differentiation period, however the expression levels in 8 days were decreased to less than half level of expressions in 4 days. In 4 days, the expression level in Japanese Black showed 1.25 times higher than that of Holstein, although the difference was not significant.

C 022

Comparison of age-dependent expression patterns of C/EBP family and PPARgamma in *Musculus longissimus* between Japanese Black and Holstein breeds

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Intramuscular fat accumulation known as marbling, is an economically important trait for the beef cattle in Japan. It is known that the expression of this trait starts at the age of around 10 months. Our objective was to obtain means to diagnose at an early stage whether an animal will produce high-marbled meet or not. We investigated age-dependent expression patterns of the adipogenic transcription factors, *C/EBPalpha, C/EBPbeta, C/EBPdelta* and *PPARgamma* in *Musculus longissimus* tissue in the vicinity of 10-months of the age. We compared the patterns of the Japanese Black known to be a breed with a rich marbling, with those of the Holstein breed, known for its poor marbling. In a competitive RT-PCR study, different expression patterns were observed between the two breeds. In Japanese Black the expression of *C/EBPdelta* and *C/EBPbeta* increased to reach a peak at 6 and 8-months of age, respectively. In the Holstein breed, they didn't increase and were lower than that in the Japanese Black during this period. Immunohistochemical analysis detected that *C/EBPbeta* and *C/EBPdelta* were mainly expressed in cell nuclei in connective tissue and muscle bundles of biopsied sample at 8-months of age. These results suggest that the increase in expression of *C/EBPbeta* and *C/EBPdelta* before 10-months of age might be associated with intramuscular fat accumulation observed in the Japanese Black breed after 10-months of age.

C 023

Identification of differentially expressed genes in distinct skeletal muscles in Hanwoo (Korean Cattle) using cDNA microarray

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Microarray technology is widely used for permitting the simultaneous analysis of transcript levels thousands of genes in different physiological states of organisms, tissues or cells. In order to identify the molecular processes that control the phenotypic characteristics among skeletal muscles of Hanwoo, we manufactured 788-gene microarray that was selected from cDNA libraries of Hanwoo loin muscle, fat tissue and liver. This microarray was used to profile the differential expression of genes between loin and round muscle of Hanwoo. The microarray analysis identified 25 differentially expressed genes between the two muscles. Fourteen genes were up-regulated and eleven genes were down-regulated in the loin muscle. With verification, muscle phenotype determination-related genes can be identified.

C 024

Comparisons between Japanese Black and Holstein cattle using gene expression profiling

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Marbling is a meat quality attribute that is important to the beef trade between Australia and Japan, because Japanese consumers associate the heritable trait with increased flavour and tenderness. Here we used microarrays in Australia to investigate the genetic basis of phenotypic differences between Japanese Black purebred cattle (Tajima strain, n=3) and Holstein pure bred cattle (n=3) grown in Japan. RNA was extracted from *Longissimus dorsi* muscle biopsy samples taken using ethically approved sampling procedures, during growth under a normal nutritional regimen. Differentially expressed (DE) genes were detected using a 9.6 K bovine muscle and fat cDNA microarray. Experimental comparisons utilised a multivariate mixed modelling approach and 162 genes were found DE for at least one of three sampling dates: approximately 12 months of age (upon recruitment into the study); 5 months later; and 9 months later. DE genes came from many metabolic pathways. The appearance of any particular gene in the DE list varied with sampling month. Some genes were DE between breeds at all three time points. These included genes for connective tissue synthesis (collagen type I), fatty acid transport (fatty acid binding proteins) and lipid desaturation (SCD). Breed-based differences in tissue cellularity cannot be excluded as an explanation for these findings.

C 025

Identification of the genes responsible for beef marbling. 1. Exploration of differentially expressed genes between high and low marbled steers

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The beef marbling is one of multifactorial and complex traits. We have been performing a strategy based on gene expression profiling, to explore the beef marbling genes. We here utilized biopsies of the *M. longissimus* in two high-marbled steers which were obtained by somatic nuclear-derived cloning of Itofuku, a super-sire with the very high breeding value for marbling, and in two low-marbled Holstein steers. Because marbling starts to appear at an age of around 10 months, biopsies were taken at 8, 10, 12 and 14 months of age. Two types of differential display-PCRs, i.e., a quantitative method with 4 times repeats (two spots per gel with two gels) for individual samples and a comparative method comparing among ages and between groups within a same gel for pooled samples, were applied to explore genes of which expression pattern is coincident with the process of intramuscular fat deposition. A total of 2,114 bands were detected on differentially displayed gels. Among these bands, 163 bands showed the expression pattern relating to intramuscular fat deposition, by utilizing the quantitative method. The expression patterns of 74 out of the 163 bands were confirmed by utilizing the comparative method. The 34, 28, and 12 among 74 bands, respectively, were shown to be significant ($P < 0.05$) in the difference between two groups of steers, in the interaction effect between group and age, and in the both.

C 026

Identification of the genes responsible for beef marbling.
2. Identification of candidates for beef marbling genes by functional retrieval and confirmation of expression pattern

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As the first step to explore the gene(s) responsible for beef marbling, we previously detected 74 differentially displayed bands of which the changes in expression pattern are coincident with the process of intramuscular fat deposition. We here identified candidates for beef marbling genes among the 74 bands by performing the retrieval of known gene function *in silico* and the confirmation of expression pattern with real-time quantitative RCRs. The 74 bands were sequenced and submitted to BLAST homology searches. Some bands matched with already known genes, others matched to ESTs or showed no significant similarity to any sequence in GenBank databases. The retrieval for function of known genes detected *SORBS1, MAF, PDHB,* and *VAPA* respectively, which are involved in insulin-stimulated glucose uptake in adipocyte, adipocyte differentiation, fatty acid oxidation, and insulin-stimulated *GLUT4* translocation. These four may be considered as functional candidate genes. The changes of expression pattern of two out of nine tested genes, i.e., *NEB*, and an unknown, were confirmed by real-time quantitative PCRs. These two may be also considered as candidate genes. Further real-time quantitative PCRs and RH mappings are in progress to detect genes likely to be involved in the intramuscular adipogenesis.

C 027

Microarray analysis of hepatic genes in pigs with extreme body composition

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All nutrients ingested pass the liver that largely contributes to the overall metabolic status of the organism. Thus genes active in liver potentially affect traits related to body condition, muscularity and obesity. We were aiming at the identification of liver ESTs with trait associated expression patterns in order to identify ESTs which represent possible candidates for carcass traits and overall body composition and condition in pigs. Microarray analysis of hepatic gene expression was employed. Eight discordant sib pairs representing extremes for the trait 'eye muscle area' were selected from a Duroc × Berlin Miniature Pig F_2 experimental cross (DUMI) in order to prepare liver RNA pools of four high and four low performing individuals. Home made pig cDNA microarrays, which allow simultaneous expression analysis some 250 liver mRNA transcripts, were used to compare the gene expression profiles of these two extreme groups. Differential gene expression detected by microarray hybridisation was independently confirmed for selected genes by real time PCR on individual RNA samples. The transcript levels of 3 liver expressed genes (apolipoprotein H, putative serine protease inhibitor I, organic anion transport polypeptide 2) were shown to be associated with the phenotype data. These data indicated that microarray analysis can complement QTL analysis to identify systems of regulation of body condition.

C 028

Analysis of caloric restriction effect on gene expression in swine muscle by cDNA microarray

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Researches of years '30 indicated that life span of rodents benefits of caloric restriction (CR) and a modification of slow and fast muscular fibres has also been reported as a consequence of CR. Present results refer to a study of differential expression of a set of genes in *longissimus dorsi* muscle of two groups of pigs (Casertana, local breed of Southern Italy) fed diets differing for composition and energy density. Two groups (AE and BE) of 15 pigs were reared in the same environmental condition from 35 to 160 kg of live weight. At the end of the trial the AE and the BE groups received a diet with 2200 kcal and 1800 kcal net pig energy per kg respectively. *L. dorsi* biopsies were sampled at slaughtering, immediately frozen and total RNA was later extracted and analyzed on a cDNA human microarray. A high level of cross hybridization between human and pig genes was observed. Data analysis of microarray allow to identify a set of genes which were up or down regulated as a consequence of the energy density of the diet. Most of these genes (as pyruvate kinase, mithocondrial genes, troponin fast and slow isoforms), were as expected but a new set of differently expressed genes (as GKAP42, telethonin and some not yet characterized) would open new perspectives in the understanding the role of energy intake in the control of muscle metabolism and aging. Possible implications of this approach will be the relationship of some differentially expressed genes with meat quality of pigs.

C 029

Analysis of Gene Expression Patterns in RN$^-$ Pigs Using a 27.742 Element Porcine cDNA Microarray

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cDNA microarrays have proven useful for monitoring global alterations in gene expression in response to various diseases and environmental conditions. We have constructed a global porcine cDNA microarray consisting of 27.742 elements spotted in duplicate. 15.943 ESTs (Expressed Sequence Tags) were selected that have human orthologs. In addition, 7.563 EST's were selected from EST clusters with no similarity to human gene transcripts and 1.783 singleton ESTs were included. Other elements on the array include control spots, labeled oligos, blanks and genomic DNA. The porcine clones used for the construction of the array were selected from 810.124 pig ESTs representing cDNA clones from 98 non-normalised tissue libraries. Clones were annotated by BLASTN searches against TIGR and NCBI databases and all clones were given unique reporter_IDs. The *RN*$^-$ phenotype in pigs was chosen as a model to test array-performance. The *RN*$^-$ phenotype is caused by an R200Q mutation in *PRKAG3*, and is characterized by a defect in glycogen metabolism. In order to study the effects on gene expression patterns in *RN*$^-$ pigs associated with the *PRKAG3* mutation, 14 wildtype (rn^+/rn^+) were compared with 14 *RN*$^-$ (RN^-/rn^+) pigs. Differentially expressed genes were verified using RT-PCR. Data presented here demonstrates the utility of the largest available porcine cDNA microarray and sheds new light on the *RN*$^-$ phenotype in pigs.

C 030

cDNA Microarray and QRT-PCR Analysis of Host-Pathogen Interactions in a Porcine *in vitro* Model for Toxoplasmosis

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Toxoplasma gondii is an obligate intracellular Apicomplexan parasite that causes opportunistic illness in immuno-compromised individuals as well as congenital disease in infants and suffers from a lack of selective chemotherapeutic agents. In infected host cells, *T. gondii* induces the up-regulation of pro-inflammatory cytokines, organelle reorganization and apoptosis inhibition. We examined the transcriptional profiles of porcine kidney epithelial cells infected with *T. gondii* using cDNA microarrays consisting of 384 known genes and total RNA isolated from *T. gondii* infected cells and uninfected control cells at 0, 1, 2, 4, 6, 24, 48 and 72 hours post-infection. Approximately 14% of all analyzed genes were significantly up-regulated (fold change in gene expression ≥ 2.0 and p-values ≤ 0.05) early during the infection (1-2h). Fifteen of the 55 induced genes (27%) encoded proteins associated with the immune response; 50% of which are cytokine related proteins. There was a significant increase in the number of up-regulated metabolic genes and cell cycle regulators later in the infection (6-24h). Towards the end of the infection (48-72h), there was sustained elevation in the number of up-regulated metabolic genes as well as apoptosis related proteins, the majority of which are known apoptosis inhibitors. Real-time quantitative RT-PCR validated the microarray generated expression pattern of 5 apoptosis related genes with a consistent and significant correlation in the differential expression of these genes ($r^2 = 0.99$, SE=0.051). Microarray and QRT-PCR analysis confirmed known changes and identified new effects of *T. gondii* on the host cell, facilitating further analysis of host-pathogen interactions in *Toxoplasmosis* that can be exploited in the search for novel drug targets.

C 031

Knock-down of BLV *tax* using siRNA and its effect on host gene expression

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We are interested in understanding the specific pathways by which bovine leukemia virus (BLV) causes lymphoproliferation, cell transformation and immune disorders in the host. To address these questions, we developed RNA interference technology to knock down the expression of the BLV *tax* gene in a BLV-infected bovine lymphocyte cell line (BL3*). A small interfering RNA (siRNA) targeting BLV *tax* was evaluated using four different cell concentrations (.75, 1.0, 1.5 and 2.0×10^6 cells in 2 ml) and two dosages of siRNA (4.0 and 5.0 μg) at 24 and 48 hr after transfection. To validate the knock-down of BLV *tax*, a SyberGreen-based quantitative PCR protocol was used to assay *tax* mRNA in cells cultured with siRNA and untreated controls. All assays were done in triplicate using beta actin mRNA as an internal control. 0.75×10^6 cells cultured with 5.0 μg siRNA for 48 hr showed the greatest knock-down percentage (46%) in comparison to all other culture conditions. The effects of the *tax* mRNA knock-down on host gene expression are currently being evaluated using a bovine cDNA microarray containing approximately 7000 genes. The siRNA knock-down of BLV *tax* in the BL3* establishes this cell line as an important resource for functional genomics of the bovine immune system.

C 032

Use of subtractive hybridisation for identification of genes expressed in horse macrophages following E.coli stimulation

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Suppression Subtractive Hybridisation (SSH) is a powerful tool for identifying differentially expressed cDNAs. Studying genes potentially involved in genetic resistance to infectious diseases in the horse, we used SSH to identify differentially expressed genes in the horse macrophage. Native monocytes were isolated from the peripheral blood by gradient centrifugation and subsequent adherence on plastic surface. Following overnight culture, monocyte-derived macrophage cells were stimulated with E.coli K12 cells. The ratio was 40 E.coli cells to one macrophage cell. After 4 hours of incubation, non-phagocyted bacteria were washed away. Macrophages were subsequently incubated in MEM alfa containing 5 μg of gentamycin in 1 ml of media for 20 hours. For RNA harvesting, macrophages were lysed in TRIReagent directly on plastic dish. Isolated mRNA was used in Clontech PCR-Select cDNA Subtraction Kit. Out of known genes, for example the interleukin 1 beta encoding gene and melanoma growth stimulating factor analogue (MGSA) gene were identified. New ESTs showing sequence similarity with immunity-related genes from other species were identified as well.

C 033

A gene exhaustive analysis of the center of the area pellucida of the blastoderm in the chicken

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Previous replanting experiments revealed that the center of the area pellucida of the blastoderm had multipotential cells. And primordial germ cells (PGCs) or the precursor PGCs is localized in the center of the area pellucida of the blastoderm in the chicken. But the research findings of molecular analysis are exiguous, so a flow of cell lineage in chick development and the mechanisms of germ cell specification remain unknown. Thus, we exhaustively searched a gene of the center of the area pellucida of the blastoderm by differential display reverse transcriptase-polymerase chain reaction (DD RT-PCR), which enabled to identify of such differentially expressed mRNA. The centers of the area pellucida of the blastoderm and whole blastodermal total RNA were extracted by TRIzol. And DD RT-PCR carried these total RNA. These results detected that 108 cDNA fragments were specific in the center of the area pellucida. This cDNA fragments were analyzed by DNA sequencing. By this means, we had gene candidates specific in the center of the area pellucida, and now we have been searching further genes specific in the center of the area pellucida.
Presentation of the day, I will explain the details of gene candidates.
This sutudy will show that how the cells of the center of area pellucida including germ line cells are characterized by molecular foundations.

C 034

Proteomic analysis using two-dimensional gel electrophoresis in muscle and fat tissue between Japanese Black and Holstein cattle

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Japanese Black is a major beef cattle breed in Japan. Their meet has very high market value and the quality is also highly evaluated on a global scale. Holstein is a well known dairy cattle but their meet quality is generally lower than that of Japanese Black. We attempted proteomic approach in order to find candidate protein spots, which indicate the different expression pattern between the breeds in muscle and fat tissue. The breed comparison experiments were carried out using three 10-month-old steers from both breeds. The animals were housed in three adjacent pens in each breed group and fed the same diets for the fattening period of 22 months. *M. longuissimus capitis* muscle and the subcutaneous adipose tissue samples were collected when they were slaughtered at 32 months of age. Two-dimensional gel electrophoresis was utilized to compare the proteome displays. The first and second dimensions were carried out on nonlinear wide-range immobilized pH gradients (pH 3-10 and 4-7; 13 cm long IPG strip) and polyacrylamide linear gradient gels (18 cm × 16 cm × 1 mm), respectively. The gels were stained by silver staining method. In total, approximately 1000 protein spots were observed on each gel. Three protein spots in muscle tissue, which indicated different expression between the breeds, were detected and all of them revealed higher expression in Holstein than Japanese Black. Two protein spots were also observed in fat tissue; one was higher in Holstein than Japanese Black and another was present in Japanese Black but absent in Holstein. Now we attempt to determine the amino acid sequence of these spots of interest by Edman degradation.

C 035

Proteome analysis of Korean Native Chicken muscles fed *Panax ginseng*: Identification of differentially expressed proteins using peptide fingerprinting

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The aim of this study was to investigate proteins affected by dietary *Panax ginseng* in Korean native chicken muscles, for influencing meat quality and productivity. 2-dimensional electrophoresis (2DE) and mass spectrometry (MS) were used to identify the differentially expressed proteins. We extracted the whole proteins from chicken muscles fed 0% (control), 1%, 3%, and 5% *Panax ginseng* in diet. More than 300 protein spots were detected on silver stained 2D gels using pH 3-10 gradients. Five differentially expressed protein spots, 4 up-regulated spots and 1 down-regulated spot, were analyzed by MALDI-TOF MS and MS/MS. The obtained Ms and MS/MS data were searched against a protein database using the Mascot search engine. Four up-regulated proteins were alpha-tropomyosin (2 spots), protease, triosephosphate isomerase and one down-regulated protein was keratin by *Panax ginseng* treatment. Further researches on the identified proteins can give valuable information of biochemical roles of *Panax ginseng* in chicken meats.

C 037

Developmental expression of paternally expressed genes from the callipyge locus of sheep chromosome 18

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The callipyge mutation occurs in an imprinted gene cluster and only paternal heterozygous (+/C) animals exhibit the muscle hypertrophy phenotype. Quantitative PCR was used to measure changes in paternally expressed DLK1 and PEG11 gene at three ages (2 weeks prenatal, 2 weeks and 8 weeks postnatal). There was a significant effect of genotype ($P<0.05$) and age ($P<0.0001$) on the expression of both genes. DLK1 and PEG11 expression in semimembranosus (SM) was not significantly different between the four genotypes in prenatal animals. At 2 weeks of age, DLK1 expression in SM of +/C animals was significantly higher ($P<0.05$) than the other three genotypes. DLK1 expression in +/C animals at 8 weeks remained significantly higher ($P<0.05$) than +/+ and C/+ animals but was not different than C/C. Expression of PEG11 in SM had a high level of prenatal expression that declined over time in all genotypes except the +/C. PEG11 expression in the SM of +/C animals increased at 2 weeks of age and was significantly higher ($P<0.05$) than the other genotypes. PEG11 expression in +/C animals declined from 2 weeks to 8 weeks to a level significantly higher ($P<0.05$) than the +/+ and C/+ animals but was not different from C/C animals. Orthogonal contrasts indicate the pattern of gene expression of both DLK1 and PEG11 is consistent with polar overdominance at both 2 weeks and 8 weeks. DLK1 and PEG11 show developmental and genotype-specific expression patterns consistent with the callipyge phenotype.

C 038

DNase I hypersensitive sites around the ovine callipyge mutation

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The callipyge (CLPG) phenotype is an inherited skeletal muscle hypertrophy described in sheep. The *CLPG* mutation, an A to G transition, was found in highly conserved dodecamer motif located in an intervening sequence between imprinted *DLK1* and *GTL2* genes. The conserved motif is thought to be a part of a locus control region (LCR), as the mutation enhances transcription level of RNAs located in the 250-kb imprinted domain in cis without altering their imprinting status. Some LCRs, long range cis-acting transcriptional elements, have been identified by hypersensitivity to nuclease. The hypersensitivity is thought to reflect relatively decondensed chromatin structures that would allow interactions of regulatory elements with their target genes via protein complex.
To elucidate the mechanism of the cis-acting effect responsible for the CLPG phenotype, we examine ovine muscle chromatin for the presence of DNase I hypersensitive sites. Here we report up-to-date results of the assay on individuals representing the four possible genotypes.

C 039

Molecular characterization of a region in *IGF2* intron 3 harbouring a quantitative trait nucleotide affecting muscle growth in the pig

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A paternally expressed quantitative trait locus (QTL) affecting muscle growth, fat deposition and heart size has been identified at the distal tip of pig chromosome 2p. We have recently shown that the QTL is caused by a G→A transition at position *IGF2*-intron3-3072. This localizes the mutation in an evolutionary conserved CpG-island that is hypomethylated in skeletal muscle. Furthermore, we have demonstrated by electrophoretic mobility shift assay and transient tranfection analyses of murine C2C12 myoblasts that the quantitative trait nucleotide (QTN) prevents the binding of a repressor *in vitro*. In the present study we report DNase I footprinting of the 300bp surrounding the QTN. The characterization of this CpG-island will help us to further elucidate the mechanisms underlying the QTL effect as well as to increase our general knowledge of the complex regulation of *IGF2* expression.

C 040

Mapping the mutation for the *Belt* allele at the porcine *Dominant white/KIT* locus

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The *KIT* gene located on pig chromosome 8, encodes the mast/stem cell growth factor receptor, which is essential in melanogenesis, hematopoiesis and gametogenesis. Seven *KIT* alleles with distinct phenotypic effects have so far been described: recessive wild-type *i, Patch* I^P, *Belt* I^{Be}, *Dominant White* I^1, I^2, I^3, and the I^L allele. We have previously reported that I^P is associated with a large duplication (~450 kb) including the entire *KIT* coding sequence while the *Dominant white* alleles are associated with the same duplication plus a splice mutation in intron 17. I^{Be}, causing a white belt across the shoulders and front legs, seen for instance in the Hampshire breed, is neither associated with the duplication nor the splice mutation. We have proposed, on the basis of similarity to some mouse *KIT* mutations, that I^{Be} is most likely caused by a regulatory mutation upstream of *KIT*. In the present study the *KIT* upstream region, corresponding to approximately −270 kb has been amplified from different *KIT* genotypes as overlapping long-range PCR fragments. The fragments will be screened for insertion/deletion polymorphisms in a first attempt to map and identify the mutation causing the Belt phenotype.

C 041

Genomic organization of tyrosinase-related protein-2/DOPAchrome tautomerase gene (*TYRP2 /DCT*) in pig

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Tyrosinase-related protein-2 (*TYRP2* or *DCT*; DOPAchrome tautomerase) is a metal-containing glycosylated enzyme that catalyzes the conversion of DOPAchrome to DHICA (5.6-dihydroxyindole-2-carboxylic acid) in melanine synthesis. The *Tyrp2* gene was mapped to the *slaty* locus in mice. Here we report the cloning, genomic sequencing of the porcine *TYRP2* gene. The total RNA was extracted from the individuals in two black coat color breeds, Hampshire and Meishan. *TYRP2* cDNA was amplified by RT-PCR and directly sequenced. We have also isolated the BAC clone that contained all of the exons of *TYRP2* gene, and determined the sequence by shotgun procedure. This gene consists of 8 exons spanning approximately 50kbp-genomic DNA, and is composed of 519 amino acids with 86.7 % and 83.6 % identity with the human and mouse counterparts, respectively. We compared the sequences of *TYPR2* cDNA among pig breeds of various types of coat colors. Some particular transcripts were detected in recessive and piebald color breeds that were shorter than that of black coat color breeds. (This work was supported by JRA and MAFF, JAPAN.)

C 042

Expression of the prion protein gene, PRNP, in different stages of pregnancy and tissues of the genotypically selected sheep ewes and conceptuses

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Scrapie is a fatal neurodegenerative disorder which is characterized by the conversion of a normal cellular prion protein (PrPc) to an abnormal protease-resistant form (PrPSc) in a kind of domino reaction. Oral inoculation is the most important mode of infection; knowledge about epidemiological importance and means of possible maternal-vertical infection are still ambiguous. Since expression of PrPc is a prerequisite for the infection to start this study aims to determine the PRNP gene expression in different tissues and stages of pregnant ewes and conceptuses. Sheep were classified as resistant (R1) or high susceptible (R5) to scrapie according to their genotype at codons 136, 154 and 171 of PRNP. Ewes were mated to rams of the same risk group. Maternal and fetal tissues were collected at the 1st, 3rd and 5th month of pregnancy and PRNP expression was determined using reverse transcription-PCR and real time PCR. The results showed that the PRNP gene was expressed as early as the first month of pregnancy in all maternal tissues investigated including ovary, oviduct, endometrium, myometrium and caruncle as well as in fetal tissues of brain, liver, lung, heart, intestine, muscle, bone marrow, skin, amniochorion, amnion, chorion, allantoic and cotyledon. Exact timing which the gene switches on during embryogenesis needs to be defined. Results of the present study will support identification of mechanisms underlying prion disease development and transmission.

C 043
Serum Nucleic Acids Directly Correlate to BSE Risk

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Etiopathologic and immune responses to prions, the agents associated with the transmissible spongiform encephalopathy (TSE) diseases, such as scrapie in sheep and bovine spongiform encephalopathy (BSE) or mad cow disease remains unclear. Germ-line mutations in the prion coding region have been described in scrapie/sheep but yet to be identified in BSE/cows. Our analysis of microvesicular associated serum nucleic acids (SNA) shows a distinct pattern of repetitive nucleic acids associated with prion-positive cattle and their associated feeding cohorts. Using a differential display PCR test of SNA, a study was conducted on confirmed prion positive cows (N=4), nine at-risk feeding cohorts of confirmed prion positive cows (N=207) and randomly selected cattle from a local slaughterhouse (N=744). Gene amplification detected unique SNA profiles in all nine confirmed prion positive associated cohorts with reactive patterns ranging from 33% to 91% of the 207 total cohort animal sera tested. In contrast, only 0.5% of controls from normal herds and randomly selected cattle from slaughterhouse were reactive ($p<0.001$). The unique SNA profiles significantly associated with BSE at-risk cows support the approach of using a SNA PCR *ante mortem* test for identifying at-risk herds.

C 044
PRNP polymorphisms in German sheep breeds

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Susceptibility to scrapie in sheep is associated with polymorphisms in the prion protein (*PRNP*) gene. We genotyped 20849 representative samples of 25 German and 8 imported breeds by direct sequencing of PCR products of *PRNP* exon 3. In addition to the five common alleles ARQ, VRQ, AHQ, ARH, and ARR at the relevant *PRNP* codons 136, 154, and 171 we identified a novel ARK allele with a lysine at codon 171 in a single land sheep. With respect to other known *PRNP* polymorphisms we confirmed the variation at the *PRNP* codons 141 (L/F), 143 (H/R), and 211 (R/Q) and identified two new functional mutations at codon 145 (G/V) and codon 168 (P/L), respectively. The low risk ARR allele was detected in each examined breed, but the breed specific allele frequencies varied between 0.4% and 79.1%. In 9 out of 9 examined German meat sheep breeds and in 2 out of 14 German land sheep breeds high ARR allele frequencies (> 50%) were observed. On the other hand 2 German milk sheep breeds and sheep from 12 indigenous land sheep breeds harboured this allele at lower frequencies (< 30%). In these breeds the wild type ARQ allele was predominantly observed. Accelerated by a decision of the European Commission selection of ARR carrying animals has started. However, there is a need for further studies as for example the inbreeding rate may increase in small endangered land sheep populations. Additionally, a focus on *PRNP* genotype alone is risky if selection towards the ARR allele is associated with other breed specific selection traits.

C 045
PrP alleles frequencies determination in 17 Italian breeds sheep

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The major genetic factors influencing scrapie susceptibility in sheep are polymorphisms in the prion protein (PrP) gene. In particular, three polymorphisms at codons 136, 154 and 171 influence the susceptibility of sheep to natural and experimental scrapie, and five alleles (VRQ, ARQ, ARR, AHQ, ARH) have been described in Europe. The VRQ and ARQ alleles are associated with susceptibility to scrapie while the ARR with resistance. Evidence to date indicates a similar genetically-determined resistance in sheep orally challenged with BSE. Due to the possible risk of BSE infection in small ruminant populations, and in order to control or eventually eradicate scrapie, the EU has recently adopted a "genetic approach" to transmissible spongiform encephalopathies (TSE) management in sheep. Under that scheme, flocks are classified into different risk categories on the basis of PrP genotype (Regulation EC 260/2003). The knowledge of PrP alleles frequencies in sheep breeds from each EU Member State (Decision 2002/1003/CE) represents the starting point for planning the subsequent breeding programmes for TSE resistance. The results of a survey on PrP alleles frequencies in 17 Italian sheep breeds (3.346 samples) are presented. PrP genotype was determined with real time PCR using the allelic discrimination technique. The VRQ allele generally shows very low frequencies (from 0% to 8%), excepted for two breeds (Merinizzata e Comisana) carrying 10% and 14% of that allele. By contrast the ARR allele is highly represented in Italian breeds (from 24% to 57%) with the exceptions of three breeds of limited numerical relevance: Bergamasca, Biellese and Pinzirita carrying 9%, 15% and 17%, respectively.

C 046
Small ruminants characterization of the *PrP* gene. Study of new polymorphisms and relationship with the resistance/susceptibility to the scrapie disease

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Scrapie is a neurodegenerative disease of the central nervous system of sheep and goats. Studies of natural and experimentally induced scrapie in sheep have shown that genetic susceptibility to the disease is modulated by allelic variation in the sheep prion protein (PrP) gene. At present, the knowledge of the genetic control of the scrapie disease in goats is limited. We report here the first *PrP* sequencing study of native and worldwide Spanish sheep and goats. In these study scrapie-affected animals, healthy animals from scrapie-affected flocks and animals from breed survey were analysed. In the sheep study, fourteen amino acid polymorphisms were detected, including the known amino acid substitutions at codons 112, 136, 141, 143, 154, 171 and 176, and new polymorphisms at codons 101 (Q→R), 151 (R→G), 151 (R→H), 172 (Y→D) and 175 (Q→E). In the goats study, although the number of animals and herds were limited, a high number of polymorphic sites can be found in the goat *PrP* gene (18, 127, 142, 154, 211, 219, 222 and 232), however, the frequencies for the rare allele are low. In summary, we describe new polymorphisms observed in the native and worldwide extend breeds and their possible relationship with scrapie susceptibility.

C 047

Study of the link between gene copy number of human mutated SOD1 and A.L.S. development in SOD1^{G93A} mice

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A.L.S. is a fatal disease which destroys upper and lower motoneurons of motor cortex, brainstem and spinal cord and finally causes death one to five years after the onset of clinical findings.
Many aspects of the illness remain undefined so that to be able to understand them a model of A.L.S. was created in 1994, SOD1^{G93A}, based on the overexpression of the human SOD1 which had a mutation in codon 93 (G->A). This is the most extended F.A.L.S. model due to high similarities to human clinical findings in A.L.S..
Due to SOD1^{G93A} mice have different human mutated SOD1 gene copy number between animals we are able to study these differences by real time PCR. Using this technology we get a relative quantification of them taking as reference standard curves created from colony founders mice.
After setting the differences on human mutated SOD1 gene copy number we will study their effect on illness development in SOD1^{G93A} mice by several behavioral tests and clinical findings.
As well as clinical interest, the knowledge of the number of human mutated SOD1 gene copy number in our animals along several litters allows us to be sure about our colony does not run the risk of losing the mutation.

C 048

Haplotype analysis of feline xanthine dehydrogenase gene

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Xanthine dehydrogenase (XDH, EC1.1.1.204) is an enzyme that has a primary biochemical role in purine catabolism. Dysfunction of XDH induced xanthine urolithiasis because of the increase of xanthine and hypoxanthine and the decrease of uric acid in urine. In order to study on feline xanthine urolithiasis, genetic markers were investigated in feline xanthine dehydrogenase gene. Two polymorphisms were observrd in an intron of feline XDH gene. One is a SNP of substitution of G-A which results in the change of a digestion site with *Pst*I. G-type has the sequence digestible with *Pst*I and A-type has not the sequences on it. In 50 cats, the gene frequencies of G-type and A-type allele in the SNP were 0.46 and 0.54, respectively, that did not derivate from Hardy-Weinberg equilibrium. A polymorphic 18bp-deletion was also observed in the same intron of feline XDH gene. Although heterozygote of alleles with 18bp-deletion and without the deletion was observed in 26 of 50 cats, homozygote of the 18bp-deleted allele was not detected in the samples. Haplotypes analysis of these two polymorphisms in feline XDH gene revealed that feline XDH gene was classified into three haplotypes and the gene without the *Pst*I site and the 18bp-deletion was estimated to be an original allele.

C 049

Linkage and physiopathological analysis of spontaneous hereditary canine disorders segregating in French pedigrees

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The Alfort Veterinary School receives approximately 30,000 animal patients annually. This extensive caseload provides an exellent basis for recruiting dogs affected by hereditary disorders. We identified a congenital myopathy in Labradors, clinically homologous to the human orphan centronuclear myopathy. We generated an experimental pedigree segregating the canine autosomal recessive disease locus and mapped the locus on the canine chromosome 2. Insertion of a tRNA-derived repeat DNA element (tRNA-SINE) in exon 2 of the canine *PTPLA* gene was identified once in heterozygotes and twice in affected dogs. We failed to detect the insertion in dogs from 10 other breeds or mongrels, strongly suggesting that two copies of the *PTPLA^{cnm}* allele cause the disease. Analysis of *PTPLA* expression in affected puppies revealed 2 wild-type alternative transcripts, detected at a very low level, and at least 5 additional aberrant transcripts resulting from unusual splicing events, leading to truncated proteins. The SINE insertion therefore induces an abnormal transcription pattern which is more complex than the exonization or the selective splicing of the SINE-containing exon, as usually described. Expression and function of the PTPLA protein in normal and affected muscles is presently under investigation. Helped by kennel clubs, we also collected pedigrees mainly segregating neurological and sensorineural diseases. Some are now subjected to linkage analyses and will be presented.

C 050

Ichthyosis disorder in Chianina cattle: identification of the inherited transmission by pedigree analysis and sequencing of TGM1 candidate gene

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Recently, in Chianina, a valuable autochthonous cattle breed, several cases of ichthyosis were reported in Italy. Ichthyosis is a disorder characterized by an excessive amount of superficial scales on most areas of the skin. Several inherited forms have been described in man. Two main forms have been reported in cattle: Foetal ichthyosis, more severe and lethal and Congenital ichthyosis, similar but less severe than the previous one. The present work represents a first step of a wider project and it had the goal to demonstrate the inherited transmission of the form, to exclude karyological defects and to obtain the sequence of the candidate gene TGM1. On 12 affected calves autoptic analysis confirmed the diagnosis of ichthyosis. Pedigree data confirmed by microsatellite genotyping the heredity of the trait as authosomal recessive and showed that all the cases are related to a single ancestor sire widely used. Karyotype was normal. We chose TGM1 as candidate gene of ichthyosis by comparison with the human disease. Being bovine sequence missing, we aligned bovine ESTs and the human AA sequence to obtain a theoretical sequence of the bovine TGM1. On this sequence, primers for retrotranscription were designed and the complete cDNA of the gene was obtained. Work is in progress to find polymorphisms of the gene in linkage with the disease.

C 051

Cloning of bovine *F11* gene and identification of a mutation responsible for factor XI deficiency of Japanese black cattle

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An inheritable bleeding disorder caused by an autosomal single recessive gene has been reported in a population of Japanese black cattle. The disease has been diagnosed as coagulation factor XI deficiency of cattle. To characterize the molecular lesion causing factor XI deficiency in cattle, we isolated cDNA clones covering entire coding region of bovine *F11* gene from a bovine liver cDNA library and their nucleotide sequences were determined. We also determined the genomic sequence of 15 exons and 14 introns of the *F11* gene. The nucleotide and deduced amino acid sequences of bovine *F11* gene had 89.6 and 90.2% identity with those of the human *F11* gene, respectively. In order to identify the mutation within the *F11* gene causing factor XI deficiency in cattle, we amplified the 15 exons of the *F11* gene from an affected animal, determined their nucleotide sequences, and compared the nucleotide sequences with that of normal cattle. Consequently, a substitution of one nucleotide with 15 nucleotides resulting in a substitution of one amino acid with six amino acids was identified in the affected animals. The genotypes of the *F11* gene were completely corresponding with the factor XI activities among 119 animals of the Japanese black cattle, indicating that the substitution is the causative mutation for the factor XI deficiency.

C 052

Prevalence of mutant alleles for seven genetic disorders in the Japanese cattle population

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Seven DNA tests for genetic disorders in cattle are currently being carried out at our institution. In order to grasp the prevalence of mutant alleles and control these genetic disorders, we are investigating the distribution of mutant alleles in the Japanese cattle population. Our study employs the Complex Vertebral Malformation (CVM) and the Bovine Leukocyte Adhesion Deficiency (BLAD) DNA tests for testing Holstein cattle. Tests employed for Japanese Black Cattle (Wagyu) include the Erythrocyte Membrane Protein Band Ⅲ Deficiency (BAND3), the Bovine Blood Coagulation Factor XIII Deficiency (F13) Claudin-16 Deficiency (CL16), the Chediak-Higashi Syndrome (CHS) and the Molybdopterin Cofactor Sulfurase Deficiency (MCSU). All genetic disorders inspected are autosomal recessive. Carriers of these disorders are phenotypically normal. The following figures include the period (years) of research, number of tested samples (proven sires and young sires) and carrier frequency (%). BLAD: 12 years, 2500, 5.8%, CVM: 3 years, 2800, 12.1%, BAND3: 7 years, 3000, 2.7%, F13: 7 years, 3000, 0.8%, CL16: 5 years, 3000, 9.2%, MCSU: 3 years, 2800, 0.1%, CHS: 3 years, 2800, 4.7%. The carrier frequencies of both CVM and BLAD are high. Carriers of the BAND3, CL16 and CHS were detected at a high frequency in the population of Wagyu sires. In addition, only a few homozygotes for the mutant allele of CL16 and the mutant allele of CHS have been found in young sires respectively, due to incomplete penetration. Since carriers of these disorders were found in the population of young sires, further surveillance is necessary.

C 053

Equine Osteochondrosis - a Molecular Genetic Approach

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The aim of this branch of a newly started project is to study genetic expression characteristic to growth cartilage in young equines, and genes involved in the development of osteochondrosis (OC). OC is one of the most frequent causes of lameness in young horses. A high prevalence of the disease (14,3%) and a heritable component have both been detected in the Norwegian Standardbred trotter. OC is a developmental disease characterized by a focal disturbance of the growth of bone. Areas of growth cartilage die, and is rendered from the normal process of mineralization. This leaves the overlying articular cartilage susceptible to damage. In this study, cartilage from the hock joint (the distal intermediate ridge of the tibia, and the lateral trochlear ridge of the talus) and samples from various other tissues are collected from 0-8 weeks old foals. The foals are individuals of different breeds being euthanized for reasons other than OC, and Standardbred trotter foals bred from parents with OC. Sampling materials from young horses where the growth cartilage is still active, enables both characterization of the growth cartilage, and the early stages in the development of OC. Genes differentially expressed in growth cartilage may be detected by comparing growth cartilage to a pool of other tissues using RDA (Representational Difference Analysis). By Comparing cartilage from affected and non-affected foals, RDA also enables detection of genes involved in OC. Studying genetic variation of the OC-specific genes on population level, facilitates detection of possible correlation between genes and OC. Cooperation with a pathological branch involved in the same project, gives useful information about morphologic and pathologic characters of the specific cartilage being studied.

C 054

Comparative analysis of gene expression in M. biceps femoris of healthy and splay leg piglets

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Congenital splay leg is the most frequent hereditary disorder observed in newborn piglets. Affected piglets have an impaired ability to move and are subject to increased losses. An obvious muscular weakness leads to the typical splaying of the hindlegs. The genetic basis of the splay leg syndrome however, is still unknown. We analyzed a set of 12 genes coding for proteins contributing either to structural composition of the muscle fibers or to regulation of muscle development and growth. Total RNA was prepared from M. biceps femoris of each 11 newborn splay leg and healthy piglets, respectively. We found no significant differences in the expression of all investigated genes between both phenotypes. However, electron microscopy of muscle samples revealed that the phenotype “splay leg” included piglets with pathological alterations as well as piglets without any findings in muscle ultrastructure. Re-analysis of the expression data according to these results demonstrated significantly increased expression of genes for different Myosin Heavy Chain (MyHC) isoforms in splay leg piglets with pathological muscle structure compared to healthy piglets. No differences were observed for regulation factors (IGF1, MyoD1, myf5, myf6). This indicates that the increased expression of structural components in skeletal muscle of splay leg piglets is part of a compensatory process rather than the cause for the disease.

C 055

Identification of genomic regions, which are associated with porcine hernia inguinalis/scrotalis by genomic mismatch scanning (GMS)

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Previously, linkage and association analyses were performed to identify loci affecting susceptibility to hernia inguinalis/scrotalis by scoring characterized sequence variations such as microsatellites and single nucleotide polymorphisms. Genomic mismatch scanning is a hybridization-based technique designed to enrich regions of identity by descent (IBD) between two individuals without the need of genotyping or sequencing. Identical DNA fragments from two genomic sources are enriched in two steps: First, DNA heteroduplexes formed between genomic DNA fragments from two individuals are purified by a procedure based on differential restriction methylation and endonuclease digestion. Secondly, heterohybrids that contain mismatches are nicked *in vitro* by the *E. coli* MutHLS mismatch repair system and are eliminated subsequently from the pool, leaving only mismatch-free heterohybrids. Here, we demonstrate the feasibility and efficiency of this kind of linkage mapping using DNA of related and unrelated pigs affected with hernia inguinalis/scrotalis. The genomic locations of the GMS-selected IBD DNA fragments were determined by PCR-screening of a Somatic Cell Hybrid Panel and a Radiation Hybrid Panel as well as by Fluorescence *in situ* Hybridization (FISH). The results of the genomic mismatch screen were compared to the results of linkage and association studies achieved by the more common method of microsatellite typing.

C 057

Establishment of cell-culture models reproducing bovine and porcine M cells *in vitro*

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The epithelium that lines the gut is impermeable to macromolecules and microorganisms, except in Peyer's patches (PP). There the lymphoid follicle-associated epithelium (FAE) contains M cells that transport antigens and microorganisms. A cultured system that reproduces the main characteristics of FAE and M cells was established by cultivation of PP lymphocytes with the differentiated human intestinal cell line Caco-2 (Kerneis *et al*., 1997). Using this coculture model it was shown by Heppner and colleagues (Heppner *et al*., 2001) that infectious prion proteins (PrPsc) were transported exclusively through monolayers haboring M cells. These findings suggest that transcytosis via M cells and subsequent delivery to gastrointestinal lymphoid tissue may be an important route of uptake of the PrPsc. The pathogenesis of TSE in human and cattle is most likely dependent on intestinal entry of orally ingested PrPsc. However in some species, such as pigs and chicken, oral transmission of PrPsc is hindered by the existance of a large species barrier, most likely located in the gastrointestinal tract. To investigate the causes of possibly diminished PrPsc uptake at the porcine FAE and to compare characteristics of human, bovine and porcine M cells we tested if the Caco-2/RajiB coculture model is applicable to bovine and porcine fetal intestinal cell lines. Here we report our results of comparing the three intestinal epithelial cell lines Caco-2, FBJ (Fetal Bovine Jejunal) and IPEC-J2 (Intestinal Porcine Epithelial Cell) using RT-qPCR and immunohistochemical methods. Furthermore we tested the transport-capacity of cocultures of FBJ/RajiB and IPEC-J2/RajiB cells.

C 058

Commercialization and Mass Production of CPP-H and β-casein H

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We confirmed through protein and DNA sequence analyses that the H variant differs at five residues from the A2 sequence: Arg25/Cys, Leu88/Ile, Gln117/Glu, Glu175/Gln and Gln195/Glu. Of these substitutions the 25th residue was contained in the casein phosphopeptide (CPP) region. In rats, calcium solubilizing effect of the CPP of bovine variant H was increased by 23% compared with that of the CPP of non-H. Using extensive Korean Bostaurus pedigrees, we confirmed that β-CN H was controlled by a codominant allele. (Animal Genetics, 2000, 31, 49~51). CPP-H gene exists in Korean cattle, but Korean cattle have low milk production (1~5Kg per day). For commercialization and mass production of CPP-H, Korean bulls were crossed with 120 heads of Holstein. In the F1 generation, 120 heads were obtained (58 cows and 62 bulls). Among them, there were 62 heads which had CPP-H (30 cows and 32 bulls). The calves produced were mostly black, some of them had white spots and only two heads were brown in color just like Korean bull. The F1 cows produced milk on an average 15.2Kg per day. CPP-H was determined by using acidic starch gel electrophoresis. The calves born in both of F1 and F2 were healthy and did not have any symptoms of diseases indicating that they have strong resistance to disease. They also had higher quality of meat. This animal factory shows the possibility that we can develop milk and meat producing breed with high resistance to the disease and mass production of milk and meat.

C 059

Relationship between restriction fragment polymorphism (RFLP) at bovine calpain and calpastatin locuses and meat quality

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Calpains - Ca^{2+}-activated cysteine neutral proteases (CANPs) - are expressed ubiquitously; μ-calpain is activated by μM and m-calpain by mM concentrations of Ca^{2+}. There is no tissue specificity for both calpains, but their expression differs in tissues. CANP activity is regulated by calpastatin (CAST) - specific inhibitor. CANP contribute to cytoskeleton remodeling and degradation of cellular proteins. The m-calpain and calpastatin genes polymorphism, the released product of which may affect the tenderization and conditioning during meat ageing and refrigeration storage, was conducted in Black-and White (B-W) bulls. M-calpain gene polymorphism was detected with *Hha*I enzyme in 61 B-W bulls. Genotype AA showed the three-band (0.2), BB – two-band (0.5), while AB animals displayed a pattern of all four bands (0.3). Allele frequencies were: A– 0.43 and B– 0.57. Colour component B differed between AA and BB genotypes (organoleptic valuation). CAST gene polymorphism was detected with *Xmn*I enzyme in 84 bulls. The genotype frequencies were: 0.2 for AA, 0.4 for BB and 0.4 for AB; allele frequencies were: A– 0.40, B– 0.60. Sequence analysis proved transversion C/G in 61 bp exon 12 position (GenBank AY 258325). The highest: protein content was in BB but fat content in AB bulls; cooking loose differed significantly between AA and BB genotypes. In animal breeding calpain and calpastatin genes affecting meat tenderness are considered as a candidate gene for quantitative trait *locus* (QTL) to speed up the selection of animals.

SECTION D

Gene Mapping
Comparative Genomics

D 001

The influence of missing pedigree, missing genotypes and occurrence of null-alleles on haplotype reconstruction accuracy

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A correct reconstruction of haplotypes is an important part of fine-mapping QTL using IBD based methods. In this study we simulated the effect of different causes of genotype data noise on the accuracy with which haplotypes can be reconstructed under a quick reconstruction algorithm. The noise factors were: missing pedigree data, missing genotype data and the occurrence of null-alleles.

Haplotypes of 20 markers were simulated assuming various levels of the noise factors and various map densities. The accuracy of haplotype reconstruction was assessed as percentage complete haplotypes correctly reconstructed and percentage correctly estimated intervals.

Map densities, which ranged from 5 cM intervals to 0.5 cM intervals, did not greatly influence the accuracy. The difference between the highest and the lowest density typically was around 5% of correctly reconstructed haplotypes.

Although both missing pedigree and the occurrence of null-alleles did negatively impact the accuracy, the reduction in accuracy was only moderate. For every percent point increase in the frequency of null-alleles the decrease was around 3%. For missing pedigree this decrease was around 4%.

The most disruptive noise factor was missing genotypes. An increase in frequency from zero to 5% missing genotypes resulted in a decrease of 12% of correct intervals (from 91% to 79%) and a decrease of correct haplotypes from a mean of 42% down to 9%.

D 002

Integrated maps and Oxford grids: maximising the power of comparative mapping

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The full power of comparative mapping is realised only if all available maps for a particular species (linkage, physical, somatic-cell hybrid, radiation hybrid) are integrated into a single map for that species. We have utilised the strategy and algorithms of the Location DataBase (LDB; Morton *et al.* 1992; Ann. Hum. Genet 56, 223-232) to create an integrated map for each of cattle and sheep from maps publicly available. These integrated maps comprise estimates of the kb location of 4610 and 1736 loci for cattle and sheep, respectively. They are publicly accessible at medvet.angis.org.au/ldb. Electronic zoomable Oxford grids (Edwards *et al.* 2003; HGM2004) have been created for each integrated map against the molecular sequence maps of human, mouse, rat, dog and chicken; and against integrated maps for non-sequenced species. These grids, which are publicly accessible at oxgrid.angis.org.au, provide powerful images of comparative maps.

D 003

Comparisons of human, mouse and cattle chromosome maps reveal the reuse of evolutionary breakpoints

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Detailed genome maps of phylogenetically distant species of mammals provide a powerful means of addressing questions relating to chromosome evolution and speciation. Cattle BAC-end sequences (BESs) were used for construction of high-resolution comparative maps for cattle homologs of HSA1, HSA3, and HSA11. The cattle BESs were selected to have spacing of ~1 Mbp along the human chromosomes and also to have significant sequence similarity in the mouse genome. The BESs were mapped to 10 different cattle chromosomes by radiation hybrid (RH) mapping and integrated with a previously constructed EST-based cattle-human comparative map. Evolutionary breakpoints in human, mouse and cattle chromosomes were identified from pairwise comparisons of the cattle RH, and mouse and human sequence-based comparative maps. These comparisons allowed for identification of lineage-specific breakpoints and conserved chromosome segments preserved in the human, cattle and mouse genomes over 90 MY of evolution. Strikingly, a significant number of reuse breakpoints were identified, thus suggesting that evolutionary breakage sites on chromosomes are not randomly distributed. These three-way genome comparisons provide clear exceptions to the Nadeau-Taylor random breakpoint model of chromosome evolution. The findings that many large chromosome segments are unbroken after >90 MY of mammalian evolution, and that there are rearrangement hot spots, suggest that some chromosome rearrangements are neutral while others may provide selective advantage/disadvantage.

D 004

Alignment of human chromosome 1 with livestock and rodent species

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At low resolution organisation of large regions of chromosomes is conserved between species, with extended chromosome segments containing many of the same genes in several species. As higher resolution data becomes available in different species it is apparent that gene order can differ within apparently conserved segments, and individual genes may be displaced to unexpected locations. EST sequence data was used to direct primer design and construct gene-based bovine and porcine radiation hybrid maps. Orthologs of mapped genes were identified and localized on the human, mouse and rat sequences producing a relatively high-resolution alignment of the cattle, pig, mouse and rat genomes with human chromosome 1. Our data shows that human chromosome 1 shows blocks of conserved synteny with 4 bovine chromosomes (2, 3, 16, and 28), 4 pig chromosomes (4, 6, 9 and 10), 3 mouse chromosomes (1, 3, 4), and 3 rat chromosomes (2, 5, 13). While the gross conservation of synteny between human, pig, mouse and rat chromosomes is continuous for each chromosomal segment represented on human chromosome 1, homologous regions between human chromosome 1 and bovine chromosome 16 are fragmented into three blocks.

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D 005

Fine mapping of EST-cDNA clones based on RFLP in the silkworm, *Bombyx mori*

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We have developed the genetic analysis by using molecular markers based on restriction fragment length polymorphism (RFLP) in the silkworm, *Bombyx mori*.. In the silkworm there is no crossover on female chromosomes, so a (RF02 × RF50) female was crossed to a RF02 male and from this BC_1 segregant more than 250 ug individual DNAs were prepared and used to know their genotypes. Every clone gave homozygous or heterozygous genotype for each individual and the patterns for each clone of 15 BC_1 segregants could be identified from each other. After the 28 patterns for every chromosome were made, every new clone was easily to know their linkage group from the genotyping of 15 individuals. After linkage analysis, all clones were mapped by using the 100 segregants of the cross between a (RF02 × RF50) male and a RF02 female. From all of the genotypes, non-recombinant individuals are those with either all A, homozygous, or B, heterozygous, but recombinant individuals have both A and B. The linear order of clones is known after data is sorted and distance between clones determined by counting the recombinants. Now about 300 cDNA clones have been mapped on molecular genetic map.

D 006

Establishment of a first generation microsatellite-based genetic map in the rabbit

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Although, rabbit (*Oryctolagus cuniculus*) has great potential as a biomedical model for man and its breeding is a small but active economic sector in Europe, rabbit genomics is far from well-developed. In addition, there is a need for specific studies on resistance to diseases and production traits. Thus, INRA has launched a program to build a rabbit genetic map based on microsatellite markers. Among the 352 newly isolated microsatellites, 141 are derived from a genomic library and 211 from rabbit BAC clones containing genes regularly distributed on the human genome Simultaneous FISH-mapping of the BACs and genetic mapping of the associated microsatellites permits direct construction of an integrated cytogenetic and genetic map. We find that 50% of the microsatellites contain a TC repeat. For genotyping, we use a technique based on a PCR with three primers, including a locus-specific right primer, a locus-specific left primer with a tail of 17 base pairs (bp) and a third dye-labeled primer specific for the 17 bp tail. To date, 190 BACs have been FISH-mapped, eight reference families of three generations (186 rabbits) have been built and 276 microsatellites have been tested, among which 166 are correctly amplified and 133 are informative in the reference families with 2 to 6 alleles. We shall present the first version of this rabbit genetic map based on at least 100 microsatellites.

D 007

The first linkage groups in the mink (*Mustela vison*)

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Genome research is progressing in an astonishing pace providing new frontiers in animal improvement. Genetic and physical maps for a large number of livestock species have been constructed during the past decade. These maps have been used for the identification of genes that modulate monogenic traits, or for the identification of chromosomal regions which contain genes having a major effect on important traits (QTLs). Despite the economic importance of mink production in Northern Europe and North America, mink genomics research is lagging far behind other livestock species. The mink industry would benefit tremendously from information derived from linkage and cytogenetic maps of the mink genome, giving an opportunity to speed up genetic improvement. A collaborative effort is aimed at creating first generation linkage and physical maps of the mink genome. The objective of our work is to create the first generation of linkage map with at least 20 cM resolution. This will serve as a basis for further refinement. Genotypes of a reference population consisting of four males, nine females and 71 F1 progeny were determined at 46 expected polymorphic microsatellite loci of which 34 were informative giving results which could be scored. Six markers were assigned to two linkage groups using Crimap software. Physical mapping of the microsatellites was also performed using a panel of mink-hamster hybrid somatic cell lines, showing consistent results with the linkage map.

D 008

Characterization of canine microsatellites isolated from BAC clones harbouring DNA sequences homologous to ten genes

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Human or canine primers specific for the genes *LEP, HBB, PAX3, ESR2, TPH1, ABCA4, ATP2A2, MAOA, POUF1* and *TECTA* were used to identify clones in a canine BAC library. Subcloning of the positive BACs in plasmids, screening with microsatellite motifs and subsequent sequencing allowed for the identification of ten novel microsatellites *ZuBeCa53, ZuBeCa57, ZuBeCa59, ZuBeCa60, ZuBeCa61, ZuBeCa64, ZuBeCa66, ZuBeCa67, ZuBeCa69* and *ZuBeCa70*. BAC end sequencing of two clones harbouring putative canine *TECTA* and *POU1F1* identified two repetitive sequences *ZuBeCa47* and *ZuBeCa48*, respectively. The presence of the gene of interest was confirmed by sequencing the PCR products amplified in the positive BACs. The PCR products were identical with the corresponding human or dog sequences, in the range of 73.4% (ABCA4) to 100% (PAX3, LEP). A total of 24 dogs, representing 24 different breeds, 10 red foxes, 10 arctic foxes and 10 Chinese raccoon dogs were genotyped with the newly developed markers. All markers showed Mendelian inheritance with the exception of *ZuBeCa57* and *ZuBeca61* — which turned out to by X-linked. DNA of positive clones was labelled and mapped by FISH to Q-banded dog and red fox metaphase chromosome preparations. The approach to develop polymorphic markers closely linked with functional genes, provides tools of interest to comparative mapping projects but also to studies aimed at finding genes responsible for disease or production traits.

D 009

Characterization of 138 novel microsatellite DNA markers and a preliminary linkage map for duck (*Anas platyrhynchos*)

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We have constructed an enriched library with the aim to identify duck microsatellites. A total of 300 pair of microsatellite primers were developed and used to detect polymorphisms. 113 loci exhibited sequence length polymorphisms and 25 loci had only one allele in 31 unrelated Peking ducks. A total of 524 alleles were observed from the novel polymorphic microsatellite markers, which ranged from 2 to 19 with an average of 4.64 per locus. The frequencies of the 524 alleles ranged 0.02 to 0.98. A total of 63 loci had heterozygosities more than 0.50. The polymorphism information content (PIC) of the 113 loci ranged from 0.03 to 0.90. The loci with a PIC more than 0.50 was 41.59% (47), with a PIC between 0.25 and 0.5 was 45.13% (51), and with a PIC lower than 0.25 was 13.27% (15). Only 1 of the 2 minisatellites could be genotyped, 9 alleles were observed and 0.50 PIC was show in the detected population. The preliminary linkage map of the duck has been developed by segregation analysis of 138 microsatellite markers using a population from the resource family consisted of 12 full sibs families with a total of 224 F_2 individuals. The total length of the preliminary linkage map for duck is 1110.7 cM (sex-averaged map), as in other species, the total of genetic map for the female with 928.7cM is shorter than that for the male with 1377.2 cM.

D 010

Mapping of plumage color and blood protein genes on the microsatellite linkage map of the Japanese quail

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The Japanese quail (*Coturnix japonica*) is valuable for egg and meat production. Recently, it is appreciated as a laboratory research animal and a pilot animal for chicken (*Gallus gallus*). To densify the linkage map of the Japanese quail, we performed linkage analysis using two resource populations maintained at INRA (n=497) and Gifu University (n=291). The INRA population was used in constructing a first-generation microsatellite linkage map (Kayang *et al*., 2004) and QTL analysis concerned with production traits. The Gifu population was investigated with classical markers including plumage color and eggshell color loci, blood group and blood proteins, in addition to microsatellite markers. A total of 15 linkage groups were constructed with five classical markers and 72 microsatellite markers and they were assigned to 14 autosomes (CJA01-07, CJA09, CJA10, CJA13, CJA14, CJA20, CJA24 and CJA27) and the Z chromosome through comparative mapping using chicken orthologous loci. The linkage groups covered a total map distance of 783 cM with an average spacing of 10 cM between loci. Two plumage color loci, black at hatch (*Bh*) and yellow/fawn-2 (*Y/F*), were mapped on CJA01 and CJA20, respectively, while three blood protein loci, transferrin (*Tf*), hemoglobin (*Hb-1*) and prealbumin-1 (*Pa-1*), were mapped on CJA09, CJA14 and CJA24, respectively.

D 011

Construction of linkage map in Japanese quail (*Coturnix japonica*) using AFLP, functional gene, and chicken microsatellite markers

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Japanese quail has been used not only as meat and egg producer but also as laboratory animal because of its small body size, short generation intervals and high egg production. However, the genetical information is so limited and the saturated linkage map has not been constructed yet. In this study, we established backcross resource family using two Japanese quail lines; a muscular disorder (LWC) and neurofilament-deficient mutant (Quv) quail line. Subsequently, the linkage map was constructed by AFLP, functional gene and chicken microsatellite markers. In total, 1933 AFLP, 25 microsatellite and 10 functional gene markers were developed in Japanese quail reference family. By linkage analysis at LOD threshold of 3.0 using these markers and three phenotypic markers (Quv, LWC and sex), 1883 of the markers were distributed into 58 linkage groups and the remaining 88 markers were unlinked. But since duplication of markers existed in many loci, the number of substantive loci was 662. The current map has a total length of 3383.1cM. The average interval between two adjacent loci was 5.1cM. Especially, strong syntecy between the linkage group 2 and chicken chromosome 2 was observed through the chromosome. The Quv was located on the linkage group 6, while the LWC was not linked with any linkage groups.

D 013

Towards a further intergration of the physical, linkage, cytogenetic and sequence maps

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A physical map of the chicken genome will not only be an essential validation of the coming whole genome sequencing assembly but also be an essential guide for selection of clones for gap closure, transgene constructs, organisation of segmental duplication and tile paths for array based comparative genome hybridisation studies. From fingerprinted BAC libraries with 20-fold genome coverage, a physical map of 260 fingerprint contigs of overlapping BAC clones was established. Of these contigs, 202 could be assigned to a specific chicken chromosome. Assignment of the remaining BAC contigs is performed by genetically mapping of SNP markers derived from end sequences of BACs located within these contigs. In addition, ongoing FISH experiments with BACs from the same contigs are used towards a further integration of the physical and sequence maps with the cytogenetic map. Currently the sequence map only covers 30 chicken chromosomes and the BAC mapping experiments ultimately will result in the availability of sequence maps for each of the individual 38 autosomes and the two sex chromosomes. The assigned BAC contigs will be key elements in the eventual completion of the sequence for the complete chicken genome a goal not only essential for understanding chicken biology but for mammalian biology as well.

D 016

High Density ESTs based Bovine Physical Maps Constructed Using a Radiation Hybrid Panel and BAC clones

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The bovine genome sequencing project started November 2003. The sequence is the ultimate genome map and can be used to identify genes and control sequences and to explore the evolution of genomes. Experience in other species, such as mouse and rat, showed that good physical maps help with the assembly of the sequence, and allow alignment of genetic mapping and QTL data with the sequence. A non-redundant set of 21K sequence confirmed bovine cDNAs has been used to build a bovine expression microarray. The Sequence information from the cDNA set was annotated and aligned with sequence trace files from the bovine sequencing project and with human sequence to identify intron/exon boundaries. PCR primers were then designed to where possible to intonic sequence or to span introns, so that products were either bovine specific or distinguishable from hamster products. Genes are currently being mapped onto both a 3000 Rad Bovine whole genome radiation hybrid panel and a bovine BAC library using these primers. The target is to produce a high-density bovine RH map, which will be used to improve the assembly of the bovine whole genome BAC physical map. The BAC contigs and RH map will be used to aid the assembly of the bovine sequence.

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D 017

A comprehensive radiation hybrid map of the bovine genome comprising 5,757 loci

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A comprehensive bovine RH map for all 29 autosomes and the X chromosome was constructed using a 7000-rad radiation hybrid (RH) panel consisting of 92 cell lines (Mariani *et al*. ISAG 2000, Itoh *et al*. ISAG 2002). The framework was constructed with 3219 microsatellites based on the scaffold of the USDA-MARC linkage map, followed by the placement of 2538 ESTs, resulting that the RH map was composed of 5757 loci. The map provides essentially complete coverage across the genome and makes clear ordering of loci whose positions were not separated on the linkage map. We constructed a cattle-human comparative map using 1830 bovine genes or sequences homologous to the human genome sequence, which refined previously reported syntenies. This map provides a framework for mapping a large collection of ESTs and BAC ends, useful to construct a transcript map and assess the assembly of fingerprint-based BAC contigs, respectively. This RH map, together with the linkage map and a BAC contig map, comprises fundamental genomic tools for positional cloning of genes of interest.

D 018

A comprehensive genetic map of the cattle genome based on 3,802 microsatellites

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A microsatellite-based high-density genetic map is essential for fine mapping of hereditary traits of interest and providing a foundation for a physical map construction. Here, we incorporated 2,310 microsatellites into the USDA MARC cattle linkage map with two-point LOD scores (>3.0), of which 2,277 were fine-mapped. The new 3160-cM map with 29 sex-averaged autosomal and a sex-specific X chromosome linkage groups includes 3,960 markers with 2,423 positions, resulting in an average interval size of 1.4 cM. More than half (51%) of the total length of the map is covered within 2.0-cM intervals and the largest gap is a 10.2-cM interval on the X linkage group. The new map should remarkably accelerate fine mapping and positional cloning of genes for genetic diseases and economically important traits in cattle, as well as related livestock species.

D 019

Detection and characterization of microsatellite loci in animals based on PCR

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Tri and tetra repeat microsatellite loci have been isolated based on PCR detection procedures in Korean cattle. The pooled genomic DNA samples, which were digested with Sau3A I restriction enzyme, were separated onto agarose gels. DNA fragments were recovered for 3 sections (200 to 500 bp, 500 to 1000, 1000 to 1,500). A total of 14 genomic libraries were constructed by a PCR-enrichment procedure with biotined oligo probes, which were used microsatellite selection process. A total of 3,800 clones were analyzed for (ATG)n, (TAA)n, (GGC)n, (CAT)n, (GCA)n, and (CTG)n repeat sequences. Most clones (89%) were contained repeat regions and more than 70% clones contained single nucleotide polymorphisms. Effective clones, which contained repeat sequences that used in this study, were 45%. On the Blast search, 65% of clones were hit, 20% of clones were identified as known microsatellite loci, and 15% of clones were identified as unknown genes.

D 020
Extent of linkage disequilibrium in Australian dairy cattle

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There is great interest in the pattern and extent of linkage disequilibrium (LD) in livestock and other species. We used data on 729 bulls genotyped for 48 markers spread over 13 chromosomes in the present analysis. The animals were considered unrelated for the purpose of this study. The order and distance of markers were based on the USDA-MARC map. The extent of LD (D´) was estimated using an EM algorithm approach and a Bayesian approach, and these were in close agreement (r = 0.9). About 47% of the syntenic pairs showed significant LD and 23% of the non-syntenic pairs also showed significant LD. The mean estimates of D´ for marker pairs with a inter-marker distance of less than 25 cM (n=20) were 0.22 ± 0.019, for syntenic marker pairs with distance more than 25 cM (n=63) were 0.15 ± 0.007 and for non-syntenic pairs (n=1045) were 0.13 ± 0.002. The Malecot model was fitted for the exponential decline of LD with distance. The asymptote (0.13) of this model fitted for syntenic markers indicated that there was no difference between the non-syntenic LD and the syntenic LD for infinitely separated markers and hence the non-syntenic LD were merged in the combined analysis. We estimated the swept radius, the distance to which LD useful for mapping extends, of 27 cM. The present analysis indicates a high level of LD in Australian dairy cattle and will aid in the development of optimum marker density and design for fine-localisation of genes affecting quantitative traits using LD mapping.

D 021
Genome-wide linkage disequilibrium in Japanese Black cattle

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In recent years, many experiments have been conducted to detect quantitative trait loci (QTL) for economically important traits in livestock. The next issue is to identify the causal genes underlying genetic variation in these traits. Exploiting linkage disequilibrium (LD) may be more effective for fine mapping the QTL. However, little is hitherto known about the degree of LD for Wagyu populations. In this study genome-wide LD was assessed in a population of the Japanese Black cattle. We used microsatellite genotypes of 162 maternal gametes sampled from the population of Oita Prefecture. LD was measured with D' and tested for significant allelic associations between syntenic and between non-syntenic marker pairs. In the analysis of syntenic pairs, high levels of LD were found, which ranged over several tens of centimorgan and declined as the marker distance increased. Significant LD was observed frequently for marker pairs <40cM apart, as the cumulative frequency of *P* values from the significance test departed largely from its expected distribution under the random allelic association. However, LD was not significant between non-syntenic loci. The difference of D' among chromosomes was significant when corrected for marker distances, which may have resulted from selection. The D' ranged from 0.5 to 0.2 for 5–40cM spaced markers. And a rapid decline of D' to 0.3 was observed from 5–15cM spaced markers. Therefore we suggest that LD mapping can be feasible in the Japanese Black cattle with moderate marker density.

D 022
DNA Sequence Analysis and Chromosome Localization of The Korean Cattle BAC Clones

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Chromosomal DNA of the Korean cattle showing the high performance in meat quality and quantity was employed to generate a total of 150,000 BAC clones. Considering average insert size of the BAC clones is 130-140kb, BAC clones contain the DNA sequences corresponding to about 6X coverage of the chromosomal DNA in Korean cattle. With the completion of the 5' and 3' terminal DNA sequencing of a total of 2,794 BAC clones using two universal sequencing primers (RP2 and T7), approximately 3.3×10^6 base pairs of the Korean cattle chromosome have been obtained. The in silico results revealed that 34.88 % of the DNA sequence contained the repeated sequence. Among those, a total of 394 microsatellites were identified. The DNA sequences, with the elimination of the repeated sequences, were compared with those of human and mouse by using BLASTN program. In addition, the same DNA sequences were applied to the COMPASS software and localized the positions of 1,429 BAC clones in the thirty bovine chromosomes. Our current efforts are being focused on the finding of the DNA sequences that are unique in the Korean native cattle and important for the economic value of the cattle production.

D 023
Comparative mapping and SNP detection of the bovine Expressed Sequence Tags

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The ESTs (Expressed Sequence Tags) are valuable resources for functional genomic studies even though most of them are not full-length coding sequences and some of them have unknown functions. We constructed three normalized cDNA libraries from the skeletal muscle, adipose tissue and liver of four Hanwoo (Korean cattle) aged 24-month old. More than 9,000 clones from the libraries were randomly selected and sequenced from 5'-end of the clone. Those ESTs were analyzed by our local database system downloaded from NCBI GenBank Database. Some of the unique and unmapped ESTs produced using cDNA microarray were predicted *in silico* method with information of human and mouse and have identified the location of the clones with a 5000 rad bovine RH panel. The putative SNPs were also detected by comparing with other bovine EST databases like TIGR cattle gene index. The expression levels of some ESTs were also performed by semi-quantitative RT-PCR with the individuals showing the different phenotypic records. These information will be provided the identification of genes responsible for the economically important traits in cattle.

D 024

Towards a bovine chromosomal linkage disequilibrium map: An integrated radiation hybrid map of *Bos taurus* chromosome 14

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Linkage disequilibrium (LD) mapping has the potential to increase the mapping resolution of quantitative trait loci (QTL) for economically important traits and provide insight regarding recombination and natural selection. We have utilized the 3000 rad bovine whole genome radiation hybrid (WGRH) panel to construct an integrated map of *Bos taurus* chromosome 14 (BTA14) that comprises markers from Commonweath Scientific and Industrial Research Organisation (CSIRO, Australia), Institut National de Recherche Agronomique (INRA, France), Meat Animal Research Center (MARC, USA) and the 5000 rad bovine WGRH map. In addition, we have identified clones from the CHORI-240 BAC library that span the length of the entire chromosome. Furthermore, we have mapped the top and bottom most clones for each of the 30 contigs from the BAC scaffold map that encompass BTA14. This newly constructed integrated map will be used in the creation of the first generation linkage disequilibrium map of BTA14.

D 025

Enhancing the Sheep Genome Map

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The sheep gene mapping community has relatively limited resources when compared to the gene mapping communities of other mammals such as humans, mice, rats, cattle, dogs and pigs. As a consequence the genome map of sheep is less well developed than the genome maps of these species. Genome scans are being undertaken in sheep for a range of traits including disease resistance, meat production, wool production, milk production and prolificacy. Consequently it would be useful to have a more highly developed sheep genome map. There is currently no plan to perform whole genome sequencing for sheep. Hence there is a need to integrate the information obtained from species where whole genome sequencing projects have been undertaken with that of sheep to create a more useful sheep genome map. The current sheep linkage map comprises 1,232 loci and spans ~3,600 cM. Many of these loci have homologues that have been mapped in other species that can act as reference positions between the sheep map and the maps of other species. A composite sheep map is being created that consists of a linkage map framework together with estimated map positions for unmapped sheep GenBank sequences. This will enable the sheep gene mapping community to easily identify the sheep sequences that are available in GenBank for a region of interest. The strategy being used is to identify homologous sequences in other species following blast searches, and then to use the positional information from these other species, in conjunction with that of the reference loci, to estimate map positions. The composite map will be accessible from the Australian Gene Mapping Web site (http://rubens.its.unimelb.edu.au/~jillm/jill.htm).

D 026

Status of the chicken radiation hybrid map

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Radiation hybrid maps are of intermediate resolution between the genetic maps and the BAC contig maps and therefore can be used, even in species such as chicken for which a draft sequence is available, to track errors in the assembly. The chicken radiation hybrid maps are built using a panel composed of 90 clones, with a mean retention frequency of 21.9 %. Over 1200 markers corresponding to microsatellites from the genetic map, BAC end sequences, ESTs or genes were mapped at the time of writing. Complete framework maps covering entirely chromosomes 2, 5, 7, 10, 13, 14, 15, 18, 19 and 28 were obtained and progress is made rapidly towards coverage of the whole genome. When comparing the framework maps of the completed chromosomes with the draft sequence assembly, we confirm its high quality, although a few discrepancies can be detected, with blocks composed of several markers sometimes inverted or placed on the wrong chromosome. In addition to this, 4.5 % of markers of the radiation hybrid maps are located in sequence of unknown location and 2 % were not found at all. Finally, we found one radiation hybrid linkage group, covering an estimated 3 Mbases of sequence, to be composed of a total of 6 markers, 2 of which correspond to sequence of unknown location and the other 4 not found in the sequence assembly. This could correspond to an entire region of poor sequence quality. Maps can be viewed and genotype submitted at: http://w3.toulouse.inra.fr/lgc/chickrh/ .

D 027

Developing a physical (BAC contig) map of the pig genome

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A physical cloned based map of the pig genome will provide a launch pad for finding genes of economic importance, targeted sequencing of specific loci or the entire genome and the development of molecular tools for marker assisted selection. Large fragment genomic clones from two BAC library (pigE BAC and CHORI-242) have been characterised by DNA fingerprinting and BAC end sequencing. The sizes of *Hin*dIII fragments generated from each clone were estimated following separation by agarose gel electrophoresis using Image. Data analysis and contig assembly were effected using FPC. Both ends of BAC clones were subjected to single pass sequencing. In initial analyses fingerprints from almost 169,156 BAC clones could be assembled at high stringency into 15,172 contigs. A total of 310,450 BAC end sequence (BES) reads have been obtained of which 263,883 passed the quality threshold. When 229,579 BES were subjected to WuBLASTN analyses against the human genome sequence 40,305 BES found matches with > 70% identity and scores > 700. The alignment of the BES reads with the human genome sequence has allowed the identification of genes in the BAC libraries that contain genes of interest. These data will be merged with those from other groups in Illinois and INRA during summer 2004 to develop comprehensive sequence ready maps of the pig genome. [This project is funded by BBSRC, Defra, Roslin Institute and Sygen/PIC].

D 028

Construction of a high resolution physically-anchored human-pig comparative WG-RH map

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Comparative mapping information can be effectively utilized for the identification of candidate genes within chromosomal regions containing ETL. The successful use of such information relies upon the construction of a detailed genome map determining segments of conserved synteny and gene order, as well as evolutionary breakpoints. The recent availability of the complete human genome sequence and thousands of homologous porcine sequences provides a tremendous resource for the construction of such a map in the pig. Using the INRA-Minnesota porcine Radiation Hybrid (IMpRH) panel, we have constructed a radiation hybrid map of the porcine genome composed of nearly 2,350 markers, including ~350 ESTs and ~2,000 porcine BAC-end sequences. The average spacing between comparative anchor loci is approximately 1.5 Mb based on human genome sequence. This radiation hybrid map has the highest resolution of any porcine genome map to date, and should greatly facilitate the positional cloning of porcine genes influencing traits of economic importance. Additionally, this map will provide a framework for anchoring contigs generated through BAC fingerprinting efforts and will allow for the determination of a minimal tiling path and assembly of the first sequence-ready map of the porcine genome.

D 029

A Porcine Physical Map Through Comparative Genomics

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Significant progress has been achieved in sequencing animal genomes. The pig (*Sus scrofa*) genome sequencing is underway and we have generated, characterized and submitted to Gene Bank 76,903 high quality BAC end sequences (BESs) from the RPCI-44 and CHORI-242 porcine BAC libraries. BESs were analyzed using BLASTn similarity searches against the human genome (build 33) (E-value $<e^{-5}$) and BESs (19,564) with single matchs within the human genome (unique BESs) were utilized to comparatively anchor the porcine and human genomes with an average density of 1 anchor per 73 Kb of human genome sequence. Approximately 64.2% of unique BESs were observed in orthologous sequences of human genes, providing potential markers for every fourth orthologous human gene (6,336). To date the pig has been characterized as a species with poor physical map, thus requiring the development of a well defined map with a high number of type I markers. Our collection of unique BESs was utilized to design primers for RH mapping. This approach to "piggy back" human sequences for construction of a high resolution map was demonstrated by mapping SSC13. The density and quality of markers allowed generation of a 1.2 Mb RH map. This approach supported the construction of high-resolution porcine physical map to permit development of a minimum tiling path for complete sequencing of porcine genome. (Supported in part by NRI-CSREES grant AG2001-35205-09965, CSREES grant AG2002-34480-11828 and ARS-USDA, AG58-5438-2-313).

D 030

Generation of a SNP map for QTL-studies in pig

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As part of an ongoing QTL-project, we are creating a SNP-map of the pig genome. The information used to detect new SNPs for this map comes primarily from the Danish-Chinese Pig Genome Sequencing Project. In this project a large number of candidate SNPs have been produced from 1 mill. EST-sequences. A subset of these has been selected for resequencing in a panel of animals including the founders of our QTL-families. SNPs verified in this panel have subsequently been genotyped in a large family material consisting of 12.000 progeny from 14 boars and 700 sows to study segregation. At the moment, more than 1.2 million genotypes have been included in the effort of generating the SNP map. Ultimately, more than 8 million genotypes will be included in the study, involving a total of 1500-2000 SNPs.

D 031

Refining the comparative maps of porcine chromosomes 9 and 10

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To identify positional candidate genes for quantitative trait loci (QTL) on pig chromosomes (SSC) 9 and 10, more refined comparative maps have been developed. The current ZOO-FISH maps of SSC9 and SSC10 provide a low resolution view of evolutionary breaks that have occurred throughout pig and human chromosomal evolution. ZOO-FISH has revealed that sections of SSC10 are orthologous to regions of human chromosomes (HSA) 1, 9 and 10, and that SSC9 is orthologous to HSA1, 7 and 11. However the boundaries of these regions are not clearly defined. To further refine the boundaries of these regions, genes from the relevant chromosomal regions of the public human genome sequence were used in a BLAST of porcine expressed sequence tag (EST) database. Matching ESTs were used to design porcine specific primers. This allowed the assignment of genes to pig chromosomes using the INRA somatic cell hybrid panel (INRA-SCHP) and/or the high resolution radiation hybrid panel (IMpRH) to determine whether gene order was conserved. Seventeen genes were mapped to SSC9, and sixteen to SSC10 as predicted. However, eleven genes were localized to SSC1, three to SSC3 and three to SSC4. The physical mapping of these genes has contributed to refinement of the comparative maps, and has also improved the identification of evolutionary breakpoints between pigs and humans.
This work was supported by Australian Pork Limited (APL) project 1756.

D 032

Construction of a high resolution comparative gene map between human chromosome 14 and swine chromosomes using RH mapping

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In swine populations of National Institute of Animal Industry (NIAI), Japan, genes responsible for vertebra and teat number have been indicated to localize in regions of swine chromosome (SSC) 1; and those for backfat thickness, in a region of SSC7. The SSC1 and SSC7 regions have been shown to correspond partly to human chromosome (HSA) 14 by bi-directional chromosome painting and other methods. Therefore, in the present study, we have attempted to construct a dense comparative map between HSA14 and swine chromosomes. Two hundred forty primer pairs were designed for porcine cDNA sequences shown to be the transcripts of the genes orthologous to those in HSA14, and applied to RH mapping using IMpRH panel. As a result, 155 genes were successfully assigned to a swine radiation hybrid (RH) map, IMpRH map, with lod scores greater than 6. Twenty-seven genes, which are localized in HSA14q21-23, were assigned to SSC1. The remaining 128 genes, which are localized in HSA14q11-21 and HSA14q23-32, were assigned to SSC7. The present study suggests that the chromosomal rearrangements have occurred in the syntenic chromosomal regions after the speciation of swine and human.

D 033

Assignment of 99 genes localized in HSA17 to a swine RH (IMpRH) map to generate a dense human-swine comparative map

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In several swine populations, growth after weaning and meat color have been indicated to be controlled by regions in swine chromosome (SSC) 12. However, there is not sufficient information for gene arrangement in these regions to select candidate genes for the traits. Comparative gene map between human and swine chromosomes would be helpful for the selection of candidate genes for the traits. Since the SSC12 has been indicated to correspond to human chromosome (HSA) 17, we have, therefore, attempted to assign the genes localized in HSA17 to swine chromosomes by using the IMpRH panel in the present study. Primer-pairs were designed for 255 genes in HSA17, and the primer pairs for 227 out of 255 genes were found to be mappable to the IMpRH map. Currently, we have assigned 99 genes to the IMpRH map. The mapping results revealed that 72 genes were assigned to SSC12 with lod-scores greater than 6.0. The correspondence between HSA17 and SSC12 was consistent with the observations obtained from bidirectional chromosome painting. In addition, 17 genes were suggested to reside on SSC12 with lod-scores less than 6; 2 genes, on SSC10; 1 gene, on SSC13; and 1 gene, on SSC16. The remaining 6 genes showed no significant linkage with any framework markers. Now we are assigning the remaining 128 genes to the IMpRH map.

D 034

A comparative radiation hybrid map of SSC17 and human homologous chromosomes HSA 20, 8 and 4

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Previous comparative maps based on bi-directional chromosomal painting experiments indicated homology between porcine chromosome 17 (SSC17) and two human chromosomes: HSA 20 and 8, with an extensive homology to HSA 20. We report here the construction of a high-resolution comparative map of SSC17 by mapping about 150 pig ESTs on the INRA-University of Minnesota porcine Radiation Hybrid Panel (IMpRH). For this purpose, we made use of swine EST sequences either available in database or developed in the Sino-Danish Pig Genome Sequencing Consortium, chosen according to their potential localisation on SSC17 on a sequence similarity basis with human genes. It allowed us to confirm the large homology with HSA 20 and to define a precise gene order within this well conserved synteny, that we showed to be organised in three blocks. In addition, we extended the homology of the pericentromeric region of SSC17 to two other human chromosomes and we defined accurately the boundaries of the chromosomal syntenic breakpoints. Indeed, this porcine chromosomal region corresponds to two parts of HSA 8p separated by a very small previously undetected syntenic region, which is homologous to the q arm extremity (3.5 Mb) of HSA 4. This work will contribute to the improvement of a porcine high-resolution map and will provide valuable molecular genetic information for mapping of economical important trait loci in pigs.

D 035

Assignment of 106 genes localized in HSA10 to a swine RH (IMpRH) map to generate a dense human-swine comparative map

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Economically-important traits such as growth and ovulation rate in pigs have been indicated to be controlled by genes in swine chromosome (SSC) 10q12-ter. Comparative chromosome/gene map between human and swine would be helpful for selection of candidate genes for the traits. Although SSC10q12-ter has been found to correspond to HSA10 based on the bidirectional chromosomal painting and gene mapping, the comparative map between SSC10 and HSA10 was not sufficient for selection of the candidate genes. In present study, to provide a dense comparative map in the region, and also to contribute to construction of BAC contig in SSCs, we have attempted to design the primer-pairs for genes in HSA10 and to assign the genes to IMpRH map. Primer-pairs were designed using porcine sequences indicated to be orthologous to those of human genes. The swine fragments amplified in the PCR using these primer-pairs were sequenced, and compared with the sequences used for primer design, revealing that 106 genes in HSA10 were mappable on IMpRH map. Then, we have attempted to assign the genes to IMpRH map: 14 genes were assigned to SSC10; 90 genes, to SSC14; one gene, to SSC3; and the remaining one was not linked to any framework markers of IMpRH map. The correspondences between HSA10 and SSC10 and between HSA10 and SSC14 were consistent with the observations obtained from bidirectional chromosome painting or other results. This study further indicated that, although the synteny was conserved between HSA10 and SSC10 and between HSA10 and SSC14, a large number of intrachromosomal micro-rearrangements occurred in the synteny conserved regions after species separation.

D 037

Comparative chromosomal studies of *E. caballus* (ECA) and *E. przewalskii* (EPR) in a F1 hybrid

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The horse family of Equidae is notable for its rapid karyotypic evolution. The chromosome numbers of the nine extant species vary from 2n=66 in EPR to 2n=32 in *E. zebra hartmannae*. However, the production of any interspecies hybrids at all implies a certain degree of genetic similarity. Unlike other equine interspecies hybrids the hybrids between Przewalski horse and domestic horse are fertile. Previous research revealed that the karyotype of ECA (2n=64) differs from EPR (2n=66) by one additional pair of metacentric chromosomes. Moreover, the formation of a trivalent during meiosis and the homologies in G-banding patterns suggest that ECA 5 corresponds to two acrocentric EPR chromosomes. Compared to results from two separate metaphases the F1 hybrid metaphase is advantageous for banding and FISH, because it combines haploid chromosome sets of both parental species. Q- and G-banding patterns of ECA, EPR and the hybrid revealed interspecies homology between all chromosome pairs except for ECA 5, EPR 23 and EPR 24, which were unique for that particular species. Moreover, they indicated homology between ECA 5p and EPR 23 as well as ECA 5q and EPR 24. FISH on hybrid chromosomes revealed hybridization of the BACs LAM B3 and LAM C2 to ECA 5p and EPR 23 confirming their homology. Contradictory to their previous assignment to ECA 5q NFIA and IGL@ mapped to ECA 7 and EPR 6, respectively. Due to this different assignment they could not support the homology of ECA 5q and EPR 24. Nevertheless, it can be concluded that the karyotypes of ECA and EPR differ by one Robertsonian translocation.

D 038

International Equine Gene Mapping Workshop Linkage Map

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Phase Ⅲ linkage map from the International Equine Gene Mapping Workshop (IEGMW) was generated from 851 genetic markers. This third generation map was produced from an enlarged data set which combined four published mapping resources. The previous generation of the IEGMW linkage map reported a low-density male map of 344 informative loci of which 310 were localized and 257 were linearly ordered. Map length was estimated at 2262 cM with an average interval of 10.1 cM. We report on the latest analysis of 851 markers that located 778 (91.5%) and linearly ordered 630 loci. The current analysis yields a map that spans 3956.7 cM with an average interval spacing of 6.61 cM.

D 039

The second generation whole genome radiation hybrid (RH) and comparative map of the horse

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The first generation RH and comparative map of the equine genome comprising 730 markers and average markers density of one marker/4 Mb was reported last year. Concerted efforts have since been made to generate the second generation map with focus on regions deficient with mapped markers. Our current aim is to produce a 1 Mb resolution physical map with markers evenly distributed over the whole equine genome. To achieve this goal a combination of strategies are being used to obtain markers typing our 5000rad horse × hamster RH panel. These include designing of primers from: our horse skeletal muscle cDNA library, conserved gene sequences from various mammals, BAC end sequences obtained from BACs isolated following overgo hybridization, screening of microsatellites from BACs, etc. Of the total ~3000 typed markers (2000 new with 100 FISH mapped), ~1100 are specific genes. Maps for selected chromosomes will be presented. The findings improve the overall status of the map by over 4-folds, refine the comparative status of the map between horse and the species with finished or 'in-progress' sequence maps (human, mouse, rat, cattle and pig) and bring us a step closer to the main goal of identifying genes governing traits of significance in the horse.

D 040

Isolating and mapping TKY microsatellite markers in the horse

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Genetic maps are extremely important tools for tracing genes governing economically significant traits. There have been significant advances in horse genome maps in recent years. The first-generation linkage map using a full-sib family was published in the year 2000 (Swinburne *et al*., 2000). Since then, another horse linkage map using half-sib families (Guérin *et al*., 2003) and a radiation-hybrid map (Chowdhary *et al*., 2003) were also developed. Isolation of new polymorphic microsatellite markers and mapping them using these resources will be critical for fine mapping of traits of economic significance in the horse. This will eventually lead to the identification and analysis of genes governing these traits. In this study, we report the isolation and mapping of novel and previously reported TKY microsatellite markers. Seven hundred and seventy-eight novel microsatellites were isolated from a microsatellite-enriched library and characterized. Of the newly isolated TKY microsatellites, 457 were assigned to specific horse chromosomes using the half-sib family or the RH panel. Of the previously reported TKY microsatellites, 145 were assigned to horse chromosomes using full-sib family analysis. In total, 602 microsatellites were assigned to horse chromosomes. The mapping data is a significant contribution towards the expansion of the horse gene map.

D 041

Targeted high-resolution comparative mapping of horse chromosomes 7p, 10p, 17, 22, and X

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The current horse (ECA) gene map requires considerable improvement in resolution to i) finely align it with the human and mouse maps and ii) facilitate the isolation and analysis of genes important to the horse industry. Our present efforts toward building such maps focus on equine homologues of human chromosomes 13 (ECA17), 19 (ECA7p+ECA10p), 20 (ECA22), and X (ECAX). Two different approaches were used to generate the maps. In the first approach, genes were selected at 1Mb intervals along HSA13, 20, and X, and primers were designed in highly conserved regions by selecting sequences with 2-3 mismatches between rodent and other mammalian species. In the second approach, overgo primers were designed for genes at 1Mb intervals along HSA20. These overgoes were radioactively labeled and hybridized to filters from the CHORI-241 BAC library. DNA isolated from the positive clones was end sequenced. The sequences were used for designing primers. Primers from both approaches were typed in duplicate on the 5000rad horse × hamster RH panel. Analysis of the results provided a map for each of the selected horse chromosomes with an average of one gene specific marker every Mb of the chromosome. The high-resolution maps thus obtained provide a blueprint for the development of gene maps for other equine chromosomes. This will facilitate targeted searches for candidate genes associated with traits of interest in the horse.

D 042

Current progress of high-resolution RH map for the horse chromosome 7 (ECA7)

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Comparative genomics is used effectively to make gene maps in domestic animals. Previously first generation equine RH map was published comprising 24 markers in the horse chromosome 7 (ECA7). The chromosome corresponds to HSA11, with small segments of correspondence with HSA19. We recently initiated the development of a high-resolution map (one marker/Mb intervals) of this chromosome. For this, primers were designed from thirteen HSA11 genes. Following optimization, the equine orthologs were typed on the Texas A&M 5000rad horse × hamster RH panel. Sequencing and BLAST search results of the amplified products indicated that all 13 genes were well matched to the selected human genes. These initial efforts will be rapidly expanded to obtain a comprehensive comparative map between HSA11 and ECA7 to i) identify evolutionarily conserved chromosomal segments on this chromosome and ii) discover ECA7 genes and their role/molecular-mechanisms in causing various diseases and inherited disorders.

D 043

A detailed physical map of the horse Y chromosome

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Stallion fertility is of significant importance in the multibillion dollar equine industry. Until now the underlying genetic causes of infertility in stallions are unknown. In humans, gene mapping studies indicate that more than 25% of male infertility cases are due to deletions/rearrangements present in the Y chromosome. These cases are routinely diagnosed for presence/absence of sequence tagged sites (STSs). In horses presently there is no map for the Y chromosome. Therefore, the primary aim of this study is to build a detailed physical map of this chromosome for identifying Y-related causes of male infertility in horse. To materialize this we constructed the first radiation hybrid, FISH, and STS map of the euchromatic region of the horse Y. Additionally, seven contigs consisting of 73 BACs were built across the euchromatic region using STS content mapping and validated by restriction fingerprinting and Fiber FISH. The map is presently the most informative among the domestic species and second to only human and mouse Y maps. The construction of this map will pave way for functional characterization of genes critical for normal male fertility and reproduction and will lead to development of a diagnostic test to facilitate early identification of deletions/rearrangements on the Y chromosome of potentially affected animals.

D 044

Mapping HSA10 genes in horse to obtain an improved horse-human comparative map

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Comparative gene map between human and horse facilitates to select candidate genes responsible for traits of interest in the horse. However, at present, the density of genes in the comparative map is not sufficient for candidate gene search. This study aims to generate a high resolution comparative map in the horse by using porcine primer-pairs designed for genes mapped on HSA10. We examined 104 primer-pairs that were previously used for assignment of genes to the pig RH (IMpRH) map. Forty primer-pairs gave horse specific amplification by PCR using equine genomic DNA as a template. The resulting fragments were sequenced, revealing that 24 primer-pairs could amplify DNA fragments derived from equine genes orthologous to the human genes. These primer-pairs were used for typing the horse × hamster RH panel. Following analysis, 10 genes were assigned to ECA1, and 2 genes to ECA29 with lod-scores greater than 12 (a threshold of significance). The chromosomal locations of the genes are consistent with previous horse-human ZOO-FISH observations. In addition, 3 genes were suggested to reside on ECA1 with lod-scores less than 12; 1 gene on ECA10; and 1 gene was assigned to ECA30. The remaining 7 genes showed no significant linkage with any framework markers in the current RH map.

D 045

Linkage disequilibrium analysis of degenerative suspensory ligament desmitis in the Peruvian Paso horse

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Degenerative suspensory ligament desmitis (DSLD) occurs in the Peruvian Paso breed of horse, resulting in a crippling condition in which the suspensory ligaments are no longer able to support the leg. The condition can occur in the hindlegs or all four legs. Available pedigree information indicates this is a heritable syndrome with an autosomal recessive mode of inheritance, but because the condition occurs in older horses, affected horses have already entered the breeding population by the time they are diagnosed with it. Therefore, identification of a genetic marker for the disease would be of benefit to Peruvian Paso breeders.
Linkage disequilibrium analysis was performed using 182 microsatellite markers to perform a low-density genome-scan. Genomic DNA from thirty-eight affected and thirty-two unaffected horses was tested against the markers using dye-labeled primers and an ABI 377 DNA Sequencer. Seven markers demonstrated statistically greater homozygosity in the affected group compared to the unaffected group. These markers map to ECA 2, 7, 9, 11, 14, 21 and 28. Additional markers on these chromosomes will be tested to confirm the significance of these regions as possible sites of candidate genes for DSLD.

D 046

Analysis of the Genetic Background of Juvenile Baldness in Pigs

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A newly arisen pig phenotype mainly characterized by juvenile baldness has been encountered in a Danish pig herd. The trait shows autosomal dominant inheritance and has great resemblance to the integrin subunit β6-/- knockout phenotype seen in mice. Integrins are a family of cell surface proteins each consisting of an α and a β subunit spanning the cell membrane. Integrins mediate cell-cell and cell-matrix interactions. Here we perform a linkage study with the integrin subunit β6 gene (*ITGB6*) as candidate gene. The comparative map predicts that the porcine ortholog of *ITGB6* maps to SSC15. Seven microsatellite markers evenly distributed in the region of interest on SSC15 have been genotyped in 6 pig families (n = 69). In each family one of the parents was affected and the trait segregated according to our hypothesis of autosomal dominant inheritance. The trait links to 4 microsatellite markers on SSC15 with LOD scores from 3.6 to 5.2. Further characterization of the gene responsible for this phenotype is in process.

D 047

A Genome-Wide Scan for the Hereditary Cutaneous Melanoma in the MeLiM Swine Model

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The MeLiM (melanoblastoma-bearing Libechov minipig) swine model was evaluated and employed, after clinical and histological phenotyping, to perform preliminary genome scan using 79 backcross (BC)-1 pigs, which derived from affected MeLiM F1 pigs and healthy Duroc founders. Four porcine chromosomal regions on SSC1, 2, 7, and 8, respectively in intervals 44-103, 1.9-18, 59-73, and 47-62 cM, as well as a fifth region close to *MC1R* on SSC6, were identified as being potentially involved in melanoma susceptibility with incomplete penetrance. We report here that with 200 additional BC-1 pigs, we were able until now to confirm the previous results on SSC1, and find also this region was associated with the coat color ($P<0.01$). In addition, the interval mapping results on the whole genome will be presented. The ongoing massive radiation hybrid mapping and fine-tuning of the corresponding genomic segments between human and swine, jointly with our QTL mapping results, might help finally to dissect the molecular pathways underlying the melanoma risk susceptibility in pigs.

D 049

Screening for polymorphisms in candidate genes for hypokalemic periodic paralysis (HypoPP) in burmese cats

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A hereditary muscle weakness called hypokalemic periodic paralysis (HypoPP) has been reported in Burmese cats all over the world. Affected animals show clinical signs of a polymyopathy, and severe attacks end lethally. Blood examination shows a low serum potassium. A similar clinical symptom complex of humans is caused by different mutations in genes for skeletal muscle voltage-dependent ion channels, namely the Calcium- (*CACNL1A3*), Sodium- (*SCN4A*), and Potassium- (*KCNE3*) channel. To assess the molecular reason for HypoPP in Burmese cats, we sampled affected and unaffected cats and built up a three generation pedigree of a family bearing HypoPP. A total of 10 microsatellites were genotyped, which were located on chromosome E1 (2, flanking *SCN4A*), D1 (5) and F1 (3). Three of these markers were monomorphic in the Burmese family, seven show 3 to 6 alleles. Candidate gene *CACNL1A3* could not be excluded or confirmed using microsatellite analysis and direct targeting of this gene was chosen next. Feline mRNA sequences for *CACNL1A3* were generated by reverse transcription and PCR. Cat specific primers were selected from the mRNA sequences to amplify five genomic DNA fragments, in which mutations associated with HypoPP have been reported in humans. Sequence analysis of the amplicon containing nucleotide 1583, one of the most frequent mutation sites in human HypoPP, shows no substitution in this position for affected cats. Comparative sequencing of the other fragments are going on in affected and unaffected cats.

D 050

Muscular dystrophy candidate gene in chicken genome

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Our recent studies revealed that the genetic locus for chicken muscular dystrophy (cMD) of abnormal muscle (*AM*) was mapped to chromosome 2q, and that the region showed synteny with the human chromosome 8q11-24.3. In this study, we attempted to map more functional genes, which located on human chromosome 8q, to chicken chromosomes. Twenty-three genes and ESTs were mapped to chicken chromosome 2 by linkage analysis using the Kobe University resource family. The *AM* locus was mapped between two genes (*IMPA1* and *CBGA2T1*) whose interval was 1.9cM, and corresponded with six loci (*FABP4, MMP16, DECR1, CALB1, CGI-77* and *MCW0166*). The functional genes in this region are the most likely candidate genes responsible for cMD. We performed northern hybridization using total RNA extracted from normal and cMD pectoralis to analyze the expression of 10 candidate genes. Three genes (*DECR1, FABP4* and *CA3*) showed different expression. Both *DECR1* and *FABP4* showed higher expression in cMD than normal. They are associated with synthesis of fatty acid. In the cMD muscular tissues convert to fatty tissues, suggesting the higher expression by fat tissue. The *CA3* expressed higher in normal than in cMD. In general, *CA3* express higher in the muscle of muscular dystrophy. Therefore, these genes were not considered as the responsible gene of cMD.

D 051

Arthrogryposis Multiplex Congenita (AMC), a hereditary disease in swine, maps to chromosome 5 by linkage and QTL analyses

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A genetic form of Arthrogryposis multiplex congenita (AMC) was recently identified in Swiss Large White (LW) pigs. At least 18 LW artificial insemination boars are carriers of the defective allele and cause considerable economic losses in the Swiss pork industry. Affected piglets exhibit a permanent joint fixation or ankylosis in the fore and/or hind limbs and in the vertebral column, as well as brachygnathia inferior. Our resource family with full or half sibs founders, included 219 pigs. Forty-one animals (18.7%) manifested the typical symptoms and were not born alive. In this study we show that the disease is controlled by a single autosomal recessive allele designated as *amc*. A comprehensive genome scan, performed with 48 informative microsatellites spread on each chromosome at intervals of approximately 40 cM, revealed that *amc* is located on pig (*Sus scrofa*) chromosome 5 (SSC5). *AMC* was further mapped by linkage analysis of 17 microsatellites on SSC5 to the relative position of 92 cM, between *SW152* and *SW904*/*SW1094*. QTL analysis results led to the same chromosomal region, which may provide an important road map for future studies in humans and other species.

D 052

Linkage mapping and identification of the causative gene for congenital ovine arthrogryposis

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Congenital arthrogryposis is characterised by immobility of multiple joints at birth or a limitation in the range of motion of the phalangeal, carpal, metacarpophalangeal, and metatarsophalangeal, metaphalangeal joints. Previous breeding studies that we carried out have shown that ovine arthrogryposis is an autosomal recessive single gene disorder. The objective of the current study is to map and identify the mutation responsible for ovine arthrogryposis. From the previous study two homozygous affected Suffolk rams remained in the UCD flock, these were used as founder males for a backcross pedigree of half-sib families. The experimental population was created by crossing the Suffolk rams with 30 Cheviot ewes, eventually producing 32 informative F_2 offspring. All F_2 animals were phenotyped at birth for the presence and severity of arthrogryposis. Currently a full genome scan is being undertaken using a panel of approximately 150 microsatellites markers. Results from the genome scan will be presented and additional homozygosity mapping data will also be discussed.

D 053

Ovine Neuronal Ceroid Lipofuscinosis: use of an indirect marker in the CLN6 gene for genotype prediction

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The neuronal ceroid lipofuscinoses (NCLs, Batten disease) are a group of fatal inherited neurodegenerative diseases characterized by the accumulation of fluorescent lysosomally derived protein aggregates in cells. At least eight different forms of NCL occur in humans and six separate disease-causing genes have been identified to date (*CLNs 1,2,3,5,6* and *8*). We are investigating several large animal models of NCL to improve our understanding of biochemical and pathological aspects of the diseases as the overall pathobiology of this group of diseases is still poorly understood. In sheep, we are currently sequencing the ovine *CLN6* gene after the disease was mapped to a region on OAR 7 in South Hampshire and Merino sheep. A disease causing mutation has not yet been identified but a silent G →A single nucleotide polymorphism (SNP) in exon 7 is successfully used as an indirect marker in South Hampshire sheep and is currently being investigated in Merino sheep. The polymorphism introduces a *Hae*II cut site, which has been used to exclude another ovine form of CLN6 in Borderdale sheep, and to determine the extent of chimerism in chimeras constructed from fused normal/affected embryos.

D 054

Genetic characterisation of two animal models for Neuronal Ceroid Lipofuscinoses (NCL): the Australian Merino sheep and Devon cattle

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The Neuronal Ceroid Lipofuscinoses (NCL) are a group of autosomal recessive neurodegenerative disorders found in several species, including humans, cattle and sheep. These disorders are characterised by brain and retinal atrophy and the accumulation of autofluorescent lipopigment granules within a number of cells. To date eight different variants of NCL have been characterised in humans (*CLN1 – 8*). Unfortunately there is no cure for this disorder. It is hoped that the investigation of animal variants of NCL will provide a greater understanding of these disorders and in time an appropriate treatment, if not cure, can be developed. Through the use of candidate gene analysis, we identified a single base pair insertion in the bovine variant of the *CLN5* gene in a family of Devon cattle known to carry a genetic predisposition for NCL. This frame shift mutation leads to a premature stop codon in affected cattle. NCL in Devon cattle is the first large animal model for the human variant, *CLN5*. The causative mutation for NCL in Merino sheep has not been identified; however the disease was mapped to a region containing the ovine *CLN6* gene. Preliminary gene expression studies have shown that *CLN6* in an affected animal is expressed at approximately five times the normal rate. However, more detailed analysis of this result is required. Continued analysis into the functional aspects of the CLN proteins in both cattle and sheep are needed before a comprehensive understanding of the pathology and biomechanisms of this group of disorders can be established.

D 055

Mapping in Australian Border Collie Dogs of Collie Eye Anomaly and Ceroid Lipofuscinosis Genes

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Collie eye anomaly is a congenital defect that is extremely common (>50%) in many collie dog breeds. Phenotypic expression is variable from mild choroidal hyperplasia with no effect on vision to detached retinas and blindness. In Australian pedigree border collies it's incidence is a few percent. Ophthalmological screening at 6 weeks and selective breeding is practiced to further reduce the incidence of the disease. We have mapped the genetic defect in 3 large Australian pedigrees with over 20 affecteds. Highly significant lod scores of over 6 were found at the distal end of CFA37 in the same location as reported by Acland and Ostrander for an experimental cross which included US border collies. We are also mapping the gene for ceroid lipofuscinosis (CL) in related pedigrees. CL is a lysosomal storage disease (known as Batten disease in humans) which results in nerve degeneration from about 18 months of age leading to behavioural abnormalities and eventually death by 30 months. Although matings are chosen by breeders to avoid the occurrence of this recessive disorder the defective allele is common in the population and several cases a year have been detected. All cases are thought to trace back to a single animal imported from New Zealand in the 1950s. It has been recently exported to Japan from Australia. There are 5 known human genes causing CL and an English setter model. We have now collected enough reported pedigrees to have the power, using mapping and comparative genomics, to determine whether the border collie CL gene is a homologue to one of these or is a different gene again. Location of the disease gene or identification of the gene will allow detection of carriers and elimination of this devastating disease by selective breeding.

D 056

Linkage mapping of the locus responsible for bovine congenital eye anomaly on the bovine chromosome 18

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Bovine congenital eye anomaly of Japanese black cattle is a hereditary ocular disorder with autosomal recessive manner showing developmental defects of lens, retina and iris, persistent embryonic eye vascularization and microphthalmia. In the present study, we mapped the locus responsible for the disorder by linkage analysis, using 240 microsatellite markers covering entire bovine genome and an inbred pedigree obtained from commercial herds. The linkage analysis revealed linkage between the locus and markers on centromeric region of bovine chromosome (BTA) 18. Homozygosity mapping further refined the critical region to approximately 7.2 cM region. The results suggest the presence of a gene affecting the ocular development and vascularization in the critical region. Comparison of BTA18 with its evolutionary ortholog, human chromosome 16, revealed positional candidate genes including c-Maf oncogene. To evaluate these genes as potential candidates for the disease, we are now performing radiation hybrid mapping to determine their localization on the critical region.

D 057

Bovine dilated cardiomyopathy: evidence for a major gene on BTA 18

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A whole genome scan in an experimental pedigree comprising 113 members, revealed the presence of a major gene responsible for bovine dilated cardiomyopathy (BDCMP) on BTA 18. A total of 196 microsatellite markers were typed leading to a threshold of 1.25. Linkages above the threshold were found for BMS4008 (BTA1, lodscore 1.62), BMS1290 (BTA9, lodscore 1.33), BMS1758 (BTA11, lodscore 1.69) and 11 markers on BTA18. RME01 (4.06), BMS2785 (3.30), IDVGA55 (3.20) and BOSBIZ6 (2.72) are forming a cluster covering approximately 9 cM in the telomeric region of BTA 18. Comparative genome analysis indicated HSA19q13.4 to correspond to this region. TNNI3 located in HSA19q13.4 was identified as a candidate gene for BDCMP as it plays a major role in different forms of human cardiomyopathy. The bovine TNNI3 gene was isolated and characterized, showing 88% identity with the human TNNI3 gene. The characterization of affected and unaffected members of our BDCMP herd with respect to TNNI3 is in progress. Preliminary results seem to exclude a structural change in the expressed sequence of TNNI3.

D 058

A 3Mb BAC contig and STS content map of the POLL critical interval on bovine chromosome one

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Carcass bruising due to horns costs U.S. beef producers more than $22 million every year. The horned condition in cattle is believed to be the wild type with morphogenesis primarily occurring after birth. The polled condition has existed since domestication and has been selected for its economic importance, because of decreased dystocia and reduced bruising. The POLL locus has been mapped by genetic linkage analysis to the proximal region of bovine chromosome one. This region coincides with the Down syndrome critical interval on human chromosome 21. As an intermediate step in our efforts to identify the POLL locus and the underlying causative mutation, we have constructed a physical map of the critical interval. Our approach was to build a contig of Angus, Longhorn and Horned Hereford BAC clones for the critical interval, in conjunction with STS mapping. We used BAC clones isolated in preliminary studies as seeds to complete the Angus contig. In addition, we contributed our BAC clones and associated mapping data to the International Bovine BAC Map Consortium and were able to recover corresponding contigs containing 186 Horned Hereford clones from the CHORI-240 BAC library. To verify the order of overlapping clones, an STS content map was constructed by screening 60 markers across the BAC clones. A single contig spanning ~3Mb was assembled. This contig of overlapping BAC clones from horned and polled breeds is currently being used as a resource for SNP discovery and characterization of three positional candidate genes.

D 059

A genome scan to identify chromosomal regions influencing bovine coat color

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As a trademark for cattle, coat color is of great interest to breed conservation. Thus, there is a practical as well as scientific interest for understanding the coat color genetics. The objective of this study was to identify genomic regions affecting bovine coat color. Mating Jersey × Limousin bulls to both Jersey and Limousin dams produced six paternal half-sib families of backcross progeny. The coat color phenotypes of 800 backcross progeny were determined as light and dark. A quantitative trait loci (QTL) scan over all the bovine autosomes using 150 microsatellite markers was performed by the interval mapping approach. Two QTL were detected, one on bovine chromosome 6 (BTA6) in the interval between microsatellite markers BMS483 and BM4621 with a LOD score of 13.5, and one on BTA18 in the interval between microsatellite markers TEXAN10 and INRA121, with a LOD score of 11.2. BTA6 has several of the tyrosine kinase receptor genes (PDGFRA, KIT and KDR) and BTA18 has the MC1R gene encoding the melanocyte stimulating hormone receptor. These genes have been associated with various coat color phenotypes in other mammals.

D 060

PPARGC1A - A Candidate Gene for a QTL on BTA6 Affecting Milk Fat Synthesis

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QTL for milk production traits have been reported on bovine chromosome 6 (BTA6) by several studies in different cattle breeds. This chromosomal region is equivalent to syntenic chromosome segments in human, pig, and mouse containing loci associated with type Ⅱ diabetes and obesity-related traits. We identified the bovine PPARGC1A gene (peroxysome proliferator-activated receptor γ coactivator 1α) as a positional and functional candidate gene for a previously described QTL for milk fat yield on BTA6 due to its chromosomal position and its key role in energy, fat, and glucose metabolism. With the aim to investigate the role of the bovine PPARGC1A in the regulation of milk fat synthesis in dairy cattle we determined the cDNA sequence, genomic organization, chromosomal localization, and expression pattern. Bovine PPARGC1A gene comprises 13 exons with a total length of 6,261 bp and is expressed at different levels in a large number of tissues. Compared to the respective orthologs from human, rat, and mouse bovine, PPARGC1A cDNA and protein sequences showed a high inter-species similarity. Screening for polymorphisms in the coding sequence, exon/intron boundaries, and regulatory regions of the PPARGC1A gene revealed a total of 10 SNPs. In order to evaluate if PPARGC1A could be involved in genetic variation underlying the QTL for milk fat synthesis on BTA6 an association study between SNPs in the PPARGC1A gene and milk fat yield was performed in a major dairy cattle population.

D 061

Mapping Quantitative Trait Loci affecting circadian rhythmicity in mice

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A new mouse strain, which becomes behaviorally arrhythmic in constant darkness was isolated from the wild mice (*Mus musculus castaneus*) captured in the Philippines. These mice can entrain to light-dark cycle but in constant darkness they show shorter circadian period for a while and then become arrhythmic. To map the genes responsible for these abnormal circadian rhythms, we performed quantitative trait locus (QTL) analysis for the persistence of rhythmicity and circadian period using F2 progeny between arrhythmic mice and C57BL/6J mice. As a result, a major QTL for the two phenotypes was detected on the central region of the same chromosome. Circadian clock genes, which are involved in the generation of circadian rhythms, were not mapped in the region of the QTL detected in this study. These results suggest that arrhythmicity in wild-caught mice is caused by a mutation of a novel circadian-related gene and also indicate that this strain can contribute to understanding the circadian clock system.

D 062
QTL analyses of viral disease resistance in fish

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Gene marker technologies can be used for genetic improvement through selection for favorable traits, such as disease resistance. These traits are generally modeled as being controlled by many genes of small additive effects, which are known as quantitative trait loci (QTL).
Construction of a genetic linkage map, based on DNA markers at a large number of sites in the fish genome is necessary to identify quantitative trait loci (QTL) controlling traits of disease resistance. Linkage maps have been published for a large number of economically important fish species, such as rainbow trout, tilapia, catfish and Japanese flounder. Among these, the genetic linkage map of the rainbow trout and of Japanese flounder have permitted the identification of the QTL for infectious pancreatic necrosis (IPN) and infectious hematopoietic necrosis (IHN) resistance in rainbow trout, as well as lymphocystis disease (LD) resistance in Japanese flounder.
By identifying markers of high performance QTL in different strains or species, it may also be possible to successfully improve the performance of such traits in other strains through introgression of the desired QTL. One of the goals of selective breeding programs is to integrate genetic marker information from pedigreed brood stock fish into the successful management and culture. Such an approach, termed marker-assisted selection (MAS) and/or marker-assited gene introgression (MAI), is expected to increase genetic response by affecting intensity and accuracy of selection.

D 063
Isolation and chromosome localization of sheep Bcl-2 family member

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Apoptosis is a physiologically important cellular suicide pathway, which has also been implicated in a number of pathological conditions. There is some evidence to indicate that the mechanism of neuronal cell death in prion diseases is apoptosis as apoptotic neurons have been observed in the brain of scrapie infected sheep. Apoptosis is widely regulated, one of the family proteins implicated in it regulation is the Bcl-2 gene family, with apoptosis being prevented by Bcl-2, Bcl-w, Mcl-1 and Bcl-x_L and promoted by Bax and Bcl-x_S. In the present study, cDNA and genomic fragments from these genes were isolated and showed a high homology with the same genes from other species. Some polymorphisms were found in Bax and Mcl-1 intron that were used for linkage mapping. These genes were mapped on OAR14 and OAR1 respectively. Three cattle BAC probes containing the homologous Bcl-2, Bcl-x and Bcl-w gene were identified and used for FISH mapping. They were localized in OAR23q27, OAR13q22 and OAR7q15-q21 respectively. We also analysed the mRNA expression of these genes in mammary gland, ovarian, intestine and brain as target tissues for the sheep pathological where apoptosis is involved.

D 064
Genetic analysis of the porcine glucocerebrosidase (*GBA*) gene

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Earlier, a human GBA (glucocerebrosidase) cDNA probe was used to detect RFLP in porcine *GBA* and the gene was mapped to the chromosome 4 linkage group *ATP1B1 – GBA – EAL* (Marklund *et al.*, 1993). *GBA* is located in a chromosome region where QTLs for growth and some carcass traits have been found. In this study the porcine *GBA* was sequenced and the genomic organization was determined. The gene harbours 11 exons and 10 intervening introns, and the genomic organization is identical with human *GBA*. There is no evidence of a pseudogene in pig. Four polymorphisms were observed within the porcine gene: insertion/deletion of one of the two SINEs (PREs) in intron 2 (locus *PREA*); deletion of a 37-39-bp stretch in intron 4 (one direct repeat and 5' end of PRE) and deletion of a 47-bp stretch in the middle part of PRE in intron 4 (locus PREB); single-base transition (C – T) in intron 6 (locus *Hae*III-*RFLP*). *GBA* was assigned to chromosome 4q21 by FISH and localized to the same region by linkage analysis and RH mapping. (Supported by the Czech Science Foundation, grant 523/03/0858).

D 065
Fine mapping and construction of a porcine contig spanning the Intramuscular fat content QTL

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We previously identified the evidence for significant quantitative trait loci (QTL) affecting intramuscular fat content (IMF) on *Sus scrofa* chromosome (SSC) 7, suggestive QTL on SSC 9 and 13 with constructed a pig F2 resource population by crossing a Meishan sow and a Duroc boar. This study has focused on the QTL for IMF on SSC 7. The QTL for IMF was fine-mapped by linkage analysis to a 12.6 – cM chromosome interval on SSC 7, flanked by microsatellite markers SJ169 and MM70 (0.1% chromosome wise level). To identify the responsible gene for IMF, we screened two porcine bacterial artificial chromosome (BAC) libraries and constructed about 5.2-Mb porcine BAC contig containing the SJ169 - MM70 interval. The BAC clones were used to develop new genetic markers in the form of microsatellites (MS). This candidate region was corresponding to human HSA14q, there were 16 genes such as *LOC283584, LOC283586, GALC, GPR65, LOC283587, KCNK10, SPATA7, PTPN21, FLJ11806, EML5, TTC8, LOC376278, C14orf116, CHES1, CAP2P1* and *PRO1768*. We mapped eight of these 16 genes in this BAC contig and confirmed gene expression by RT-PCR with mRNA from several porcine tissues.

D 066

Physical mapping of the QTL region related to intramuscular fat content on SSC6q using a BAC contig

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Since intramuscular fat content (IMF) has been a major concern in high quality pork production, it is considered the most important trait in modern pig breeding. The main focus of our research is to positionally clone trait gene(s) of the QTL for IMF. The QTL region related to IMF had been identified on pig chromosome 6q28-6q32 by analyzing a reference family crossed between Korean native boars (KNP) and Landrace sows. A fine linkage map of this region had been also reported. Using the information from the fine linkage map, a BAC contig is being constructed using the KNP BAC library that had been pooled for 4-dimensional PCR screening. Seventeen MS sites located in 6q28-6q32 region were used for start sites of the contig building. The BAC end sequences of all screened clone were analyzed and used for designing the primers for the next screenings. An expected total length of the contig map is about 11 cM. A comparison of genetic map between porcine and human indicates that SSC6q28-6q32 corresponds to two segments of HSA18q11-q12 and 1p31-1p36. A break point of the two segments was estimated to be between the MS S0228 and SW2098. The location and order of genes on the contig will be presented.

D 067

Genome Sequencing and Gene Annotation on 6q28-32 region related to QTL in Pig

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Previously, the region of porcine Chr 6 between 6q28 and 6q32 (about 20cM) was reported as quantitative trait loci for intramuscular fat. Massive sequencing on that region was performed and 3.3 Mb genomic sequence could be obtained. 30 BAC clones of minimal tiling path were selected through BAC physical mapping between SW71 (98.6cM) and SW1881 (121.1cM) using by BAC End Sequencing(BES) method, and the genomic sequences of selected clones were analyzed by shotgun strategy with 8 fold. The genomic sequences were processed with Phred/Phrap assembling and repeat masking through RepBase database and the information of high homologous genes was obtained by browsing on the blastn mode of NCBI GenBank. Also, the results of blastn were compared with the results of gene prediction by GenScan web service. As the results of this study, 16 genes were predicted through comparing genetic maps between porcine and human. There are 8 genes derived from HSA1 between 1p31.1 to 1p31.2 as like LEPR, PIGK and are 8 genes derived from HSA18 between 18p11.21 to18p11.22 and 18q11.2 to 18q12.3 as like WCH4, Brunol4 on 6q28-32 in porcine. The structures of the putative genes on those regions were identified and characterized with Rapid Amplification of cDNA Ends (RACE).

SECTION E

Polymorphism
Biodiversity

E 002

Variability of candidate genes for meat production in cattle breeds of different purposes

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The progress in molecular genetics allows the study of an increasing number of genes that, for the physiological role of their products, can be potential candidates in determining the variability of economic traits. As regards beef production and quality, hundreds of candidate genes have been suggested, but only few of them have been tested for their effect on trait variability, often with inconsistent results. The aim of this study was to verify if breeds of different purposes had a different genetic structure for some of the candidates previously investigated. The selected loci were: *GH1*-exon V, *GH1*-intron 3, *GHR, IGF-BP3, LEP* and *POU1F1*. By ASM-PCR and PCR-RFLP techniques, a total of 250 subjects from 5 breeds were analysed: Aosta Red Pied (42), Belgian Blue (17), Blonde d'Aquitaine (60), Friesian (52), Piemontese (79). All the breeds were polymorphic for all the loci. The main differences concerned the low frequency of the *GHR*G* allele in Friesian (0.15 vs 0.55 ÷ 0.59 in the others) and the relatively high frequency of *LEP*T* allele in Aosta Red Pied and Friesian (0.38 and 0.47 respectively vs 0.12 ÷ 0.28). The mean expected heterozygosity ranged from 0.36 in Belgian Blue to 0.43 in Blonde d'Aquitaine. As regards loci, the lowest mean variability was observed for *IGF-BP3* (He = 0.24), while the highest one for *GHR* (He = 0.50). The comparison of the genotypic distribution for each population pair across loci showed highly significant differences between Friesian and all the other breeds ($P << 0.001$). On the basis of the genetic distances, the Neighbor-joining tree discriminated two groups, one including the beef breeds (Belgian Blue, Piemontese, Blonde d'Aquitaine), the other the dairy breeds (Aosta Red Pied, Friesian).

E 003

Allele frequency of the extension locus encoding melanocortin-1 receptor (MC1R) in Japanese Brown (Kochi variety) and Japanese Black

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Japanese Brown (Kochi variety) has brown coat color with black eyelash, hoofs, horns and tail. Here, we investigated the gene frequency of the extension (E) locus encoding MC1R of Japanese Brown (Kochi variety) and Japanese Black. The two different regions of the E locus, region I for E^D and E^+ alleles, region Ⅱ for E and e alleles, were amplified for PCR-RFLP. Amplified PCR products in the region I were digested by *Msp* AI and those from the region Ⅱ were digested by *Msp*I, respectively. Two hundreds fifteen Japanese Blacks and 145 Brown animals were tested in this study. In Japanese Black, alleles of E^D, E^+, E and e were observed. At the region I, allele frequencies of E^D and E^+ were 0.481 and 0.519, respectively and in the region Ⅱ, those of E and e were 0.995 and 0.005, respectively. On the other hand, in Japanese Brown (Kochi variety), E^+, E and e were also observed. In the region I, all animals were homozygous for E^+allele, while in the region Ⅱ, the gene frequencies of E and e were 0.962 and 0.038, respectively. In the region I of the E locus, E^D and E^+ allele shows the same frequency in Japanese Black, while E^+ allele has 100% in Japanese Brown. In the region Ⅱ, both breeds have similar gene frequencies of E and e. Thus, gene frequencies of the regions I and Ⅱ of E locus could not explain the difference of coat color between Black and Brown (Kochi variety).

E 004

Sequence and polymorphism of *Bos indicus* Glucose-6-phosphate-dehydrogenase (G6PD) gene

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Glucose-6-phosphate dehydrogenase (G6PD) is an ubiquitous enzyme which by determining the NADPH level has a crucial role in fatty acid biosynthesis and detoxification of organic peroxides in all cells. In this study for the first time coding sequence of bovine (*Bos indicus*) G6PD mRNA (Acc no: AJ566767) was reported. It was found to have 1548 bp encoding 515 amino acids. It was 81.4%, 81.8% and 85.3% similar to the G6PD mRNA coding sequences of rat, mouse and human respectively. The 11 residue tryptic peptide structure containing the reactive lysine assigned for the catalytic activity in G6PD is strikingly similar in baker's yeast, bovine, human, mice and rat G6PD. Also, four fragments of bovine (*Bos indicus*) G6PD gene viz.118 bp, 319 bp, 683 bp and 408 bp were amplified and sequenced for the first time (Acc no: AJ566639, AJ567903, AJ567904, AJ567905). Restriction digestion of 319 bp amplicon with *Hae* III enzyme revealed three patterns AA (79 bp, 109 bp, 30 bp, 66 bp, and 35 bp), BB (188 bp, 30 bp, 66 bp, and 35 bp) and AB (188 bp, 79 bp, 109 bp, 30 bp, 66 bp, and 35 bp). However, no polymorphism of 319 bp amplicon with *Alu* I and 408 bp amplicon with *Hae* III was observed. PCR-SSCP analysis of 408 bp, 319 bp, and 118 bp amplicons revealed 5, 6, and 7 SSCP haplotypes respectively. Relationship between 319 bp SSCP haplotypes and nucleotide substitution was established by sequencing. This work is a first step for the understanding of this enzyme at the molecular level in bovines.

E 005

The discovery of single-nucleotide polymorphisms (SNPs) for the Stearoyl-CoA Desaturase gene in Hanwoo (Korean Cattle)

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The Stearoyl-CoA Desaturase (SCD) is a key enzyme, which converting palmitic acid (16:0) and stearic acid (18:0) to palmitoleic acid (16:1) and oleic acid (18:1) in lipid metabolic pathway, respectively. Oleic acid (18:1) from *M longissmus dorsi* has a positive relationship and stearic acid (18:0) has a weak negative relationship with marbling score. The bovine SCD gene is placed between 20cM and 30cM in bovine chromosome 26 and this region was reported as a region of QTL for fat yield. In this study, we screened 7800 bp (1.8 kb of promoter region, six exons and five introns) using direct cycle sequencing with 24 unrelated Hanwoo and could found seventeen single-nucleotide polymorphism in Hanwoo SCD gene; ten SNPs in introns, three in exon 5 and two in promoter region. In promoter region, we have detected some novel SNPs that were in transcription factor binding sites: MZF-1 and NF kappa-1. In exon 5, two SNPs were synonymous SNPs and one was nonsynonymous SNP (Ala→Val). One synonymous SNP in Exon 5 was genotyped with 161 Hanwoo steers and analysed the relationships between genotype and carcass traits. The result, homozygotes for the A allele constituted 28% of the Hanwoo steers and homozygotes for the B allele 25%, while heterozygotes were 47%. The AA genotype was significantly higher with carcass weight and marbling score than BB and AB genotype ($P<0.05$). Therefore, it is possible that a SCD Exon 5 genotype would be a genetic marker related marbling score and carcass weight.

E 006

Identification of SNPs in GHRH and IGFBP3 genes of Korean cattle (Hanwoo)

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The GHRH (growth hormone releasing hormone) and IGFBP3 (insulin-like growth factor binding protein 3) have been suggested as candidate genes for the identification of genetic markers for meat production traits, because of the essential role of their products in the physiological mechanisms related to growth and carcass traits in livestock. Single nucleotide polymorphisms (SNPs) are the most frequently found DNA sequence variations in the animal genome and can be used as genetic markers for association analysis with economic traits. The primary aim of this study was to identify SNPs in GHRH and IGFBP3 as potential candidate genes for growth and meat quality traits in Korean cattle. Genomic DNA for sequencing was extracted from twenty-four unrelated individuals. These candidate genes were amplified by PCR and directly sequenced. In the IGFBP3 gene, twenty-two SNPs were identified. Five SNPs were selected for large scale screening on the basis of the location and frequency. The frequencies of each SNP were 0.357 (G -854C), 0.472 (G -100A), 0.418 (G +421T), 0.363 (T +1636A) and 0.226 (C +3863A), respectively. Fifteen SNPs were also identified in the GHRH gene. Frequencies of the allele and haplotype were estimated and linkage disequilibrium coefficients (| D |) between SNP pairs were also calculated. The information of SNPs and haplotypes identified in these candidate genes might be used to detect genetic markers associated with growth and carcass traits in Korean cattle populations.

E 017

Gene frequencies of Bovine Lymphocyte Antigene *DRB3.2* locus in Yaroslavl Russian cattle

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Yaroslavl breed was formed in 19th century in the territory of the Yaroslavl province of Russia as the result of native selection and has a series of valuable traits that discriminate his as a unique breed, which is well able to competite with west analogue. On the other hand, to date has not analysed his gene pool with molecular markers. Yaroslavl Russian cows (n=120) were genotyped for the Bovine Lymphocyte Antigene (BoLA)-DRB3 allele by PCR-RFLP analysis. Bovine DNA was isolated from aliquots of whole blood. A two-step polymerase chain reaction followed by digestion with restriction endonucleases RsaI, BstyI and HaeIII was conducted on the DNA from Yaroslavl cattle. Thirty-five BoLA-DRB3.2 alleles were identified with frequencies ranging from 0.31 to 14.69%. Allele types include: BoLA-DRB3.2*2, 3, 6, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 31, 32, 33, 36, 37, 40, 42, 43, 44, 48, 50, 51 and 54. Their frequencies were 1.25, 1.88, 0.63, 0.94, 1.88, 0.31, 8.44, 7.81, 3.44, 6.25, 4.34, 0.31, 1.25, 1.25, 0.31, 2.19, 3.44, 14.34, 0.63, 1.25, 0.63, 14.69, 4.34, 0.94, 0.31, 1.25, 0.63, 3.75, 2.81, 0.31, 3.13, 0.94, 0.63, 2.5 and 0.94% respectively. Of the allele types detected, BoLA-DRB3.2*28, determining resistance of cattle to leukemia occurred at the highest frequency (14.69%). Results of this study demonstrate that the BoLA-DRB3.2 locus is highly polymorphic in Yaroslavl Russian cattle. This cattle has a great value for practical selection.

E 019

Molecular differentiation of European cattle breeds

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The project CT98-118 *Towards a strategy for the conservation of the genetic diversity of European cattle* has been sponsored by the European Commission and involved the active participation and collaboration of 30 European laboratories. One of the outputs of this project is a data set of genotypes for 30 FAO-adopted microsatellites, typed in 25 to 50 animals from 64 European cattle breeds. Typings with subsets of the 30 markers were available for 58 additional breeds. Model-based clustering and Neighbor-Net graphs of genetic distances allowed the definition of the following nine breed clusters: Podolian, Iberian, Alpine Brown, Alpine Spotted (Simmental-like), French Brown, Lowland Pied (dairy black- and red-pied breeds), Baltic Red (Red Danish, etc.), Nordic, and British Isles. The breeds from the last cluster are relatively distinct. The Northern part of France and the Alpine regions appear to be transition zones with breeds (e.g., Charolais, Piemontese) that are intermediate between surrounding clusters. Both Podolian and Iberian breeds are relatively close to African and zebu cattle, while two other major groups are formed by the French and Alpine clusters and by the Lowland Pied, Red Baltic, Nordic and British Isles clusters, respectively. We propose that the relations of the clusters reflect the gradual spreading of cattle husbandry from ca. 6000 years B.C. onward. During the last centuries genetic isolation and selection have accentuated the molecular differentiation at the breed level, while also import of sires from other breeds has changed local allele frequencies. These findings may have implications for conservation policies.

E 020

Microsatellite DNA polymorphism of Holstein cattle in Slovakia

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In this work we typed a random sample of 58 Slovak Holstein cattle with a set of 11 microsatellites, including nine international minimum standard panel (BM1824, BM2113, ETH3, ETH10, ETH225, INRA23, SPS115, TGLA53, TGLA122, TGLA126, TGLA227). DNA extracted from blood was amplified in one multiplex PCR (StockMarks for Cattle, AB). Amplified PCR products were separated by ABI 310 sequencer. Microsatellites were highly polymorphic, with a number of alleles ranging between 5 (BM1824) and 15 (TGLA122). For each locus, heterozygosity, allelic frequencies, polymorphic information content (PIC) and probability of exclusion (PE) were estimated. The observed heterozygosities were ranged from 0.465 (SPS115) to 0.845 (TGLA122) and expected heterozygosities were ranged from 0,705 (ETH10) to 0,865 (TGLA53). Observed PIC was from 0.672 (ETH10) to 0,852 (TGLA53) and mean PIC was 0,743. The PE was observed from 0,480 (TGLA126) to 0,734 (TGLA53). The combined probability exclusion using 11 microsatellites was estimated as CPE = 0,999. These results demonstrate that this combination of 11 microsatellites is effective enough for Holstein cattle parentage testing in Slovakia.

E 021

Microsatellite DNA polymorphism of four beef cattles in Hungary

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The 11 microsatellite markers (TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225, BM1824) were analysed for four beef cattle breeds (Hereford, N=20; Limousin, N=20; Blond' dAquitaine, N=27; Hungarian Spotted, N=36). The samples were analysed by ABI PRISM^{TM} 310 Genetic Analyzer and Genotyper Software (Applied Biosystems, USA) was used for the evaluation of analyses.

On the basis of the examination of genetic markers based on microsatellite investigation, numerous calculations can be executed and in addition to the establishment of their frequency, additional information may be obtained on the breed and in the evaluation carried out within the herd (e.g. heterozygosity, inbreeding, presence of alleles within each microsatellite). Consequently they can be used in the comparison of herds and the estimation of genetic distance (similarity).

The results verified that DNS markers can be adopted in many fields of animal breeding. The genetic distance, the variability of populations and breeds were clearly provable.

E 024

Kinship analysis of Ugandan Ankole cattle populations using autosomal microsatellite markers and indigenous knowledge

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This study aimed to determine the degree of relatedness between and within Ankole cattle populations and individuals using microsatellite markers and to assess the accuracy level of relationship assignment based on farmers' memory information. Eight Ankole cattle populations of Mbarara district in Uganda containing four farmer assigned relationships were examined in this study. Nineteen microsatellite markers were used to type DNA isolated from blood of 304 individuals. Pairwise relatedness comparisons between the populations ranged from 0.38 (Kasiisi and Nasasira) to 0.421 (Kaibanda and Tayebwa). The mean within herd relatedness coefficients (related pairs excluded) was 0.416 ± 0.02, while mean relatedness across all populations was 0.388 ± 0.01. Likelihood tests performed on 285 pairs of individuals generally agreed with farmer assignment of relationships. A total exclusion probability (PE) of 99.9% was observed for both sire–offspring and dam-offspring relationships. High accuracy in kinship assignment by farmers was observed for sire-offspring and dam-offspring dyads (88.6 ± 12.4) although it varied between farmers, while for grand dam - grand offspring and sibs, accuracy was lower. Assignment errors were 8.2, 14.3, 41.1 and 44% among dam - offspring, sire - offspring, grand dam - grand offspring and sib dyads respectively. These results indicate that oral herdsmen information can be used to trace relationships and reconstruct parentage within Ankole cattle herds.

E 025

Intrabreed diversity and genetic differentiation among Estonian dairy cattle breeds

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Analysis of 20 microsatellites, 10 blood group systems, two milk proteins (κ-*Cn*, β-*Lg*) and one blood protein (transferrin) was applied to assess genetic diversity and population differentiation of three Estonian dairy breeds (Estonian Holstein, Estonian Red and Estonian Native). Pedigree data was used to deduce genotypes for the blood group systems. Observed and expected heterozygosity (H_{obs}, H_{exp}), variance based *F*-statistics (Weir & Cockerham 1984) and exact tests for population differentiation were computed to assess genetic diversity of the breeds. The present data showed that the blood group systems B and C had higher values of H_{obs} and H_{exp} than the microsatellites (H_{exp} breed average 0.971 in B blood group, 0.949 in C blood group and 0.720 in microsatellites). Although census sizes of the breeds varied considerably, from 500 in Estonian Native to 75 000 in Estonian Holstein, the breeds showed similar level of within-population genetic variation. This indicates that the endangered Estonian Native Cattle have retained reasonably high genetic diversity. As indicated by the overall θ estimate computed across all loci, 5.9% of genetic variation was due to breed differences. 95% confidence interval for the θ estimate (0.043-0.076) showed that the subdivision of the Estonian breeds into discrete breeds is statistically significant. In general, the present data indicated that the gene pools of the Estonian cattle breeds are developed through a breed-specific evolution.

E 026

Genetic structure of Mexican local and exotic cattle breeds

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Hierarchic structure was constructed in order to study genetic differentiation of 4 mexican local cattle breeds and other exotic cattle breeds of Mexico. DNA samples from 168 animals from 4 Mexican Criollo cattle populations, Fighting bull or Lidia (n=24), Centralamerican Milking Criollo (n=24), Holstein (n=32), Brown Swiss (n=32), Hereford (n=24), Guzerat (n=32) and Brahman (n=32) were used to amplify 9 microsatellite markers (ETH152, ETH225, INRA54, INRA63, TGLA53, TGLA57, TGLA122, TGLA227 and BOLA-DRBP1). The genetic groups/breeds were assigned to their ancestral trunks. The first level is conformed by two. The second level consisted by three ancestral groups: iberians, european and zebu. The third level, is conformed by the eight groups, one of these was the mexican criollo. And the fourth level is conformed by the eleven groups. In each level F statistics were computed (F_{IT}, F_{IS} and F_{ST}). In level 1, subspecies vs. total, the average genetic differentiation (F_{ST}) between the two species was 2.5%. In level 2, ancestral groups, F_{ST} was 2.7%. In level 3, F_{ST} was 7.7%. Finally, in level 4 (with 11 subpopulations), the average heterozygous deficit was 46.8% for each group, while for the whole population it was 51%. The average genetic differentiation was 7.9%. The F_{ST} values increased with level. This suggests that the different levels are an agglomeration of subpopulations. The mexican criollos cattle are formed by different subpopulations, and the varieties, meanwhile, are formed by breeds. The general value of the F_{ST} 0.079 suggests that the proportion of the genetic variation caused by the difference between the 11 groups is of approximately 8 % of the total genetic variation; and the remaining 92% corresponds to the differences between the members.

E 027

Analysis of the variability based on mtDNA among Iberian Cattle

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The common origin for all cattle was estimated of only 10000 years BP and it was one of the most significant achievements of Neolithic peoples in different regions of Euroasia. From this zone, emerged several strains to Asia, Africa and Europe by subsequent movements of people in their migrations during the Pleistocene that diverged into two subspecies; *Bos taurus*, are predominant cattle of Europe, north and west Africa and the Middle East, and *Bos indicus*, are found mainly in eastern Eurasia and eastern Africa.

The origin of present-day Iberian cattle is controversial, but the analysis of morphological evidence indicates that all entrances of cattle were from the *Bos Taurus* linage. A significant number of animals were probably brought, first by the Proto-Indo-European peoples, into northern Iberian during the fifth millennium BP, and second by the Roman invasions in 2300 BP. A more recent and sporadic introduction of cattle from North Africa may have occurred during the Moorish occupation. All these historical facts highlight the importance of the geographical position of the Iberian Peninsula, which has played a Key role in human movements between Europe and Africa.

At present, the Iberian breeds are classified into three main groups: the Iberian, the Blond-Brown Cantabrico and the Turdetano; defined by their geographical position, related origin, and morphological characteristics.

This is a study to determine genetic diversity based on the mtDNA D-loop sequence variation among Iberian cattle breeds and check to existence mitochondrial haplotypes of African origin.

Total DNA was extracted from blood samples and was amplified through PCR and subsequently cycle-sequenced using an ABI PRIMS 377 DNA sequencer. The study was performed on 637 pb of the mtDNA control region, which is known to be more variable that other sequences.

E 028

Mitochondrial DNA polymorphisms of *D-loop* and three coding regions (*ND2, ND4, ND5*) in three Philippine native cattle: *indicus* and *taurus* maternal lineages

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To reveal mitochondrial DNA (mtDNA) diversity and *indicus* / *taurus* type maternal lineage of the three major Philippine native cattle breeds (Batangas, Ilocos & Iloilo), we sequenced the *D-loop* region (436bp) and three protein coding regions (*ND2, ND4* and *ND5*) of mtDNA in these breeds together with other nine cattle breeds (Angus, Hereford, Swiss Brown, Jersey, Japanese Black & Japanese Brown *etc*.). Phylogenetic analyses confirmed that the Philippine native cattle breeds were divided into two distinct *indicus* and *taurus* types. There seems to still exist several unique maternal lineages in the three Philippine cattle breeds. To distinguish between both mtDNA types, we developed a simple PCR-RFLP method based on a sequence difference of the *Sca*I site in the *ND4* region between them. A total 19 substitutions in *ND5* between the *indicus* type mtDNA (Iloilo cattle) and *taurus* type mtDNA were observed. Four of the 19 substitutions caused amino acid replacement. One of the *indicus* types of mtDNA in Batangus cattle is supposed to derived from Dehon cattle in South China by the results of RFLP analysis of the LA-PCR product (15,393bp) of mtDNA.

E 029

Mitochondrial DNA evidences for multiple maternal origins of domestic yak

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The entire D-loop (897 bp) of the mitochondrial (mt) DNA was sequenced for 393 domestic yak samples from 27 populations collected in China, Mongolia, Bhutan, India, Kyrgyzstan, Nepal, Pakistan, and Russia. Ninety-two haplotypes were defined with polymorphisms at 78 sites, excluding six insertions/deletions. Phylogenetic analysis clusters these haplotypes into two distinct mtDNA lineages: mt-I (68 haplotypes) and mt-II (24 haplotypes). The mt-I haplotypes are the most frequent, being present in all populations and in 305 samples. The mt-II haplotypes are observed 88 times and in 23 populations, being absent in two Chinese, one Bhutanese and one Mongolian populations. The mean D-loop nucleotide diversity (π) in domestic yak is 1.3×10^{-2} nucleotide differences per site. Mean pairwise sequence divergences were calculated as 5.2×10^{-3} and 8.3×10^{-3} substitutions per site for the mt-I and mt-II haplotypes, respectively. An average of 4.2×10^{-2} substitutions per site is obtained between the mt-I and mt-II lineages. A divergence time of ~ 120,000 years before present (BP) is estimated between the mt-I and mt-II lineages using a mutation rate of 3.1×10^{-4} substitutions per site per year as established for other bovine species. The domestication of yak is presumed to have taken place on the Qinghai-Tibetan Plateau circa 4000 BP. Our results suggest two distinct mtDNA lineages of pre-domestic origin in today domestic yak.

E 030

Analysis of Genetic diversity and relationship in the Korean Cattle(Hanwoo) using AFLP Marker

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In order to determine the genetic diversity and relationship amplified fragment length polymorphism (AFLP) analysis was performed on ten cattle breeds in the Korean cattle. Although AFLP has been considered as a complex technique compared with microsatellites and RAPD, it is likely that AFLP is a reliable approach to fingerprint the genome in search of polymorphism. Using these polymorphic bands, the three estimates (percentage of polymorphic loci, Neis expected heterozygosity and Shannon index) of genetic diversity, Genetic subdivision (Gst) estimate, Neis genetic distance and two indices of genetic similarity were calculated. From all the calculations of genetic diversity, Korean Cattle (Hanwoo) showed higher than diversity Yenbian Cattle (China), Angus, Charolais and Holstein. Given the mean Gst Value (Gst=0.53) across all cattle examined, levels of apparent breed subdivision were considerable. A UPGMA tree showed that the Korean cattle (Hanwoo), Yenbian Cattle, Charolais, Angus, Wagyu (Japan) formed a distinct cluster from the other five cattle (Brown swiss, Hereford, Limusine, Simmental, Holstein). In particular, genetic differentiation among cattle populations of the East Asia region, indicated that Korean cattle have closer relationship with Yenbian cattle (China) than Wagyu (Japan). In conclusion, there is extensive gene flow between Korean cattle (Hanwoo) and Yenbian cattle (China).

E 031

Breed identification of beef cattle using SNPs markers derived from AFLP

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Japanese Black (JB) cattle is famous as a high quality beef producer. In retail markets, it is not rare that F1 (Japanese Black and Holstein hybrid) beef is sold as JB beef, because JB carcass is sold higher price than F1 carcass in a wholesale market. Therefore, breed discrimination especially between JB and F1 beef is important which prompt us to develop DNA markers to discriminate JB and F1 cattle. Amplified fragment length polymorphism (AFLP) method was employed to search DNA markers those are absent in JB cattle, but present in Holstein cattle. We found 11 candidate DNA markers from about 2400 three selective primer combinations of *Eco* RI and *Taq* I. Then we converted those AFLP markers into single nucleotide polymorphisms (SNPs) markers for high-throughput genotyping. The allele frequencies in both breeds were estimated for discrimination. In consequence, combination of four or six SNP markers is highly effective for beef breed discrimination and when using four markers the probabilities of F1 detection were 91.7% with0.66% error and using six markers it is 96.7% with 2.36% error. This result showed that those SNP markers could be useful for discriminating major beef breeds in Japan contributing to eliminate suspicious beef from retail markets.

E 032

Application of DNA Test for Individual Traceability in Hanwoo (Korean cattle)

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Identification of animals has been made with an ear tag with dummy code, and blood typing has been used for paternity and individual identification in live animals. As various genetic markers are for different cattle breeds vary, the discrete genetic markers are necessary to identify Hanwoo. A total of 740 progeny testing Hanwoo were used to identify Hanwoo specific markers. To examine traceability of individuals by using breed specific genetic codes, four animal were randomly sampled, and traced from live animals to post-slaughter processing stages. The candidate genetic makers used in the study were 16 DNA microsatellites which were identified in chromosomes 1 and 14. The number of alleles of those DNA microsatellites ranged from a minimum of 3 to maximum of 12. The heterozygote frequency ranged from 0.022 to 0.824. Effective number of alleles for each DNA microsatellites were 3 to 6. Six selected candidate genetic markers were able to trace individual cattle with an 100% confidence level.

E 033

Evaluation of a panel of SNP markers for the traceability of cattle

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We have developed and tested a set of 22 SNP markers for individual cattle identification that could be used to replace existing microsatellite markers. SNPs have some inherent advantages over microsatellites. They are binary and many high throughput technologies exist for SNP genotyping, they can be read fully automatically, do not require size calibration, and thus inter-laboratory analyses are easy. Markers were selected from 70 previously published SNPs and tested in a wide variety of cattle breeds. The criteria for selection were their heterozygosity and quality of genotyping performance. The DNA panel tested consisted of between 12 and 16 animals each from 20 different races, whereof 18 were of European, one of African, and one of Indian origin. In the European cattle races the 22 selected SNPs provide a power of identity between 1:280.000 for Chianina and 1:14,9 million for Reggiana breeds. The quality of our genotyping was tested by the clustering of duplicated samples. Further the cluster analysis showed that our set of markers did not result in clustering of European breeds, while the African (N'dama Guinean) and the Indian (Sahiwal) did. As anticipated, the power of exclusion was lower for N'dama Guinean (1:53.000) and for Sahiwal (1:3.300) breeds. This set of 22 SNPs constitutes our proposition for an individual traceability panel in cattle breeds of European origin.

E 034

Genetic variability among four cattle breeds prevalent in the Italian beef market and the traceability of the individual multilocus profiles

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Assignment tests based on multilocus genotypes are becoming increasingly important to certify origin of livestock products. We typed 19 STRs in 269 animals from four cattle breeds present in the Italian beef market. Based on Wright's F-statistics, four loci were discarded, and the remaining 15 loci (F_{IT} = 0.101, F_{ST} = 0.089, and F_{IS} = 0.013) were used to compute the likelihood that each animal was drawn from its true breed instead of any of the others. To avoid occurrence of zero likelihood when one or more alleles were missing from a tested breed, a conservative approach in estimating sample allele frequencies was adopted. Log-likelihood ratio distributions of the individual assignments were determined for all possible breed contrasts, and their means and SDs were used to infer the true-positive and false positive rates at several values of the log(LR). The posterior probability that the animals of a presumed breed were actually drawn from that breed instead of any of the others was then calculated. Given an observed value of log(LR) > 0 and assuming equal priors, these probabilities were > 99.5% in 10 out of the 12 possible breed contrasts. For the two most closely related breeds (F_{ST} = 0.041), this probability was 96.3%. The power of excluding the origin of an animal from an alleged breed when it was actually derived from another breed was comparable.

E 036

PCR_RFLP detection for bovine β-Casein and β-Lactoglobulin in Egyptian buffalo

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Since Egyptian Buffalo milk has a major importance in cheese processing. Polymorphism of milk protein variants are of most importance. In this study the existence and polymorphism of Bovine β-Casein (β-CN) and β-Lactoglobulin (β-LG) using PCR-RFLP were tested in a total number of 147 performance-recorded Egyptian buffalo (Bubalus Bubalis) lactating females that belong to the Animal Production Research Institute (APRI), Ministry of Agriculture and Land Reclamation (MoALR), Egypt. Genomic DNA was extracted using Chelex resin and proteinase K. PCR was performed using primers of bovine sequence. PCR product was digested by Msp I, and Hae Ⅲ for the β-CN and β-LG, respectively. The result was directly analyzed by electrophoresis in 3% agarose gel stained with ethidium bromide, followed by ultraviolet exposure. The highest successful amplification and digestion of the studied buffalo samples was shown for the β-LG (being 45%; 66 samples) resulting two bands with size range of about 100 and 120 bp. Less successful percentage was reported for the β-CN (being 33%; 49 samples) showing homozygous allelic pattern, with one allele with approximate molecular weight of 220 bp. Further GeneScan and sequencing are to be performed foe accurate allelic sizing and determination of sequence.

E 037

A new protocol for identification and parentage test in buffalo

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Knowing the exact genealogy is very important in animal production systems, especially when genealogy information is included in a herd book. Genetic value estimates accuracy is largely dependent of the herd book correctness. Buffalo breeding is on a large scale managed by using natural mating in herds with more than one sire present at the same time. Genetic and Service Lab (LGS) defined a new working protocol to verify, through DNA microsatellites analysis, the genealogy for each individual. 12 microsatellites in one PCR-multiplex were tested for this purpose (INRA006, CSSM42, CSSM47, CSSM19, D5S2, MAF65, RM4, CYP21, BM1013 CSSM70, CSSM60, INRA026, BM0922, BM0922 and BM1706). The exclusion probability (PE) was estimated by the frequency estimates of alleles for each single microsatellite. The combined PE for the used microsatellites was 0,9999. The method is nowadays largely used to verify the real sire in all buffalo herds whose animals are registered to the Italian National Buffalo herd book.

E 040

Phylogenetic studies of Bangladeshi water buffaloes estimated by blood protein and mitochondrial DNA polymorphism

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Twenty five blood protein loci and nucleotide sequence of mitochondrial DNA *cytochrome b* ((mtDNA*cytb*) gene were analyzed to study the phylogenetic relationship among different populations of Bangladeshi water buffaloes. A total of 151 animals from six populations were studied. 7 blood protein loci, i.e. albumin, transferrin, alkaline phosphatase, hemoglobin-α, hemoglobin-β, carbonic anhydrase and peptidase-B showed polymorphism. Blood protein analysis revealed significantly higher heterozygosity in the populations of eastern and southern part ($\overline{H}$±SE = 0.0933±0.0399 and 0.0816±0.0363 respectively). In phylogenetic trees, the populations of eastern part and southern part grouped in a separate cluster from other Bangladeshi populations but belonged in the cluster of river buffalo type. Population of eastern part, however, positioned separately from other river buffalo populations and stayed with the crossbred (swamp buffalo × Murrah breed) buffaloes in three dimensional projections. Complete sequence (1140 bp) of mtDNA*cytb* revealed the existence of 13 haplotypes (A-M) in all populations. Haplotypes A -K showed high homology with that of typical river buffalo. Haplotypes L and M showed high homology with that of typical swamp buffalo. The phylogenetic trees constructed on sequence divergence data indicated two maternal origins of Bangladeshi water buffaloes. Eastern part of Bangladesh possessed both river and swamp buffaloes as provided by the genetic information on maternal origin.

E 041

The complete mitochondrial DNA sequences of Asian water buffaloes

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The complete mitochondrial DNA (mtDNA) molecules of domestic swamp and river water buffaloes, Sri Lankan wild buffalo and African buffalo have been sequenced. The complete mtDNAs are 16,355, 16,357, 16,357 and 16360 bp, respectively. The differences in length are caused by variations in the D-loop region. Comparison between individual genes shows that rate of base substitution within the swamp buffaloes varies from 0 to 2.61%, while that within the river buffaloes varies from 0 to 1.73%. This difference in range may reflect genetic diversity, and may suggest that populations of swamp buffaloes have higher genetic diversity than the river buffaloes. Amino acid sequences between swamp and river buffaloes show low homology for NADH4L (95.92%) and NADH5 (98.18%) but are conserved for CO2, NADH3 and NADH6. Phylogenetic analysis by the neighbor-joining method places the Sri Lankan wild buffalo in the same cluster as the river buffalo on the basis of nucleotide divergence. These results suggest that the river buffalo was domesticated from a breed closely related to the Sri Lankan wild buffalo.

E 046

Study on the genetic relationship among Brazilian, Spanish and Portuguese goat breeds using microsatellite DNA markers

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Genetic relationship among 6 Brazilian, 9 Spanish, 1 Portuguese and 1 French goat breeds has been investigated using microsatellite markers. 728 caprine samples belonging to the following breeds have been analysed: Moxotó, Canindé, Serrana Azul, Marota, Repartida and Graúna from Brasil; Blanca Andaluza, Blanca Celtibérica, Malagueña, Murciana, Granadina and Retinta from the South of Spain; Palmera, Majorera and Tinerfeña from Canary Islands (Spain); Serpentina from Portugal and Saanen. 27 microsatellites has been typed of those recommended by the ISAG. The average alleles number found in these populations varies between 4.22 and 8.00. The average of direct count heterocygosity varies between 0.53 and 0.70. In the neighbour-joining trees from both D_S and $D_{Reynolds}$ distances it is possible to observe a clear separation between Brazilian breeds and the rest of the populations. All breeds from the South of Spain clustered together without a clear separation between them, but clearly separated from breeds from Canary Islands. In the individual tree it can observe a clear grouping of the individuals belonging to each population, except for the breeds from the South of Spain.

E 047

Genetic characterization of indigenous goat populations of Ethiopia using microsatellite DNA markers

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Eleven indigenous Ethiopian goat breeds, defined by their phenotypes, and nine reference populations from Europe, Asia, Middle East and other African countries were characterized using 15 autosomal microsatellite loci. A total of 159 alleles were identified. Molecular diversity indexes of Ethiopian goat populations were intermediated between the trypanotolerant West African dwarf goats and the non-African goats. The mean number of alleles per locus (*MNA*), in Ethiopian goats, ranged from 4.65 ± 2.09 (Abergalle) to 5.28 ± 2.9 (Long-ear Somali). Expected heterozygosity (*He*) ranged from 0.55 (Gumez) to 0.61 (Arsi-Bale). Most genetic variations were present within breeds (av. *Fst*: 0.016 and *Gst* 0.026). Phylogenetic trees, population structure and principal component analyses showed that all the Ethiopian populations are genetically distinct from the populations of Europe, Asia, Middle East and other African countries. The 11 Ethiopian populations can be grouped as nine distinct genetic entities: Arsi-Bale, Gumez, Keffa, Long-Ear Somali, Woyto-Guji, Abergalle, Afar, Highland Goats (previously separated as Central and North West Highland) and the goats from the previous Hararghe province (Hararghe Highland and Short-Ear Somali). Weitzman analysis indicates that more than half of the total genetic diversity of the Ethiopian goats is present in two breeds, Afar and Keffa, with marginal loss of diversity of 26% and 25%, respectively.

E 048

Genetic characterisation of the West African Dwarf goat

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The West African Dwarf goats (WAD) are trypanotolerant. Due to increasing human activities, the northern limit of tsetse distribution is moving southwards in West Africa. Consequently, trypano-susceptible Sahelian goats have been introduced into southerly areas where traditionally the WAD goats are found. To assess the extent of crossbreeding between the two breeds, we determined the genetic diversity, population admixture and relationships of 20 goat populations from five West African countries (Senegal, Guinea, The Gambia, Mali and Guinea Bissau) using 10 microsatellite markers. Genetic diversity was low with a mean number of allele (MNA) per locus ranging from 3.70 to 4.54, observed heterozygosity (*Ho*) from 0.44 to 0.55 and expected heterozygosity (H_E) from 0.46 to 0.54. Principal component and admixture analyses support a gradient of introgression of the Sahelian goats into the WAD goat populations possibly following two directions: a North-South gradient from North Senegal to West Guinea and a Northeast-Southwest gradient from Mali to Guinea. Gene differentiation (F_{ST}) among populations was low (5.8%) suggesting gene flow between populations, a result confirmed by genetic distance (D_A) and phylogenetic analysis (Neighbor-joining tree).

E 049

Genetic diversity in Asia, Africa and Australia goats

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In general, with blood type and/or blood protein type analysis, the genetic diversity of goat populations is less branched than for other mammal livestock species. This present study elucidates the degree of goat genetic diversity using the microsatellite marker (MS marker) analysis. DNA samples were collected in Korea, Australia and The Gambia and in total 143 individual from 7 populations were genotyped. The 15 MS markers were analysis with both ABI377™ and ABI3100 Genetic analyzer™. The genetic variability (*He*), gene diversity (*Ht*), effect of population subdivision (*Fst*) and genetic distance (*Ds*) were estimated with DISPAN, FSTAT computer programs. Ninety-seven alleles were detected in this study, including 29 common alleles. The largest allele variation was observed in the Australian Feral breed (88 alleles); for the Australian Boer breed only 49 alleles have been observed. The estimated *Ht* values in 7 populations ranged between 0.4 and 0.6 and the values for allelic richness ranged from 2.5-3.0. *Fst* and *Ds* values indicated that the genetic relationships of these goat populations were comparatively segregated. These things strongly suggested that the analysis of MS marker is a powerful tool for clarifying the genetic diversity of goat population.

E 050
Mitochondrial DNA diversity in East Asian native goats

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Domestic goats (*Capra hircus*) are deeply related with human life, and located as one of the most important livestock. Despite it, the origins of domestic goats remain uncertain. In addition, it is necessary to estimate the genetic diversity to conserve the genetic resource. In this study, we sequenced a hypervariable segment of the mtDNA D-loop region in 12 Myanmar, 34 Chinese, 34 Mongolian and 10 Laostian, 57 Pakistan natives. The neighbor-joining phylogenetic tree was constructed by these sequences in conjunction with that of our previous studies. The tree illustrated five divergent mitochondrial lineages of domestic goat (*mt-lineage A-E*). Subsequently, we estimated the genetic frequencies of the lineages by PCR-RFLP and mismatch PCR method in 180 Myanmar, 34 Chinese, 100 Mongolian and 76 Laostian, 60 Northern Vietnam, 56 Japanese, and 133 Cambodian native goats. The *mt-lineage A* was observed in all countries and most frequent in this study, and the *mt-lineage B* was detected at high frequencies in Southeast Asia. The *mt-lineage C* was observed in Chinese and Mongolian, *mt-lineage D* in Pakistan and Mongolian, and *mt-lineage E* in Chinese native goats at low frequencies. In the *mt-lineage B*, the frequency differences were observed on the basis of geographical situation in Southeast Asian countries. High frequencies were observed in the remote mountain area, while relatively low frequencies of *mt-lineage B* were detected in urban area developed transportation device such as roads or harbors. The results suggested that the *mt-lineage B* had been majority *mt-lineage* and the *mt-lineage A* derived from recent international shipping of goats in Southeast Asia.

E 051
Nucleotide Diversity on the Ovine Y Chromosome

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In order to investigate the structure of male lineages within ovine populations, a sequencing approach was used to search for single nucleotide polymorphisms within the non recombining region (NRY) of the sheep Y chromosome. Nine fragments from five NRY genes were amplified and sequenced from a set of sheep representing seven different breeds. A total of 4380 bp was screened which resulted in the identification of a single polymorphism. The resulting estimation of nucleotide diversity ($\pi_Y = 0.90 \pm 0.50 \times 10^{-4}$) was compared with an estimate made from the autosomal component of the genome. Sequence analysis of eight autosomal genes revealed 17 segregating sites within the 2933 bp investigated ($\pi_A = 2.15 \pm 0.27 \times 10^{-3}$). This level of nucleotide diversity is higher than previously reported for sheep. Following adjustment for the influence of effective population size and a male biased mutation rate, comparison revealed that less than 10% of the expected nucleotide diversity is present on the ovine Y chromosome.

E 052
A map of diversity of European and Mediterranean sheep as revealed by AFLP markers

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ECONOGENE project combines a molecular analysis of biodiversity, socio-economics and geostatistics to address the conservation of sheep and goat genetic resources and rural development in marginal agrosystems in Europe. Here we present the preliminary results of the sheep dataset comprising 1770 animals belonging to 53 authoctonous and one cosmopolite (Merino) sheep breeds sampled across Europe, middle East and north Africa.
The animals were screened with three high polymorphic AFLP primer combinations that revealed more than 100 polymorphisms scored as dominant markers. On the binary matrix produced we calculated the summary statistics and the genetic distance graphically represented with the PCO analysis The partition of the total genetic variance into the between individuals, sampling areas, breeds and Countries components were investigated using AMOVA. Exploiting the GIS information collected during sampling, we also explored the geographic clines of diversity and we produced geographic maps of markers frequencies.
This work is funded by the European Commission project QLK5-CT2001-02461.

E 053
Genetic differentiation of autochthonous Austrian sheep breeds

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Genetic variability of 21 microsatellite loci was analyzed for eleven sheep breeds, mainly from the eastern part of the Alps (Tiroler Bergschaf, Braunes Bergschaf, Tiroler Steinschaf, Alpines Steinschaf, Juraschaf, Montafoner Steinschaf, Waldschaf, Zackelschaf, Krainer Steinschaf, Kärntner Brillenschaf and Texel as out-group). A total of 720 animals was analyzed. The shortest genetic distance was found between Alpines Steinschaf and Waldschaf. Surprisingly, the Montafoner Steinschaf showed a large genetic distance to the other breeds in the "Steinschaf-Group" (Alpines Steinschaf, Montafoner Steinschaf, Krainer Steinschaf and Tiroler Steinschaf) although prior to molecular analysis, this breed has not been acknowledged as an independent breed. A correct breed assignment, using a Bayesian approach, was possible for only 66% of individuals belonging to Alpines Steinschaf, but for at least 90% up to 100% of individuals for all other breeds investigated.

E 059

Identification of SNPs in the CD61 (*ITGB3*) porcine gene

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The CD61 (*ITGB3*) gene codes for the $\beta 3$ integrin, that forms in the cell surface of platelets and other cells, together with α_{IIb} (CD41) and α_V (CD51) integrins, the receptor of the fibrinogen, vitronectin and other ligands. In pigs the *ITGB3* gene has been mapped on chromosome 12p11-2/3p13. The knowledge of the complete sequence of the coding region of this porcine gene, described by our group, has allowed us to begin works to identify SNPs in pig populations. The strategy followed has been to identify the different exons in the cloned cDNA sequence, based on its high homology with the human sequence, to design primers to amplify fragments of these exons in DNA samples from commercial Landrace × Large White (L × LW) populations, detection of electrophoretic variants by the SSCP method and sequencing of these variants. We have studied amplified fragments corresponding to the largest exons (B, C, E, I, J, K) as well as to the 3'UTR. We have found five SNPs, two of them located on the coding region (exon E: 847T→C; exon I: 1609C→T), which affects to the third position of the codon without changing the aminoacid sequence, and three others located on the 3'UTR (2925C →G; 2984A→G; 3089G→C). Frequencies and heterozygosities of these SNPs in samples from commercial L × LW populations ranged from 0.62 – 0.79 and 0.33 – 0.76 respectively. Frequencies and heterozygosities in other pig populations (Duroc, Iberian, Chato Murciano) were also obtained. An alternative way to identify the SNPs of the coding region by the PCR/RFLP method have been developed, using the *Tsp*RI and *Hae*III restrictases.
(This study has been supported by the Spanish Ministry of Science and Technology, contract BIO2000-1636-CO2-02)

E 060

Evaluation of Toll-like receptor 4 (*TLR4*) genetic diversity in pig populations

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Toll-like receptors (TLRs) are a family of cells surface receptors that have a crucial role in recognition of pathogen ligands and in activation of innate and adaptive immune responses. Actually 11 TLRs have been described and sequenced in mammals. Increasing evidence suggests that the polymorphisms observed at these genes, mostly localised in the extra-cellular domain, could have an important role to genetically differentiate host response to invading pathogens. *TLR4* recognizes the lipopolysaccharide (LPS) component of Gram-positive bacterial cells, and is currently considered an important candidate for natural resistance/susceptibility to several livestock infectious diseases. The aim of this study was to investigate the genomic sequence diversity of *TLR4* in pig populations, and to evaluate a possible functional role of mutations as markers for disease susceptibility. We had previously reported physical localization of *TLR4* gene and other TLRs family's members in pigs based on a porcine radiation hybrid map. For this work, the *TLR4* full coding region was sequenced in a panel of animals belonging to commercial breeds (Large White, Landrace, Duroc) and autochthonous Italian populations (Calabrese, Cinta Senese, Casertana, Nera Siciliana). Swine *TLR4* gene, based on its similarity to other sequences posted in database, is supposed to have three exons. Most polymorphism occurs at exon 3, which codes for the putative ligand-binding region and the signaling domain. Additional animals are being used to investigate haplotype segregation.

E 061

A simple and cost-effective procedure to analyze genomic characteristics of porcine *ACADM*

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We chose porcine *ACADM* was selected as a candidate of a QTL for intramuscular fat content (IMF). In order to get genomic information of porcine *ACADM* such as possible exon-intron organization, partial intron sequences, SNPs *etc*., a simple procedure excluding high throughput DNA sequencing was designed. The procedure uses comparative sequence analyses and simple BAC-based experiments. A BAC containing full porcine *ACADM* was screened from the KNP BAC library. Intron sizes and partial intron sequences of the gene were decided by inter-exonic PCRs and sequencing using the BAC. Direct BAC sequencing was performed to know DNA sequences of the unknown region which were promoter and two long introns. SNPs were detected from four breeds by PCR-SSCP and sequencing analysis for the regions of interest. Five substitution mutations and an insertion/deletion mutation in the promoter region, and a missense mutation in the coding region of porcine *ACADM* were obtained. A mutation in the promoter region was used for testing relationship with growth and fat depot traits. The *22* genotype of the polymorphism showed a highly significant effect ($P < 0.0001$) on IMF.

E 062

Highly efficient and precise genotyping of pigs with a new set of polymorphic tetranucleotide microsatellites

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Tetranucleotide microsatellites were identified in the genome of *Sus scrofa* domestica by sequencing inserts from four selectively enriched recombinant plasmid libraries, and by analyzing public DNA sequence libraries. One hundred markers were chosen for analysis of polymorphism in each 50 German Landrace, German Large White and Pietrain pigs, respectively, by polymerase chain reaction (PCR) and by capillary gel electrophoresis. This study revealed 25 tetranucleotide markers which exhibited at least 6 alleles in the investigated populations. The chromosomal location of 22 new markers was assigned by radiation hybrid mapping. The set covers the chromosomes 1, 3, 6, 7, 8, 9, 13, 14, 16, 17, 18 and X (pseudoautosomal region). Multiplex reactions were set up with 10 and 12 markers from different chromosomes and proved to be a very versatile tool for efficient genotyping and paternity testing of commercial breeds and wild pigs. The high discrimination power, the absence of stutter peaks, and the possibility for automated allele calling recommend the described markers as a new standard tool for genotyping and monitoring of biodiversity in pig populations. A test kit based on our findings will be made available by Biotype AG in the near future.

E 063

Evaluation of the microsatellite markers used for parentage control in pigs in the Czech Republic

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A unified DNA panel of ten porcine microsatellite markers (*SW24, S0386, S0355, SW353, SW936, SW72, S0068, S0070, S0107* and *TNFB*) is used by laboratories providing the service for breeders in the Czech Republic. The genotype determination and parentage control for elite breeding stock is performed according to law in the Czech Republic. Recently this panel of microsatellite loci is utilized by the herd book of Pig Breeders Association in Bohemia and Moravia. The length polymorphism of microsatellite sequences was examined by the multiplex PCR method followed by fluorescent fragment analysis of the amplified products (ABI PRISM 310 Genetic Analyzer). The panel of porcine microsatellites was evaluated in 418 individuals of five different breeds. The material consisted of randomly selected animals from various breeding farms encompassing Large White (n=220), Landrace (n=112), Duroc (n=30), Piétrain (n=34) and gene reserve Czech Black Pied Prestice (n=22). The probabilities of paternity exclusion/one parental genotype unavailable/and parentage exclusion were 99.91%/98.18%/99.99% in Large White; 99.53% / 94.46% / 99.99% in Landrace; 97.74% / 85.45% / 99.83% in Duroc; 99.71% / 95.97% / 99.99% in Piétrain and 99.94% / 98.62% / 99.99% in Black Pied Prestice, respectively. These results confirmed that our microsatellite panel is enough effective for performing paternity and parentage control in the breeding stock kept in the Czech Republic. (This work was supported by the grant No. MSM43210001)

E 064

Analysis of mitochondrial DNA control region of a wild boar population in North Italy

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We investigated the mtDNA control region of a group of wild boars hunted in North Italy, to evaluate if there are molecular evidences of a possible hybridization with domestic pig breeds. In fact the presence of a large number of wild boars in several territories in Italy it is producing many agriculture damages and environmental problems. We sequenced 466 bp of mtDNA control region, from position 15619 to position 16085 (reference sequence AJ002189), of 28 wild boars (North Italy), and a wild boar sample hunted in a Tuscany protected area, where a population of Italian wild boars (*Sus scrofa majori*) it is conserved. We detected five different haplotypes, four in North Italy samples and one in the Tuscany sample, with a minimum of 2 and a maximum of 8 transition substitutions and an insertion in all samples. The alignment of our sequences with those found in GenBank shows that there are many similarities between our North Italy wild boar population and European domestic pig breeds, while only the Tuscany sample has a 100% of identity with the pure Italian wild boar (GenBank AB015094). Also the phylogenetic tree constructed with the obtained sequences supports these findings. Our results suggest that a hybridization with the domestic pig occurred in this North Italy population of wild boars. This is a preliminary study to evaluate the genetic structure of such wild boar populations that could give important tools for management and conservation plans.

E 065

The complete mitochondrial DNA sequence of Jeju native pig (*Sus scrofa*) and phylogenetic relationship of the pig relative to seven other mammalian

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The complete nucleotide sequence of the mitochondrial genome of Jeju native pig (JNP) was determined. We cloned and sequenced the complete mitochondrial DNA of Jeju native pig raised in Jeju province in Korea. The total length of the sequence was about 16,750 bp. This length varied, however, due to pronounced heteroplasmy caused by variable numbers of a repetitive motif (5'-TACACGTGCG-3') in the control region. The number of the repeat in JNP ranged from 6 to 27. Genes responsible for 2 rRNAs, 22 tRNAs, 13 peptide coding regions in mitochondrial DNA were found. Three regions of the peptide-coding genes, four regions between peptide coding and tRNA genes were partially overlapped. The occurrence frequency of the start codon, ATT, was specifically showed by 29.2% in *ND2* of JNP. This codon was found previously in two European breeds, Landrace and Duroc. Distinct mutations of two tRNA genes (tRNA-Ala and tRNA-His). We estimated the phylogenetic relationship between JNP and other pig breeds as well as between JNP and other domesticated animal such as cattle, sheep, donkey, dog, cat and horse. From the analysis, JNP was classified in *Artiodactyla* together with cattle and sheep.

E 066

Phylogeny of the Suidae family based on mitochondrial and nuclear DNA sequences

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Classical taxonomy suggests that the Suidae family comprises three subfamilies; 'true' pigs (Suinae), warthogs (Phacochoerinae) and babirusa (Babyrousinae). Molecular data from some species of these subfamilies have been studied previously, and have proven consistent with the traditional classification. In the present study, we assessed the phylogenetic relationships of nine species of African, Asian and European suids, using mitochondrial 12S rRNA and control region, and nuclear PRE-1 P27 and P642 sequences. Preliminary analyses show that European Wild Boar (*Sus scrofa*), Bearded pig (*Sus barbatus*), Javan Warty pig (*Sus verrucosus*) and Sulawesi Warty pig (*Sus celebensis*) clustered in a clade distinct from other Suids including common warthog (*Phacochoerus africanus*) and Babirusa (*Babyrousa babyrussa*), which is consistent the classical taxonomy. The Red River hog (*Potamochoerus porcus*) and Bushpig (*Potamochoerus larvatus*) clustered in a clade distant from the *Sus* but close to the warthog. This suggests that a common ancestor gave rise to these two African lineages. The present results are consistent with paleontological studies but not with the traditional classification which places the 'bush pigs' (*Potamochoerus*) as a sister clade of the 'true pigs' (*Sus*) within the Suinae subfamily. This study implies that the taxonomy of the Suidae family requires review. Further analyses are underway to confirm these preliminary results.

E 067

Distribution of endogenous retroviruses in the Suidae family

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All Vertebrate species contain multiple copies of endogenous retroviruses (ERVs) which represent ancient retroviral infection of the germ line cells. The potential medical significance of porcine endogenous retroviruses (PERVs) has lead to considerable interest in determining their origins and relationships. In the present study, we assessed the phylogenetic relationships of endogenous retroviruses from European Wild Boar (*Sus scrofa*), Bearded pig (*Sus barbatus*), Javan Warty pig (*Sus verrucosus*), Sulawesi Warty pig (*Sus celebensis*), common warthog (*Phacochoerus africanus*), Red River hog (*Potamochoerus porcus*), Bushpig (*Potamochoerus larvatus*) and Babirusa (*Babyrousa babyrussa*) using reverse transcriptase (*pol*) gene sequence. Approximately 850 bp of the *pol* gene was detected in all species, which clustered in two major clades, suggesting the presence of two different types of ERVs and a possible single infection event of these species before they diverged from the common ancestor. Further analyses of ERV *pol* sequences and additional studies of envelope genes type A, B and C are underway to understand the distribution and relationships of ERVs in the Suidae family. Possible scenarios of parasite and host co-evolution will also be described.

E 068

Comparison of endogenous retroviruses in peccaries (Tayassuidae) and pigs (Suidae)

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Endogenous retroviruses (ERVs) are copies or remnants of exogenous retroviruses that integrated into a host germ line cell genome at some stage in the past. ERVs are inherited in a Mendelian way and are not normally pathogenic. In the present study, we assessed the presence of ERV DNA sequences in Collared, Chacoan and White-lipped peccaries, using degenerate and specific primer sets for the reverse transcriptase and envelope genes. Sequence search and phylogenetic DNA analyses demonstrated the presence of both *gammaretrovirus* and *betaretrovirus* endogenous retroviruses in peccaries. Given the relationship of peccaries and pigs, it could be hypothesised that peccary retroviral sequences would cluster more closely with porcine ERVs (PERVs), than with ERV sequences from other species. However, it is clear that many peccary retroviral sequences do not preferentially cluster with PERVs although some do. Analyses of ERV and pig/peccary divergence indicate that most endogenous retroviral sequences detected in these two lineages correspond to very different retroviruses which probably independently infected the Suidae and Tayassuidae families after they diverged from the common ancestor with only a few consistent with possible infection before these two host families diverged from the common ancestor. Comparisons of ERV from Collared and White-lipped peccaries indicate that extant peccary species could have been infected before they diverged from a common ancestor. Better understanding of the evolution of ERV between the Suiformes and within the Tayassuidae family requires precise mapping of ERVs so that orthologous loci can be compared.

E 069

Identification of PERVs (Porcine Endogenous Retroviruses) from Korean Native Boar BAC library

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Xenotransplantation is the transplantation of living cells, tissues and organs from one species to another. Recently, pigs (*Sus scrofa*) have been considered as possible organ donors for humans. PERVs are a possible problem for xenotransplantation even though no *in vivo* PERV infection has ever been reported in humans. About 50 proviral copies are present in the pig genome and the chromosomal insertion sites are different among pig breeds. To examine PERV copy number and integration sites in the Korean native pig genome, we screened a BAC (Bacterial Artificial Chromosome) library with PERV specific protease primers to identify PERV positive clones and used three envelope specific primer pairs for the identification of PERV types. Currently 72 PERV positive clones, 20 PERV-A and 52 PERV-B, have been identified with 90% of the library screening completed and the end-sequences of the PERV positive clones are being generated. The precise PERV integration sites in Korean Native pigs will be determined using the IMpRH panel and primers designed from the BAC-end sequences. These results will contribute to understanding the origins and timing of PERV integrations into the pig genome.

E 070

Insertional variation of two Porcine Endogenous Retroviruses (PERVs) in Korean native pigs and Asian Wild Boar

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Pigs are possible sources of organs and tissues for xenotransplantation into humans, based on safety, financial, ethical, practical considerations. Previous research has shown that porcine endogenous retroviruses (PERVs) can infect human cells *in vitro*, raising issues about the safe clinical application of xenotransplantation. PERVs are present at about 50 copies in the pig genome, although many of these are non-functional and the chromosomal insertion sites differ between pig breeds. We examined the presence and absence of a PERV-A at SSC1q2.4 (known to be replication competent in Large White pigs) and a PERV-B at SSC1.1-2 (known to be replication incompetent in Large White) in 9 Korean native pigs and 7 Asian Wild Boar using PCR primers specifically amplifying the genomic flank sequences. Preliminary results show that either insertion may be present in some Korean native pigs and Asian Wild Boar and absent in others. This insertional variability indicates that it would be possible to select animals free of these loci as part of a general strategy to generate PERV-free lines of pigs suitable for xenotransplantation.

E 071

Comparison of four mitochondrial gene (*ATPase8/6*, ND3 and *ND4L* genes) in Cervidae: *Cervus, Muntiacus* and *Rangifer*

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Cervidae is classified into four subfamilies, which are composed of Cervinae, Munticinae, Hydropotinae and Odocoilinae. Cervinae and Odocoilinae are partially utilized as livestock, but there is less molecular genetic information in Cervidae than the other livestocks. In this study, we determined the DNA sequences of mitochondrial F_0-ATP synthase subunit 8/6 (*ATPase8/6*), NADH dehydrogenase subunit 3 and 4L (*ND3 and 4L*) genes in Cervinae (*Cervus*) and Odocoilinae (*Rangifer*), and compared DNA and amino-acid sequences among *Cervus, Rangifer* and *Muntiacus*. We identified the amino-acid substitutions that affect the protein secondary structure of *ATPase8* and *ND3* among them. In *ATPase8*, five amino-acid substitutions that change secondary structure were recognized; the position of substitutions were 34, 39, 40, 48 and 49. Similarly, two amino-acid substitutions were recognized at 45 and 85 in ND3. The secondary structure of *ATPase8* was largely changed by 34His→Tyr and 49Thr→Ile between *Cervus* and *Muntiacus*. Although 45Ala→Gly of *ND3* was also a substitution that largely affects secondary structure, the Yakushima Sika deer that belongs to *Cervus* was 45Gly as well as *Muntiacus* and *Rangifer*, in contrast with the Yeso Sika deer, Honshyu Sika deer and red deer that were 45Ala. From these results, the amino-acid substitution site that affect protein secondary structure in *ATPase8* and *ND3* was predicted, and *ND3* was a unique gene in the Yakushima Sika deer.

E 072

Analysis of genetic variation of mtDNA D-loop regions in Sika deer lived in Nikko National Park for 1996-2003

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The total amount of damage caused by deer to agriculture and forestry has reached huge figures in resent years because of increasing number of Sika deer living in Nikko National Park in Tochigi Prefecture. The ordinance of Tochigi prefecture authority has permitted adjustment of population since 1996. Search of a hereditary feature of Sika deer in Nikko National Park and its neighboring areas has been achieved using base arrangements of mtDNA D-loop regions by authors since 1996. Genetic variation in base sequences of the mtDNA D-loop regions was found in two groups formed in Nikko and Ashio in winters from 1996 to 2003 classified into three times, A:1996-1999, B:2000-2002, and C:2003, in the present study. The nucleotide sequences (about 400bp) of the mtDNA D-loop regions of 80 Sika deer were decided and classified into six haplotypes (named as Nikko 1 to 6), and they were registered in DDBJ (AB089273-AB089278). Incidence of Nikko 1 was 83.3% in A, 92.6% in B, 96.6% in C, and 91.3% in the total, showing tendency toward increase year by year without significant difference. Increase of Nikko 1 with the times suggests that rapid population growth happened in the areas during the times as a maternal bottleneck. However, the two groups were defined as discrete maternal populations because Nikko 4 and 5 were detected only in the Ashio group and Nikko 2, 3, and 6 only in the Nikko group.

E 074

Genetic Characterization of Biodiversity in Chinese Local Chicken Breeds by Microsatellite Markers

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China is rich in chicken genetic resources and there are many local breeds with great phenotypic variation in size, plumage color and other characteristics. Most of them are often maintained in small populations given their medium to low performance in egg production and growth rate. The Chinese Ministry of Agriculture recently initiated a large-scale investigation on the Chinese poultry genetic resources. Seventy-eight local chicken breeds have been surveyed and their blood samples collected. Approximately 3,000 individuals were genotyped for 27 microsatellite markers on 13 chromosomes. Of the 27 microsatellite loci, the number of alleles ranged from 6 to 51 per locus. The average heterozygosity value (H, 0.622) and polymorphism information content (PIC, 0.573) suggests that the Chinese local chickens maintain more genetic diversity than that in many other countries. Most populations studied were generally under Hardy-Weinberg equilibrium at most loci, indicating that these Chinese local breeds are conserved with sufficient population size and limited degree of inbreeding. By using Nei's standard distance and the Neighbor-Joining method, the local Chinese chickens were classified into eight categories that were generally consistent with their geographic distributions.

E 075

Polymorphism of mitochondrial D-loop region of Silkie fowls

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Silkie fowl (*Gallus gallus* var. *domesticus*) is distinct from the other chicken breeds in that the Silkie fowl has silky plumage, purple-colored skin and the bluish meat, purple to dark blue colored wattles, beak and comb, the fifth hind toe pointing slightly upwards, etc. The Silkie is one of twenty-seven Japanese native breeds, which are defined to have habituated in Japan before the beginning of Edo era. Concerning the origin of the Silkie fowl, although it has been considered that the fowl has originated in India and that the breed of Silkie fowl has been established in China and Japan, its original habitat in Asia has not been clarified. In this study, the nucleotide sequence of mitochondrial D-loop region of 16 Silkie fowls was determined. In the mitochondrial D-loop region of the 16 Silkie fowls, 23 sites of single nucleotide polymorphism and one site of single nucleotide insertion were found. Average nucleotide divergence ratio, average rate of transition, and average rate of transversion were 0.006489, 0.006284 and 0.0002, respectively. Phylogenetic analysis revealed that the 16 Silkie fowls were divided into four clusters and that Silkie fowls in a population were divided into different clusters. These results suggest that the genetic divergence of the present Japanese Silkie fowl populations is very high.

E 076

Molecular evidence for hybridization of species in the genus *Gallus*

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Mitochondrial D-loop analysis has provided a phylogenetic tree for fowls in the genus *Gallus* including chicken, that supports the hypothesis based on phenotypic features that Red Junglefowl (RJF) is the direct ancestor of the chicken. The phylogenetic positions of the chicken and the other fowl species in the genus *Gallus* are considered to be of great importance in the maintenance and improvement of chicken breeds through introgression of genetic variations from wild type genomes. However, since the phylogenetic analysis based on the DNA sequences is not sufficient to conclude the phylogenetic positions of the fowls in the genus, in the present study, we have determined the sequences of whole mitochondrial DNA (mtDNA) and nuclear genome [intron 9 of ornithine carbamoyltransferase gene, *OTC*, and 4 chicken repeat 1 (CR1) elements] for the species in the genus. The phylogenetic analyses based on mtDNA sequences revealed that two Grey Junglefowls (GyJF) were clustered in a clade with RJFs and chicken, and that one GyJF was located in a remote position close to Ceylon Junglefowl (CJF). The analyses based on the nuclear sequences revealed that alleles of GyJFs were alternatively clustered with those of CJF and with those of RJFs and chicken. The alternative clustering of RJF and chicken alleles were also observed. These findings taken together strongly indicate that inter-species hybridizations have occurred between GyJF and RJF/chicken and between GyJF and CJF.

E 077

Characterisation of six Asian local chicken breeds: performances and polymorphism from microsatellite markers

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In the early 1980s, National Chung-Hsing University launched a project for conservation of local chicken. The conserved breeds were named according to their geographic origin, Hsin-Yi, Ju-Chi, Hua-Tung, Quemoy from Taiwan area, Shek-Ki from China, and Nagoya from Japan. Most of them have never been selected for production efficiency. The survey was aimed at measuring growth, immunocompetence, egg production traits, heat tolerance and gene diversity of these chickens. Hsin-Yi had the shortest and Hua-Tung had the longest incubation period ($P<0.05$). Nagoya had the heaviest mature body weight and Quemoy had the lightest ($P<0.05$). Quemoy had the highest antibody titer response to both ND and SRBC. Shek-Ki and Quemoy showed the earliest AFE and Nagoya the latest. At 40 wk of age, Quemoy had the highest number of eggs laid and Nagoya had the lowest one. Shek-Ki had a higher frequency of heat panting when the mean chicken house temperature was 34.5°C, varying between 31°C and 36°C. Each of 288 individuals (47-49 per breed) was genotyped for 25 microsatellite loci. All breeds were polymorphic ($P_{0.95}>88$ %) and had mean number of alleles per locus from 2.7 to 3.9. Gene diversity range from 0.42 to 0.57, Shek-Ki had the lowest gene diversity and Hua-Tung the highest one. Hua-Tung had 15 specific alleles which represent 9 % of allelic forms for all loci. Shek-Ki and Nagoya were the least polymorphic compare to breeds from Taiwan area. Factorial analysis of individual multilocus genotype showed that the 6 breeds are well discriminated and the most separated from the others were Shek-Ki and Nagoya.

E 078

Genetic variability and relationships of Japanese native chickens based on microsatellite DNA polymorphisms - Focusing on the breeds established in Kochi Prefecture, Japan

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Blood samples were collected from eight Japanese native breeds (Miyadi-dori, Ohiki, Onaga-dori, Shoukoku, Tosa-Jidori, Tosa-kukin, Toutenkou, and Uzurao) and two foreign breeds (White Leghorn and Rhode Island Red) to investigate the genetic variability and relationships by using microsatellite DNA technique. Excepting the Shoukoku breed, the other Japanese breeds are those established in Kochi Prefecture. Ohiki, Onaga-dori, Tosa-Jidori, Toutenkou, and Uzurao are fancy fowl, and Miyadi-dori and Tosa-kukin are utility fowl. Among the fancy fowl, Ohiki, Onaga-dori, and Toutenkou males have rich and long feathers in the tail and saddle hackle. Genetic variabilities of 20 microsatellites examined varied depending on the breeds, as the mean number of alleles per locus, proportion of polymorphic loci, and the average expected heterozygosity ranged from 2.05 to 3.90, 0.75 to 1.00, and 0.330 to 0.607, respectively. Microsatellite alleles being unique to a particular breed were detected in all breeds. Using the neighbour-joining method, phylogenetic trees were constructed based on genetic distances D_A and D_{ST}. Among the breeds established in Kochi Prefecture, fancy and utility breeds belonged different clusters. Among the fancy breeds, those having rich and long feathers in the tail and saddle hackle showed close relationship to the Shoukoku breed which also has rich and long feathers in the tail and saddle hackle.

E 079

Genetic Relationships among Japanese and Indonesian native breeds of chicken based on microsatellite DNA polymorphisms

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Genetic relationships among Japanese and Indonesian native breeds of chickens were studied on the basis of microsatellite DNA polymorphisms. DNA samples from thirteen Japanese native breeds, six Indonesian native breeds and one imported breed (White Leghorn) were analyzed using a set of microsatellites including twenty ABR, one ADL, one LEI and two MCW markers. These markers were typed by PCR amplification and electrophoresis using a DNA sequencer. Genetic differences among breeds were studied by calculating the D_A distance (Nei 1983). From the distances, a majority-rule consensus tree bases on 1,000 bootstrap replicates was constructed by the neighbor-joining method (Saitou & Nei 1987). The native chicken breeds and White Leghorn were clearly separated from each other. Two big groups that correspond to Japanese native chickens and Indonesian native chickens were recognized. One Japanese breed (Small Game) that is thought to be derived from Malay-type chicken introduced into Japan from Thailand in the 16th or 17th century for cockfighting was in the middle between the Japanese and Indonesian native groups. It is strongly suggested that microsatellite markers in this study are useful for studying the genetic relationships among chicken breeds, since our results reflect geographical and historical background of each breed.

E 080

Phylogenetic analysis of genetic structure of Korean native chickens

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Korean native chicken classified 5 lines by trait of character. The present study was preformed analysis microsatellite markers in the Korean native chicken and the Foreign chicken.
This study used 4 kinds Korean native chicken and 5 kinds foreign chicken. Chickens were genotyped for 7 microsatellite markers. This data was use to phylogenetic analysis, relationship coefficients and genetic distances between individuals. Phylogenetic tree was constructed from microsatellite and pedigree information using the UPGMA method. These results could be useful for substantially of genetic character in the Korean native chicken, and it may by necessary further investigation with well unequivocall assigned the chickens.

E 081

Discrimination of the chicken breeds using AFLP technique

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Amplified fragment length polymorphism (AFLP) technology is technique for fingerprinting genomic DNA, based on the selective PCR amplification of restriction fragments. These fingerprints are used as a tool for determining the identity of a specific DNA sample or to assess the relatedness between samples. In order to establish the method of detecting breed specific polymorphisms simply by PCR, we applied the AFLP technique to nine chicken breeds (White Cornish, Red Cornish, White Plymouth Rock, New Hampshire, Rhode Island Red, Barred Plymouth Rock, Hinaidori, Tosajidori, Tsushimajidori) to search molecular markers able to discriminate each chicken breed. Among the nine breeds, different bands considered to be breed specific were observed. Detection of these bands was confirmed on all individuals of corresponding breeds. Several DNA fragments were obtained. These DNA fragments were isolated, cloned and sequenced.

E 082

Biodiversity in chicken genome of Bangladesh

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The genetic diversity of various local chicken populations derived from different parts of Bangladesh was evaluated using RAPD-PCR technique. The results indicated that gene diversity within a population was large in Bangladeshi indigenous chicken, intermediate in layers and low in broilers. The UPGMA dendrogram based on genetic distance indicated that the native chickens were grouped in one cluster whereas imported exotic chicken formed another cluster. The wide genetic diversity of Bangladeshi indigenous chickens can meet different requirement of breeding for chicken quality in Bangladesh. The contribution of the determination of genetic diversity with genetic markers to the decision on conservation and/or further use of the populations in breeding programs designed to create genetic stocks with improved adaptability and productivity in tropical countries is discussed.

E 083

Gene constitution of four calpain genes using PCR-RFLP of native chickens in Southeast Asia and commercial chickens

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This study aims to analyze gene constitution of native chickens and commercial breeds by using PCR-RFLP of four calpain (mu-calpain, m-calpain, p94, and mu/m-calpain) genes. In 1996 to 2000, 517 blood samples of native chickens in Southeast Asia (Thailand, Laos, Myanmar, and Indonesia) were collected at 16 areas, and 201 samples of commercial breeds (broiler and white leghorn) were used. As a result, polymorphism was detected at all genes. The expected average heterozygosity was estimated as 0.357 – 0.438 in native chickens and 0.509 – 0.538 in commercial breeds. A dendrogram was drawn according to the genetic distance, which was calculated by the Nei's genetic distance matrix. The populations of native chickens and commercial breeds were clustered of one group. This result supported to the result reported by our laboratory using blood protein polymorphism.

E 085

Polymorphism of the tyrosinase gene in chickens carrying the recessive white mutation for plumage colour

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A restriction fragment length polymorphism (RFLP) was observed with 3 different enzymes in all chickens carrying the recessive white mutation at the C locus, by using a total chicken tyrosinase (*TYR*) cDNA probe. This result suggested an insertion/deletion type rearrangement in the *TYR* gene. The purpose of this study was to localize the rearrangement region of the gene and to investigate its consequences on the function of the gene. Several partial cDNA probes were used to localize the rearrangement region, the partial probe designed to cover exon5 identified the diagnostic RFLP band specific to the mutation. This suggested that the structural difference of the *TYR* gene between recessive white mutant and wild-type chickens was located in the 3' terminal region of the gene. Amplification of the 5 exons, either by using genomic DNA or messenger RNA, showed the expected fragment size for all exons in both genotypes. The sequencing results showed that there were no changes in sequence for all exons as compared to the published cDNA sequence for chicken *TYR*. Current results suggest that the rearrangement may take place in intron 4, the sequence of which is not yet fully available on the chicken genome website. A RT-PCR study showed that the *TYR* gene was transcribed in skin samples for both the recessive white and the wild-type chickens, but with an apparently lower intensity in the recessive white genotype. Quantitative PCR studies are undertaken to measure the gene expression level. These results should help to identify the causal mutation in the *TYR* gene, which could explain the recessive white phenotype.

E 086

Evaluation of avian leukosis virus subgroup A (ALVA) and subgroup B (ALVB) Receptor Genes in Recombinant Congenic Strains of Chickens

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To dissect the genetic makeup of complex traits we are developing 19 recombinant congenic strains (RCS) of chickens. Inbred lines 6_3 and 7_2 were crossed, followed by two consecutive backcrosses to line 6_3. Then sib matings were applied within 19 families for 13 generations to form the 6C.7 RCS. Line 6_3 is susceptible to ALV of subgroups A, B, and E whereas line 7_2 is resistant to these viruses, which reduce productivity. To examine genetic resistance to ALV approximately 9 adults from lines 6_3 and 7_2 and each of the RCS were subcutaneously injected with subgroup A Rous sarcoma virus (RSV) in one wing-web and subgroup B RSV in the other wing-web. DNA typing by a SNP assay was developed to assess the *TVB* receptor alleles. All of the chickens in line 6_3 and in 18 of the RCS were homozygous for *TVB* susceptible alleles, i.e., *TVB*S1/*S1*. All of the line 6_3 and 94% of the RCS chickens from the 18 RCS had tumors 12 days after subgroup B injection. In contrast, the line 7_2 and 7 chickens from the RCS 6C.7G were homozygous resistant, i.e., *TVB*R/*R*, and they did not develop tumors. Two additional chickens of the RCS 6C.7G were heterozygous, i.e., *TVB*S1/*R*, and developed tumors as expected. After subgroup A RSV injection all the chickens in the line 6_3 and in 16 RCS had tumors. However, line 7_2 and 22-45% of the chickens in 3 other RCS lacked tumors. A molecular assay is in development to analyze the *TVA* receptor allele in these chickens. We conclude that the *TVA* and *TVB* receptor genes from line 7_2 were randomly assorted in the 19 RCS and remain in 1-3 RCS. These data establish the value of developing the 6C.7 RCS for identifying, evaluating and characterizing genes underlying traits of economic interest.

E 090

Individual Identification of Ducks with 13 Novel Microsatellites

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Ducks (*Anas platyrhynchos*) have been used for meat and egg production in Asia and other countries because of their fast growth rate, high feed efficiency, and good egg production. However, in ducks, genetic improvement has not been sufficient to date. One of promising tools to accelerate genetic selection is the use of Marker Assisted Selection (MAS). We have isolated and characterized microsatellite markers from ducks. In this study, we utilized 13 novel microsatellites to identify individuals. In details, 96 ducks were selected randomly among commercial hybrid ducks, and genotyped with microsatellites. Genomic DNA was prepared from dried blood cells. DNA sequence containing (CA) repeats were amplified by PCR using microsatellite primers. The PCR products were electrophoresed in automated capillary DNA sequencer and analyzed by GeneScan Software version 3.7 to determine the size and to detect polymorphism. Then, the number of alleles was counted manually. Statistical analysis was performed to calculate the allele frequency, the expected heterozygosity, the Polymorphism Information Contents (PIC), and the Power of Discrimination (PD). The number of alleles per locus ranged from 3 to 9. The expected heterozygosity were ranged from 0.430 to 0.769. The PIC were ranged from 0.403 to 0.733. The PD per locus were 0.583 to 0.910. Combined PD with 13 microsatellites was 0.999999 ($1\text{-}3.05 \times 10^{-9}$) in this population. Based on these results, 96 ducks were completely discriminated with 13 microsatellites. In conclusion, 13 novel microsatellites were sufficient to identify individuals of ducks at acceptable accuracy.

E 091

Genetic conservation of Co duck breed for biodiversity and sustainable agricultural ecological in Vietnam

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Animal genetic conservation is an urgent and pressing global problem for biodiversity and sustainable ecological environment. Native Co duck breed (anas platyrhynchos platyrhynchos) traditionally symbiotically related to the rain-feed rice agriculture (as Igamo ducks in Japan). Numbers of Co duck had been reducing quickly from 85% population (1970) to 63% (1999) and 43% (2003). It will be in endanger if there is no plan for conservation. Based on the conservation steps: survey - investigate; collection - conservation; assessment - documentation; develop and use, Co duck breed has been conservating through 9 generations. Characteristics are stabilized such as: pure individuals reach 98%; mature body weight at 17-18 weeks is 1,1 kg (female) and 1.2- 1.3 kg (male); reproduction rate at 90% prolongs 3-4 month sequently; male/female = 1/9 - 1/10; egg production is 225 - 235 egg/female/52 weeks laying; egg weight is 64.4g; fertility is 94.1%; hatchability per total eggs is 85.6%; HU = 87.3 (day 3). Some of correlations between characteristics had been calculated and analysed; most of hematology and cytology criteria are identified and accessed. The conservative Co ducks have been develop-using effectively by duck-rice; duck-fish; duck-rice-fish; duck-rice-fish-fruit trees systems to contribute to the preservation of sustainable agricultural ecological; environmental protection; for food safety (without insecticides) and become a way of poverty alleviation in rural Vietnam, appreciated by some of farmers now.

E 092

Isolation and sequence determination of novel microsatellites from duck (*Anas platyrhynchos*)

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Microsatellites, highly polymorphic short tandem repeats, are distributed throughout most eukaryotic nuclear genomes and have been utilized as DNA markers, for example, in QTL analysis. In chickens, swine, and cattle, a large numbers of microsatellites have been isolated. However, in ducks, only 197 microsatellites have been reported to date. Because of the fast growth rate, high feed efficiency and good egg production, ducks have been used for meat and egg production. In the world poultry meat consumption, ducks are positioned in the second, next to chickens. In this study, we constructed CAG- and AGG-enrichment libraries from duck genomic DNA, picked microsatellite clones, and determined their nucleotide sequences. Genomic DNA was prepared from a female commercial hybrid Pekin Duck and digested with *Sau*3AI. DNA fragments were ligated to a pair of adapters to be used as primers for PCR amplification. Then, PCR products were probed for CAG- or AGG-repeat with $(CAG)_{10}$ or $(AGG)_{10}$ biotin-labeled oligonucleotide. They were retrieved using streptavidin coated magnet beads. Released from streptavidin in alkaline buffer, microsatellites were amplified by PCR and were subcloned into the T-vector. Microsatellites plasmids were introduced in *E. coli*, JM109 by electroporation. In total, more than 200 clones have been picked and sequence determination is in progress. The sequence determination has been completed more than 100 clones and almost all included microsatellites.

E 093

Genetic variability of mitochondrial DNA D-loop sequence in 8 populations of Japanese native horses

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Genetic variability of Japanese native horses was examined in 412bp sequences of mitochondrial DNA D-loop region using 345 blood samples from 8 populations of Japanese native horses. A total of 14 haplotypes was observed (5 in Hokkaido population and Kiso population, 4 in Misaki population, 2 in Taisyu population, Miyako population and Yonaguni population, 1 in Noma population and Tokara population, respectively). Among them, 2 haplotypes were common to 4 populations from Hokkaido (north) to Okinawa Islands (south), one was common to 3 populations from Honshu (central) to Tsushima Islands (west). Haplotype diversity was 0.831 in 8 populations. It was high (0.754) in Kiso population, but low (0.000) in Noma population and Tokara population. Viewed from Nucleotide diversity, Kiso population showed high diversity (2.0%) which was higher than 8 populations (1.6%), whereas Noma population and Tokara population did not show any diversity (0.0%). The haplotypes common to several populations in Japanese native horse were also reported in Thoroughbred horse and Arabian horse. This suggests that these haplotypes have been conserved from the origin of these populations or introduced afterwards by regional trade among the horse-breeding areas.

E 095

Genetic variation analysis of the Korean native horse using microsatellite markers

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The genetic structure of the Korean native horse (n = 41) including Thoroughbred horse (n = 44), Hokkaido native horse (n = 5), Quarter horse (n = 11), Mongolian horse (n = 39) and Cross breed horse(Korean native horse × Thoroughbred, n = 56) were investigated, by means of 13 microsatellite markers (AHT4,5, ASB2,17, CA425, HMS3,6,7, HTG4,10, LEX33, TKY321 and VHL20) proposed by Equine Genetics, International Society for Animal Genetics(ISAG).

Genetic similarities was calculated with 13 microsatellite allele frequencies in comparison of six horse breeds, and were used to estimate expected heterozyosity(He), genetic distance (Ds). This matrix was used to generate dendrogram by UPGMA methods. The observed number of alleles per locus ranged from 7(HMS6) to 16(ASB17). The observed heterozygosity and the expected heterozygosity ranged 0.639 ~ 0.797 and 0.649 ~ 0.804 in the horse breeds, respectively. Genetic variation and relationship of Korean native horse were compared with other horse breeds.

E 096

Studies on microsatellite polymorphism of Cheju native horse and Cheju racing horse

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We have investigated the polymorphism of the microsatellite markers in three horses group (36 Cheju Native Horses, CNH; 40 Cheju Racing Horses, CRH; 40 Thoroughbreds, TB for control) in Jeju. Eleven microsatellite loci (AHT4, AHT5, ASB2, HMS2, HMS3, HMS6, HTG4, HTG6, HTG7, HTG10, and VHL20) were typed, and their allele frequency, heterozygosity, polymorphic information content (PIC) and exclusion probability (PE) were estimated. Alleles observed with high frequency (>0.5) were HTG4 (131 bp), HTG6 (96 bp), HMS6 (164 bp), ASB2 (245 bp), and HTG7 (125 bp) in the CNH group, HTG4 (131 bp) in the CRH group, HTG6 (86 bp), AHT5 (132 bp), HMS6 (166 bp), HMS3 (149 bp), and HMS2 (225 bp) in the TB group. The numbers of alleles for eleven loci were 5 to 10, and the expected heterozygosity and PIC value were 0.364~0.836 (mean 0.699), 0.341~0.801 (mean 0.656) in the CNH group. The numbers of alleles in the CRH group were 5 to 11, and the expected heterozygosity and PIC value were 0.601~0.861 (mean 0.777), 0.550~0.833 (mean 0.735). And the numbers of alleles in the TB group were 4 to 8, and the expected heterozygosity and PIC value were 0.450~0.834 (mean 0.638), 0.405~0.801 (mean 0.580). The combined PE of 11 markers in the CNH, CRH and TB group were 99.95 %, 99.99 % and 99.73 %, respectively. The results showed that the polymorphism of eleven loci in Cheju horses was higher than that in the TB group, suggested that these eleven loci could also be used in parentage test of Cheju horses.

E 097
Genetic diversity of Zemaitukai horse

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A program for the preservation of the genetic resources of different farm animals was developed in Lithuania. The Zemaitukai horse is one of the native Lithuanian horses. In this study wide range of genetic markers (12 microsatellites, 7 blood groups loci, 10 biochemical loci) and mitochondrial DNA (mtDNA) sequencing were used to access genetic diversity in Zemaitukai horses. The mtDNA study was performed on 421 bp of the mitochondrial DNA control region, which is known to be more variable than other sequencies. Samples from each remaining mare family lines of Zemaitukai horses and three random samples for other Lithuanian (Lithuanian Heavy Draught, Zemaitkai heavy type) and ten European horse breeds were selected. Five distinct haplotypes were obtained for five Zemaitukai mares' families supporting the pedigree data. A total of 20 nucleotide differences compared to the reference sequence were found in Lithuanian horse breed. All detected mutations were transitions. Total of 37 haplotypes were found out of 13 breeds investigated. High levels of genetic variation for both genomic and mitochondrial DNA within the Zemaitukai horse breed was observed. Phylogenetic analysis of mtDNA haplotypes and microsatellite data was performed in order to derive genetic relationships between Lithuanian and other horse breeds. Based on genetic distance values, Zemaitukai do not show close relationship to any breed that we tested, except for those of other Lithuanian horse breeds. Zemaitukai represent unique genetic and cultural resource and conservation efforts should be continued.

E 098
Genetic analysis of Thai native horses using microsatellite markers

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A program for the preservation of the genetic resources represented by farm animal species has been developed in Thailand. Samples of 41 Thai native horses were investigated for genetic variation using 11 microsatellites markers (VHL20, HTG4, AHT4, HMS7, ASB2, AHT5, HMS6, HTG10, HMS3, LEX33, ASB17). Amplified PCR products were separated and visualised by an Automated Laser Fluorescent DNA sequencer (ALFexpress Ⅱ DNA Analysis System, Amersham Bioscience, UK). Alle frequencies were calculated and heterozygosities and exclusion probabilities were determined. The number of alles of the markers was varied. The results showed high polymorphic information content. These results can give basic information for developing parentage verification and individual identification system in Thai native horses.

E 099
Genetic diversity in the Polish Heavy Horse and other heavy draft breeds

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After World War Ⅱ, there was a great need for horses for use in field work on small farms in Poland. The draught power of horses also was used for transportation at that time. Although the need for horses in agriculture has decreased since that time, they still have a role on small farms. Currently, "cold-blood" type horses make up about 60% of the total horse population (~400,000 animals) in Poland. In addition to their use for draught power, heavy horses have an important role as slaughter animal for export to western Europe and are an important source on income for small farmers. In this study, we examine genetic variation in the Polish Heavy Horse based upon seven blood group loci, 10 biochemical loci, and 12 microsatellite loci. Genetic variation in the breed is slightly greater tan mean levels for domestic horse breeds. The draft horses form a distinct cluster that pairs with true pony breeds in genetic distance analysis. Within this "cold-blood" group, the Polish Heavy Horse clusters with the Posavina breed from Croatia and the Belgian Draft Horse breed. Patterns of variation within the Polish Heavy Horse breed are typical of those seen for other draft horse breeds.

E 100
Genetic diversity of the Faeroes pony and the relationship to other breeds

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The Faeroes pony is a unique breed of the Faeroes Islands, situated in the North Atlantic. These horses were originally brought to the islands by Celtic and Scandinavian settlers about 200 A.D. The population has recently gone through a severe bottleneck where a few animals founded the present the population. The genetic diversity of the Faeroes pony population was investigated using 12 microsatellites. The results were then compared with 14 other breeds. As expected from the severe bottleneck, there is little allelic variation among Faeroes ponies, When compared to other breeds, the lowest degree of population differentiation was estimated between the Faeroe pony and the Icelandic pony (F_{ST}=0.17). When compared to the other breeds, the F_{ST} estimates ranged from 0.22 - 0.34. Thus, the Faeroes pony appears to be a very unique breed, and the Icelandic pony seems to be the closest relative when studying genetic markers, in these 14 selected breeds.

E 102

Loss of genetic variability through time in the American Standardbred horse

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Genetic variability based upon seven blood group and 10 biochemical loci was examined in 74,833 American Standardbreds and 37,258 American Saddlebred horses born in 1970 through 1996. Variation based upon 12 microsatellite loci was measured in 65,361 Standardbred and 9,660 Saddlebred horses born in 1975 through 2002. Standardbred samples were analyzed separately based upon their racing gait, either Trotting or Pacing. Variation measures were calculated for each year of birth cohort. There was a statistically significant reduction in heterozygosity and effective number of alleles in Pacers and Trotters over the time period but not in Saddlebreds. In Trotters, the change in observed heterozygosity based upon biochemical loci was 15.4% over the total time period. Inbreeding levels in Standardbreds born during this period were calculated from pedigrees. Inbreeding increased in Pacers from 0.05 to 0.07 and in Trotters from 0.10 to 0.11 over this time period, however, inbreeding level alone did not account for all of the loss of variability. In Standardbreds, but not in Saddlebreds, a small proportion of stallions sire the majority of the offspring. This produces a large variance in the male reproductive contribution which results in a reduced effective population size. This appears to be a major contributor to the loss of variation.

E 103

Sarcidano horse gene frequencies in a ten years period

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The aim of this work was to evaluate the genetic evolution of Sarcidano horse (SRH), a small endangered Sardinian horse population of interest for its biodiversity. The original population was typed in 1991-92 (SRH1). Since 1997 this population was divided in to three groups never again crossbred. To investigate the evolution of the genetic structure of the population, 61 subjects taken from two out of these three groups (SRH2, SRH3) were collected ten years later from the first typing. They were analysed either for protein polymorphisms and microsatellite markers in order to evaluate the polymorphism within the population. Allele frequencies, genetic equilibrium according to Hardy-Weinberg law and inbreeding coefficient ($F_{is} \equiv f$) were calculated. The phylogenetic tree using UPGMA algorithm was obtained from the genetic distances calculated using the Nei's distance, comparing the three Sarcidano groups, 8 Italian breeds and the Spanish Pure Breed. The Principal Components Analysis (PCA) on genetic frequencies was performed. The results suggested that the genetic variability was still conserved, as with respect to the 84 alleles found in the original population SRH1, 73 alleles were found in the SRH2 and 85 in the SRH3 groups. Rare alleles primarily identified in the SRH1 population were confirmed in both of the two other groups. The phylogenetic tree and the PCA showed the isolation of the three Sarcidano horse groups with respect to the Italian breeds and the Spanish Pure Breed.

E 106

Screening of sex chromosome aberrations in horses based on the parentage test and the analysis of genetic X- and Y-linked markers

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Sex chromosome aberrations are often associated with clinical symptom that affects equine health and reproduction. However, abnormal manifestation with such abnormalities usually appears in adult and the occurrences of such abnormalities are not always clinically apparent in the newborn. Therefore, an available survey at an early stage is needed even when adverse symptoms cannot be identified. We conducted a screening by combined application of the routine parentage test with 17 microsatellites including a X-linked marker (*LEX003*) and the analysis with additional X- and Y-linked markers. In this study, 17,777 newborn foals, 9,125 males and 8,652 females, of the light breed horses, Thoroughbred and Anglo-Arab, were investigated and 19 cases with sex chromosome aberrations involving 63,XO, 65,XXY and 65,XXX could be found. The XO, XXY(pure 65,XXY and/or mosaics/chimaeras) and XXX were found among 0.15%, 0.05% and 0.01% of the available population, respectively. These incidences are minimum estimates, which would give attention to clinic treatment and breeding for horses. In addition, the parental origin of X chromosome of each disorder could be proved by the present results, which could also provide available information to cytogenetic characterization. This screening method must be useful for not only general diagnosis in equine clinic but also cytogenetic study.

E 108

Allelic Variation of *DRD4, 5HTR1A* and *AR* in Asian Dogs

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We previously examined the dopamine receptor D4 gene (*DRD4*) polymorphic regions that is associated with canine behavioral trait in Japanese native breeds (7 breeds; *n*=374) and found that Japanese Chin is distinct from the other breeds. In this study, polymorphic regions of three genes, *DRD4*, serotonin receptor 1A (*5HTR1A*), and androgen receptor (*AR*) were analyzed for a total of 1,050 Asian dogs originated from Japan (7 breeds and 3 populations; *n*=706), Korea (3 populations; *n*=96), China (5 breeds; *n*=78), Taiwan (*n*=21), Mongol (*n*=32), Thailand and Laos (*n*=32), Indonesia (3 populations; *n*=65), Sri Lanka (*n*=20), 501 European dogs (36 breeds), and 32 wolves (2 subspecies). Two alleles were observed in *DRD4*exon1, *DRD4*intron2, and *5HTR1A*. In *DRD4*exon3, *AR*dog1 and *AR*dog2, 8, 3 and 6 alleles were observed, respectively. Wolves shared common alleles with dogs except allele 27 of *AR*dog2 (22-27 repeats of glutamine). In a neighbor joining tree based on the allele frequencies of 3 regions of *DRD4*, breeds, populations and subspecies were divided into three groups, each including Asian dogs, European dogs and wolves. In the *5HTR1A* (A808C), most dogs had the *C* allele at high frequency, while the *A* allele was predominant in wolves. In the *AR*dog2, Korean and Indonesian populations showed a relatively high frequency of alleles *22* and *25*, respectively.

E 109
Microsatellite polymorphism in Japanese mongrel dogs

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Genetic variability of 178 unrelated Japanese mongrel dogs was studied by collecting blood cards from various areas of Japan, Hokkaido to Okinawa. The 10 microsatellite loci were chosen from different chromosomes, including 4 loci (AHT121, AHTk253, PEZ03, PEZ12) adopted by the ISAG Canine Genetic Workshop. Multiplex PCR products were analyzed by PRISM 310 Genetic Analyzer with GeneScan software (Applied Biosystems). Hardy-Weinberg equilibrium was confirmed in 9 loci and in one locus (Ren277K09) after combining a few rare adjoining alleles to suppress the influence of rare genotypes. The power of discrimination (PD) of loci, Ren37A11, Ren48E01, Ren277K09, ZBC30, Ren42N13, AHTk253, PEZ12, AHT130, PEZ03, AHT121 was 0.79, 0.84, 0.88, 0.88, 0.89, 0.90, 0.96, 0.96, 0.96, 0.97, and the polymorphic information content (PIC) was 0.61, 0.66, 0.68, 0.71, 0.74, 0.72, 0.83, 0.84, 0.84, 0.86, respectively. The combined probability of exclusion (PE) was 0.99990, indicating that these microsatellite markers are useful for paternity testing of dogs.

E 110
Genetic structure of Portuguese autochthonous dog breeds

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Recent applications of Bayesian inference on population genetics have considerably changed our ability to detect cryptic population structure in both wild and domestic species. In this work we used a set of 10 microsatellites to characterize Portuguese dog breeds. Genetic diversity values were found to be moderate to high when compared to other dog breeds, while the observed FST (≈ 0.10) was lower than those reported in recent publications. The assignment of individual dogs to their breeds of origin varied widely and was above 90% only for three out of nine breeds analysed in this study. A subset of four breeds consisting of cattle dogs was additionally characterized with nine more microssatellites and compared with a sample of Portuguese wolves. While the results suggest the absence of hybridisation between the wolf and the dog, the gain in clustering power with the additional set of microsatellites did not result in unambiguous breed identification. Our results indicate that different patterns of historical gene flow between breeds may explain the limited success obtained with the application of Bayesian inference methods to Portuguese dogs.

E 111
Evaluation of 13 microsatellite markers for canine parentage verification in Japan

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We are currently undertaking canine parentage verification using microsatellite markers in Japan. For the purpose, PIC, probability of exclusion (PE) and combined probability of exclusion (CPE) of eleven microsatellite markers chosen from ISAG panels and additional two markers were calculated in 3,939 dogs from 3 popular breeds in Japan, Miniature Dachshund (DHM), Chihuahua (CI) and Toy Poodle (PT).

Locus	DHM, n=1,522		CI, n=1,421		PT, n=996	
	PIC	PE	PIC	PE	PIC	PE
AHT121	0.7965	0.6599	0.7794	0.6337	0.8393	0.7115
AHTh171	0.7100	0.5308	0.7926	0.6422	0.8455	0.7196
AHTk211	0.5207	0.3190	0.6016	0.4012	0.5977	0.3986
AHTk253	0.6109	0.4049	0.5951	0.3875	0.6616	0.4773
C22.279	0.5906	0.3926	0.7427	0.5632	0.6272	0.4216
FH2001	0.7666	0.6002	0.8193	0.6847	0.6684	0.4766
FH2054	0.8106	0.6653	0.7819	0.6249	0.7679	0.6119
FH2289	0.8544	0.7363	0.8476	0.7292	0.8553	0.7403
FH2326	0.7806	0.6248	0.8501	0.7304	0.8167	0.6784
FH2328	0.7278	0.5526	0.8242	0.6850	0.7098	0.5328
FH2611	0.8459	0.7224	0.8158	0.6822	0.8737	0.7682
INU042	0.7452	0.5706	0.8606	0.7432	0.7660	0.6025
INU055	0.6846	0.4932	0.4956	0.3284	0.7517	0.5867
CPE	0.999986		0.999997		0.999996	

These data demonstrated that the set of the 13 markers was highly efficient for the routine parentage verification for the dogs in Japan.

E 112
Routine parentage control in Berger Blanc Suisse dogs

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In 1996 the breed club for Berger Blanc Suisse dogs established a mandatory routine parentage verification program for all their animals. A Blood sample of each dog has to be deposited at our Institute. We currently use microsatellite genotypes (ten selected microsatellite markers) to verify the relationships of the dogs.
During the process of selecting microsatellite markers for this purpose, we identified several markers which displayed high mutation rates in this population, and therefore had to be replaced. As some of these microsatellite markers (e.g. FH2247) are still included in the international panel for the standard dog parentage test, it will be important to compare our result with the findings of other laboratories. If similar high mutation rates of these markers are present in other dog populations the composition of the international panel would have to be reviewed.

E 113

RAPD analysis of genomic DNA of Asian Elephants (*Elephas maximus indicus*) using OPG series of Primers

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The molecular genetic structure of Asian elephant (*Elephas maximus indicus*) of Assam, India was studied in a herd of elephants rescued from different areas of hills, north of Assam in the Himalayan range and then domesticated and used as work animals keeping in captivity. The study was made for tracing their phylogeographic origin and genetic structure by DNA analysis. Genomic DNA was isolated from whole blood of the elephants of either sex by Wizard Genomic DNA purification Kit. Purity was checked by Agarose Gel electrophoresis. For RAPD-PCR amplification, 5 number of 10-mer primers from OPG series according to the manufacturer's recommendation were used. The random primers used for polymerase chain reaction typing was obtained from Operon Technologies Inc., USA. Amplification reactions were carried out in a final volume of 25 ml using the primers OPG1 to OPG5.

Four of the five primers used in the study revealed unique pattern of amplified fragments with all the blood samples. This indicated that the samples obtained from the individuals were clonally same and they may be from the same origin. However, Primer OPG 1 could differentiate the individuals showing two distinct patterns of amplifications for the male and female animals. Primer OPG5 gave better resolution of bands and genomic DNA of the Asian elephants were recorded and studied by using the same.

E 114

Phylogenetic relationship of wild musk shrew (*Suncus murinus*) populations in Asia based on morphological, mitochondrial DNA and protein variation

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A musk shrew is a small mammalian species belonging to Insectivora and has been domesticated as a new experimental animal. This animal is widely distributed in tropical and subtropical areas in South and Southeast Asia. Surprisingly, it's body size, coat color and chromosome number vary largely according to locality, but this animal has been classified only as one species, *Suncus murinus*. Previously, the wild musk shrew populations in Asia were reported to be classified into 3 groups based on mitochondrial DNA (mtDNA) variation and inter-subspecific level of differentiation was found among the groups. In this study, we investigated the morphological and mtDNA variation of wild shrew populations in Myanmar and Cambodia where are surrounded by the 3 groups. The result was that Cambodian populations fell into the Islands' group, but the populations in Myanmar were divided into 2 different groups according to locality (North and West Myanmar and South Myanmar). From the result, musk shrew populations were globally classified into 2 main groups (Continental group in South Asia and Islands' and Southeast Asian group) and 2 unique groups (Malay and Myanmar). To more clarify the relationship among these groups, we proceed to investigate protein polymorphism as a bi-parental marker in addition to mtDNA as a maternal marker.

E 116

The imminent extinction of the Australian dingo

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Is the dingo destined to extinction in the wild? Recent contact between European domestic dogs and dingoes has lead to a large amount of hybridisation. Dogs and hybrids are gradually replacing the dingo. The hybrids have a tendency to be larger and breed more often than dingoes, thus increasing pressure on other endangered native species. These wild dog populations are an agricultural pest, and managing wild dog populations while trying to conserve the dingo is a fine balancing act for wildlife managers. Because the dingo is so closely related to the dog, there are few practical solutions to save the dingo in the wild.

To examine the extent of hybridisation, 20 microsatellites with different allele profiles in dogs and dingoes have been typed in 1500 wild canids from across Australia. In most areas only 10 to 15% of animals show NO evidence of European dog ancestry. The Fraser Island population is the only east coast population tested with very low levels of dog introgression.

E 117

Microsatellite Marker Panel for Puma (*Puma concolor*): Development and Applications for Ecology and Forensics

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Polymorphic microsatellite DNA markers (commonly called Short Tandem Repeats, STRs, in forensic literature) have become important tools of molecular ecology and wildlife forensics by providing accurate means of identifying individuals and characterizing the genetic structure and variation of wildlife populations. Microsatellite markers are particularly useful for studying threatened wildlife species such as puma (*Puma concolor*, also called mountain lion and cougar), which are highly secretive and dangerous to handle. Accurate census and genotype data are needed to properly manage and conserve this protected species. An accurate method of identification is also essential in cases of livestock predation and public safety incidents, as well as cases of poaching and illegal capture. Employing markers originally developed for domestic cats (*Felis catus*) by the Laboratory of Genetic Diversity, National Cancer Institute, Frederick, MD, we have developed a panel composed of polymorphic microsatellite markers to differentiate individual pumas and provide parentage, kinship, and population structure information. The panel differentiates samples originating from puma from other felids, and provides discriminatory forensic match probability (i.e., likelihood that identical DNA genotypes would be drawn at random a second time from the population).

E 118

Space use and foraging strategy of pine marten (*Martes martes*) and stone marten (*Martes foina*) identified by genotyping of DNA isolated from faeces

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Two species of martens, *Martes martes* and *Martes foina*, occur sympatrically in Central and Eastern Europe. The knowledge on the differences between ecological niches of the two species is very limited, because martens are difficult to observe and because it is impossible to distinguish between the scats of both species on the base of their morphological features. To resolve this problem, we used DNA extracted from faeces for species identification. We analysed two microsatellite markers: Mel10, described in the literature as a marker that allows for genetic distinction of marten species, and Ma18, originally developed in *Martes americana*. On the basis of 33 tissue samples: 16 of pine marten and 17 of stone marten, we showed that the marker Ma18 also allows to differentiate the two European species of martens. The size range of alleles is 150-152 bp in pine marten and 178-198 bp in stone marten. The simultaneous application of these two markers allows to obtain reliable results. For the identification of individuals we analysed eight microsatellite markers: Gg7, Gg14, Gg454, Ma1, Ma2, Ma18, Ma19 and Lut604, which were polymorphic in pine and stone marten (in total, 43 and 34 alleles respectively). The possibility of assigning the faeces to particular individuals let us to evaluate of the diet, to determine the home ranges and to estimate the density of both marten species in different environment variants.

E 120

Parentage testing and linkage detection in crocodiles

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To ensure that correct pedigree information was available for a genetic improvement program for saltwater crocodiles, a parentage determination kit was assembled. The kit consisted of fourteen microsatellite loci and the number of alleles ranged from 2 to 16, with observed heterozygosities ranging from 0.219 to 0.875. The cumulative exclusion probability for all fourteen loci was 0.9988. The eleven loci that showed the greatest level of polymorphism were used for parentage testing with an exclusion probability of 0.9980. Using these eleven markers on 107 juveniles from 16 known-breeding pairs, a 5.6% pedigree error rate was detected. This level of pedigree error, if consistent, could have an impact on the accuracy of genetic parameter and breeding value estimation. In addition, these markers were found to be useful for assigning parentage in situations where maternity and/or paternity may not be known, thus further extending the usefulness of a genetic improvement program. The DNA samples available are a valuable resource for future construction of a framework marker map which will enable identification of QTLs for economically-important traits in crocodiles.

E 121

Natural clones of the loach *Misgurnus anguillicaudatus* (Teleostei: Cobitidae) identified by DNA markers

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Our previous studies reported that natural triploid loaches frequently occurred in northern area of Hokkaido island and these triploids arose from the accidental incorporation of sperm genome into unreduced diploid eggs of the diploid clone, which normally reproducing gynogenetically. The clonal lineage was verified by experimental crosses as well as molecular genetic analyses. However, presence or absence of different types of clonal lineage other than "clone 1" has not been examined yet.
In the present study, diploid loaches which had mitochondrial DNA haplotype identical to common haplotype of natural triploids were screened as a candidate of putative clonal individual among specimens collected from 23 localities in Japan by analyzing RFLPs of PCR product (about 1600bp) in the control region of mtDNA, because triploid individuals should appear from eggs of clonal diploids and have same maternal origin. Clonal nature of these candidates was then identified by microsatellite genotyping in several loci, RAPD analyses using highly useful 5 random primers and DNA fingerprinting using $(GGAT)_{13}$ probe.
Different type of clonal diploid (clone 2) was discovered in specimens from the other place of Hokkaido island. Two types of clonal lineage which were different from clones 1 and 2 were also discovered in Ishikawa prefecture, Honshu (main) island, Japan.
We sequenced and analyzed 950bp of the control region of mtDNA in 119 individuals and found that Japanese loaches could be categorized into two groups A and B. The group A has been detected in only northern Hokkaido and Ishikawa pref., whereas the group B has distributed all over Japan. Clonal lineages occurred in places including loaches of both group A and B.

E 122

Microsatellite DNA markers as a genetic tag in the stock enhancement program of the rare species Barfin flounder

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Barfin flounder fisheries situation has been declared to be threatened, as the amount of wild individuals has been drastically reduced since 1975. The National Centre for Stock Enhancement in Japan (Akkeshi-Hokkaido) started the stock enhancement program of this species in 1987. In the present study, one hundred fifty individuals, captured at Akkeshi and Kushiro (Hokkaido) in 2003, were examined in order to elucidate whether they were hatchery produced or wild specimens. Microsatellite loci were used to establish the origin of the captured individuals, matching or not the genotypes of the captured samples with the genotypes of the parentals that were used to produce the released juveniles in Akkeshi-station. Besides, hatchery-progeny that was produced in Akkeshi station in 2001, and posteriorly released in Akkeshi bay, were monitored before the release in order to examine their pedigree. Family survivability and growth rate by family were estimated after determining the pedigree of the released and recaptured stock. Microsatellite markers successfully identified the pedigree of the hatchery stock and the origin of the captured stock. All captured individuals were apparently of hatchery origin. Nearly all families present in the released juveniles were found in the recaptured samples. Distinct difference of growth rate among families was not observed.

E 123

Domestic animal diversity studies – the global perspective

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About ten years ago ISAG and FAO proposed the MoDAD (Measurement of Domestic Animal Diversity) recommendations as standards for genetic diversity studies. In 2003 a survey was conducted, to verify the degree of familiarity and acceptance of these recommendations in practice. Information on 87 domestic animal diversity studies covering breeds from 13 mammalian and avian species was obtained. Breeds from 40 % of all countries have already been subject in diversity studies, with a certain concentration on Europe. The size of projects varied between one and 120 breeds originating from up to 33 countries. In 95 % of all projects it was aimed to fulfil the minimum requirement of sampling 25 animals per breed. In 95 % of all projects blood samples were taken, in 90 % microsatellite markers were used. However, in only 23 % of all projects all markers were taken from the recommended marker list. The degree of acceptance of the recommendations was highest in pigs and poor in chicken. The main reasons for not using recommended markers were: preference of already available markers, higher suitability for multiplexing, and cross species amplification. Only 7 % of the researchers were not aware of the existing recommendations. Researchers favour Nei's standard genetic distance, and PHYLIP and DISPAN are the most widely used software packages. The outcome of this survey is taken as a starting point for the development of improved MoDAD recommendations.

E 124

Choice of conservation strategy and optimum allocation of resources in livestock conservation schemes

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First steps were taken to assess the worldwide biodiversity, but there still is a lack of appropriate methodology for the efficient management and allocation of limited conservation funds. This study aimed at defining a methodology for assessing the optimum number of breeds to be included in conservation programs and the choice of the most efficient conservation program for each of these breeds. In a second step, shares of the total funds were allocated optimally to the breeds chosen for conservation in order to get a maximum of expected future diversity. The methodology was illustrated with data on 23 African cattle breeds.

Marker information was used to calculate the present and expected future diversity with the Weitzman approach. For each breed, cost of four different conservation programs and their effects on the breed's extinction probability were estimated: herdbook and promotion of the breed (HB), in situ conservation (IS), cryoconservation of semen (CC) and IS and CC combined (IC). With a newly developed iterative algorithm an assumed total budget of US$ 2'000'000 was allocated to the breeds.

CC, HB and IS proved to be more efficient than IC. With the optimized allocation of funds to eight breeds, 64% of the present diversity could be conserved. This is an increase of 13% compared to the scenario of no action being taken.

E 125

Optimum selection and mating system for purging inbreeding depression and maintaining genetic diversity in conserved animal populations

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Although the primal goal of conservation of animal populations is to maintain the genetic diversity, the purging of inbreeding depression due to deleterious recessive genes will also be an important aspect for the future use of the populations. These two objectives, however, may involve trade-off, because a higher rate of inbreeding increases potential opportunities for purging deleterious recessive genes through the increased homozygosity, but accelerates the decreasing rate of genetic diversity. In the present study, computer simulations were carried out to assess the optimum selection and mating system to solve the trade-off. Combinations of two selection methods ('random' and 'minimum coancestry' selection) and three mating methods ('random', 'minimum coancestry' and 'maximum coancestry' mating) were compared under two genetic models with different mutational parameters for deleterious recessive genes affecting viability. Population with 40 breeding animals was maintained over 20 generations. The results generally showed that the combination of minimum coancestry selection and maximum coancestry mating could purge efficiently the inbreeding depression with a relatively small loss of genetic diversity. A drawback of this system, i.e., a higher rate of inbreeding, could be largely solved by switching the mating method to minimum coancestry mating at the final generation of conservation.

E 126

Genetic management of the subdivided structure in domestic animal populations

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For the genetic improvement toward high aggregated performance and the maintenance of the genetic diversity in domestic animal populations, population subdivision into several breeding herds would be one of the most efficient strategies. The success of this strategy will critically depend on three problems, (1) the restriction of inbreeding, (2) the maintenance of genetic diversity in the whole population, and (3) the maintenance of genetic diversity among the herds. In this study, numerical computations with the finite island model were carried out to optimize the subdivided structure of breeding populations in terms of the number and size of breeding herds and the migration rate among herds. We assumed a situation, where 4 or 5 breeding herds were constructed each from the five traditional subpopulations in the population of Japanese Black cattle. It was suggested that about 5 males and 200 females would be required for solving the problems (1) and (2), and the migration rate of males should be restricted to below 0.1 to balance the problems (1) and (3). The effective migration rate of males in the actual population (the migration rate in the finite island model, which gives the rate of inbreeding observed in the actual population) was estimated to be 0.24, indicating that under the current migration pattern, the genetic diversity among the subpopulations would be rapidly decayed.

E 127

Forensic Identification of Wildlife for Conservation and Enforcement

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The use of genetic markers for identification of individuals, populations and species is a critical analytical capability that supports enforcement of national and international wildlife laws and treaties. Often the only material remaining from which to discern population or species origin, or with which to compare to a crime scene is a processed item, gut pile or trace blood stain, and morphological discrimination is impossible. The Genetics Section of the U.S. Fish and Wildlife Service's National Fish and Wildlife Forensic Laboratory is uniquely poised to address such law enforcement concerns for a variety of animals of commercial and sport interest. We have used mtDNA sequence and 8 to 12 robust simple tandem repeat (STR) loci and Y-chromosome markers in more than 350 cases for population assignment of North American Black bear, Gray wolves and White sturgeon and for individualization of wolves, bear, moose, elk, white-tailed deer, mule deer and eagles. Reference databases for canid, ursid and cervid analyses are comprised of representatives from throughout the species' geographical distributions in the U.S. Average heterozygosity (0.60 – 0.85) and number of loci used in forensic identifications are sufficient to support conservative probability of identity (PID) estimates of one in more than ten thousand individuals.

E 130

New MoDAD marker sets to be used in diversity studies for the major farm animal species: recommendations of a joint ISAG/FAO working group

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About ten years ago, MoDAD (Measurement of Domestic Animal Diversity) recommendations of markers to be used in farm animal diversity studies were released by a joint ISAG and FAO working group. The suggested marker lists have now been revised and substantially extended. The overall aim of the MoDAD initiative is to promote the use of standardized sampling and experimental designs and common marker sets in farm animal diversity studies. Lists of 30 microsatellite markers were compiled for each of the farm animal species: cattle, sheep, goat, yak, buffalo, horse, ass, pig, camelids, chicken, and duck. This compilation reflects development of and practical experience with marker sets in numerous large studies conducted by research consortia in the different species. The lists take into account technical features, e.g. degree of polymorphism, good readability and suitability for multiplexing or multi-species studies, as well as pragmatic aspects like widespread use in past or present major farm animal diversity studies. The revised marker lists will be made available on the internet. The URL http://dad.fao.org/en/refer/library/ guidelin/marker.pdf gives information on marker names, links to mapping information and to Genbank, primer sequences, annealing temperatures, range of allele sizes, and references to studies in which the respective marker has been used. Where available, information on multiplexing is added. The suggested markers are arranged in a ranked list: if in a diversity study only a subset of N < 30 markers are to be used, the top N markers from the list should be selected. A maximum degree of acceptance of the recommendations and suggested marker sets will allow researchers and FAO the development of a more complete picture of farm animal genetic diversity by combining results of separate but overlapping studies.

E 131

SNPs and SSR as markers for biodiversity study

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In a recently completed biodiversity project (AVIANDIV), genetic diversity was studied on a subset of ten populations. Sequencing of 6952 bp from 15 genomic DNA fragments in non-coding regions yielded 145 SNPs. In a follow-up project the same individuals were genotyped at 29 microsatellite loci and 214 different alleles were observed. Based on the genotypes information at SNP and microsatellite loci, the 100 individuals were analyzed for clustering into 10 groups using the computer program STRUCTURE. We examined two measures of clustering success. (a) Percentage of individuals that were correctly assigned to their populations based solely on their genotypes information; 99% using microsatellite data, as compared to 86% using SNP data (not significantly different) (b) Average genome uniqueness of a population; microsatellites (94,1%) was significantly higher than that of SNPs (72,4%). As a case study, sequence polymorphism was analysed in a gene coding a flavin-containing monooxygenase 3 (FMO3). Using the same set of 100 individuals, we sequenced 703 bp exon and 195 bp intron. We identified 19 SNPs; 13 within the exon, 7 within the intron regions but only one changed the amino acid sequence.

SECTION F

Association between Markers and Traits

F 001

Behavior genetics: a search for temperament associated genes in horses

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Domestic horses are unique in that we tend to place more emphasis on their temperament than that of other livestock species. During the last decade considerable progress has been made in understanding the genetic background of behavior traits. This study aims to elucidate the genetic basis of equine temperament. In the present study, we used two groups of 2 years old thoroughbred horses (69 in 2002 and 67 in 2003) that were kept in the Hidaka Training Farm of the Japan Racing Association. In order to assess the behavior traits of individual horses, we carried out a questionnaire survey of the caretakers (3 persons for each horse). Some behavior tests were also conducted on the 2003 group. The temperamental scores for each horse assessed by the two approaches were relatively consistent, suggesting that the professional caretakers' assessment could be used as a reliable measure of equine temperament. Factor analysis was carried out on the questionnaire data and several behavior traits were extracted. In parallel with temperamental assessment, blood samples were obtained for genome DNA analyses. As candidate genes, four neurotransmitter-related genes were chosen, namely serotonin transporter (5HTT), catechol-O-methyltransferase (COMT), dopamine D4 receptor (DRD4) and monoamine oxidase A (MAOA) genes. DNA sequence of each gene was determined based on available information for other species, and polymorphisms in the translated region of each gene were identified using the brain cDNAs obtained from genetically unrelated nine horses. We found one SNP causing amino acid substitution in each of the 5HTT, COMT and DRD4 genes, but not the MAOA gene. Analysis of the association between these SNPs and temperamental scores is still underway, but an interesting correlation of the genotype of DRD4 with the temperamental trait 'Curiosity' has been found and is consistent in the two separate horse groups.

F 002

Linkage mapping of susceptive loci for recurrent exertional rhabdomyolysis (RER) in Thoroughbred

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Tying-up, the common muscle problem in horses, has also been called azoturia, or paralytic myoglobinuria. In some breeds, i.e. heavy draught, the causative mutations in genes responsible to carbohydrate storage and metabolism have been identified. In Thoroughbred racehorses, recurrent exertional rhabdomyolysis (RER) is another typical form of tying-up, and more than seven per cent of racing Thoroughbreds, especially young, nervous fillies, are affected. Since clinicians commonly experience familial occurrences of RER, it may involve a genetic predisposition. In 2001, we collected random blood samples from 3500 racehorses at an annual medical checkup in the racehorse clinics of JRA's two training centers. Out of the total 6543 JRA registered racehorses (at the time of sampling), 502 individuals (7.67%) were diagnosed as paralytic myoglobinuria or paralytic myoglobinemia during the years from 2000 to 2003. We had selected 10 male-driven half-sib families to analyze their linkage between RER and the 131 polymorphic microsatellite markers on the linkage map from the International Equine Gene Mapping Workshop. DNA typing with microsatellite markers were performed only in affected individuals (n=72) and their unaffected siblings (n=406), and analyzed by Bayesian Markov Chain Monte Carlo (MCMC) linkage package LOKI together with their pedigree data. Finally, LOKI showed two candidate loci on ECA4 and ECA12 from a single stallion family, but no any significant linkage were shown in the other 9 stallion families.

F 003

Prospects for whole genome linkage disequilibrium mapping in thoroughbreds

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Linkage disequilibrium (LD) mapping is often used in searches for genes governing economically significant traits and diseases. The D' coefficient is a commonly used measure of the extent of LD between all possible pairs of alleles at two markers. This study aimed to test the utility of the D' coefficient for LD mapping of a trait in a thoroughbred population. Microsatellite genotype data and grey coat colour as a trait model in a thoroughbred population were used to assess the extent of LD. We demonstrated that LD mapping was a reasonable approach for initial genome-wide scans in a thoroughbred population. Significant LD was demonstrated at approximately 7 cM, implying that roughly 430 appropriately spaced microsatellites were needed for systematic whole-genome LD mapping in this model. LD mapping methods using D' in a thoroughbred population were useful for identifying the chromosomal regions for diseases and economic trait loci. It was suggested that a thoroughbred population represented a population particularly suitable for LD mapping.

F 004

HEXA – A locus affecting predisposition for BSE in cattle?

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Bovine Spongioform Encephalopathy (BSE) continues to be a concern in Europe, especially in protecting the consumer against prion-contaminated meat. To date, a causal mutation influencing genetic susceptibility to BSE has not been identified in cattle. Literature analysis for candidate genes indicated that the lysosomal enzyme Hexosaminidase A (*HEXA*) might affect the development of the disease: *HEXA* shows increased expression in PrP^{sc}-infected brains of mice and is located both close to a marker on BTA 10 that is associated with BSE and within a homologous prion incubation time QTL in mice. We tested the candidate status of *HEXA* by genotyping SNPs to derive haplotypes spanning the gene and neighbouring region in both BSE-affected UK animals (n= 350) and a control group of halfsibs (n= 270). We used the genotype information to perform an association study and found significant discrepancies in the allele frequencies for one loci. In the BSE-affected group, we detected a significantly lower frequency of the T-allele (p= 0.0003) in an intronic G/T polymorphism. For four SNPs, we found four different haplo-types, one of which was significantly underrepresented in the BSE cases (p= 0.0358). A genomic control consisting of genotypes of 35 randomly chosen SNPs excluded the possibility that the observed associations between each marker and BSE susceptibility arose from the structure of population. However, the causal mutation underlying the associations has not yet been identified, such that further analysis of sequence variation in *HEXA* and neighbouring region is required.

F 006

Relationship between genetic variants of mitochondrial DNA and growth traits in Hanwoo

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Genetic variants of Hanwoo mt DNA in the region of cytochrome oxidase subunit I, Ⅱ, and Ⅲ complex were detected using restriction enzymes. PCR primers were designed based on the bovine mt DNA sequence, and 6 primer sets (Mt4, Mt5, Mt6, Mt7, Mt8, and Mt9) were used. A total of 20 restriction enzymes were used, and 6 restriction enzymes, which were Pst I, Pvu Ⅱ, Rsa I, Eco RI, Bgl Ⅱ, and Msp I, showed genetic polymorphisms. Significant associations between genetic variants and weight traits were observed at WT15 ($P<0.05$) and WT18 ($P<0.01$) with Pvu Ⅱ restriction enzyme for Mt9 segments in the region of cytochrome oxidase subunit Ⅲ complex. No significant associations were observed at Mt9-Pvu Ⅱ, Mt6-Bgl Ⅱ, and Mt8-Rsa I segments for WT9 ($P=0.07$), WT15 ($P=0.06$) and WT15 ($P=0.06$), respectively. These results suggest that genetic variants of mt DNA in the region of cytochrome oxidase subunit Ⅲ complex may be candidate segments for improvement of animal growth as weight traits.

F 007

Effect of sequence variation in mitochondrial DNA D-loop region on economic traits for Hanwoo and Holstein

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This study was performed to analyse the sequences of variations of mtDNA D-loop and their effects on economic traits in Hawoo (Korean cattle) and Holstein. The resulting sequences were compared with sequences previously published for other cattle breeds (GenBank J01394). The PCR was used to amplify a total of 964 bp between nucleotide 15758 and 383 within the D-loop region of mtDNA using specific primers. Group 1 (Hanwoo progeny testing bulls) showed 25 polymorphic sites by nucleotide substitutions. Group 2 (94 Hanwoo) showed 27 polymorphic sites by nucleotide substitutions. Group 3 (Holstein) showed 37 polymorphic sites by nucleotide substitutions. High levels of sequence polymorphism frequencies were detect at 169, 16042, 16093, 16119, 16255 and 16302 nt. The substitution effect at 169 and 16119 nt was found significant ($p<0.05$, $p<0.01$) on marbling score in herd of Hanwoo progeny testing bulls. Also substitution effect at 169 and 16042 nt was highly significant ($p<0.01$) on backfat, thickness. The substitution effect at 106, 16185 and 16119 nt was found significant ($p<0.05$, $p<0.01$) on milk in herd of 94 Hanwoo. The substitution effect at 169 nt was found significant ($p<0.05$) on milk in herd of Holstein. Polymorphism of mtDNA sequence in the D-loop region can be useful for the analysis of cytoplasmic genetic variation and associations with the other economically important traits and maternal lineage analysis in Hanwoo and Holstein.

F 008

FEZL affects somatic cell count through SEMA5A in Holstein-Friesian cattle

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Mastitis is the most frequent and costly disease in Holstein-Friesian cattle. Since a relatively strong genetic correlation between somatic cell count (SCC) and mastitis has been reported, we used SCC as a phenotype parameter for linkage analysis to find genes responsible for mastitis resistance. We collected DNA from 478 daughters of 6 half-sib families having the same grandfather, including 181 daughters whose average SCC during their first lactation period was more than 200,000 per ml and 297 daughters less than 25,000 per ml. A whole-genome scan showed significant linkage of a SCC locus to a 7.7-cM interval in BTA22, equivalent to HSA3p14.2, with LOD score of 20.24. The critical region harbors 6 known genes without any single nucleotide polymorphism changing amino acids except for *forebrain embryonic zinc finger-like* (*FEZL*). FEZL contains a C2H2 type zinc finger domain and a glycine stretch, where high SCC daughters tend to have 13 glycines while low SCC daughters 12 ($p = 9.48E-05$). *FEZL* expressed in brain, kidney and mammary gland. Transfection of the two variant cDNA accumulated FEZL protein in the nucleus of COS7 and OCUB-M (a human breast cancer cell line) cells. Chromatin immunoprecipitation assay revealed FEZL binds to *Semaphorin 5A* (*SEMA5A*). FEZL with 12-glycine stretch activated *SEMA5A* more than FEZL with 13-glycine based on luciferase assay ($p = 0.0036$). Since SEMA4A, one of the Semaphorin family, enhances T-cell activation, impaired immune responses by lower transcription activity of FEZL may underlie the pathogenesis of mastitis.

F 009

Detection and mapping of an AFLP marker for clinical mastitis in Canadian Holsteins

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Mastitis is the most frequent and complex disease and estimated to cost dairy farmers approximately $200 per cow annually. In the present study, a total of 200 cows, including 100 resistant and 100 susceptible to mastitis, were selected from a population of 3314 Canadian Holsteins on the basis of clinical mastitis occurrence. Genome-wide QTL screening was carried out using selective DNA pooling and amplified fragment length polymorphism (AFLP) techniques. Using 89 selective primer pair combinations, 27 significant AFLP markers were found (FDR < 0.05). The most promising AFLP fragment of 155 bp where fluorescence intensity was significantly higher in the mastitis resistant group was obtained using primers *Eco*RI + AGG and *Taq*I + CAG. This association was confirmed by individual genotyping. The 155bp AFLP fragment was sequenced and flanking genomic sequences were obtained. A transition single nucleotide polymorphism (A⇔G) was detected and named CGIL4. This marker was then converted into a co-dominant marker through PCR-RFLP. The frequency of the *A* allele was significantly higher in the resistant group as compared to susceptible group (0.42 *vs*. 0.26). CGIL4 also has effects on first lactation milk yield, fat percent, and first lactation and overall somatic cell score. Radiation hybrid mapping suggested close linkage of CGIL4 with the bovine lactoferrin gene, which has been assigned to BTA22. These findings have potential use in marker-assisted selection for clinical mastitis resistance and may also be relevant to the study of human mammitis.

F 010

Association of the single nucleotide polymorphisms from the MCP-1 gene with milk somatic cell counts (SCC) in a Canadian Holstein cattle population

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The increase in SCCs during intramammary infection is largely attributed to an influx of blood leucocytes into the mammary gland. Although these cells can contribute to tissue damage during acute clinical and chronic mastitis, they also play an important role in the host resistance to intramammary infection. Little is known about the genes that regulate the trafficking of blood leukocytes to the mammary gland. The chemokine, MCP-1 has been detected in both human and mouse mammary glands, is known to regulate the trafficking of monocytes and lymphocytes, and is therefore a good candidate for regulating SCCs. We hypothesize that polymorphisms in the bovine MCP-1 gene and its promoter regions may contribute to variation in SCCs within the Canadian Holstein cattle population. To test this hypothesis, the estimated breeding values (EBVs) for Canadian Holstein bulls were used to construct two phenotypic extreme groups, one containing bulls that produced cows with high (H) SCCs, the other containing bulls that produced cows with low (L) SCCs. Bull semen DNA was pooled within each H and L group, and scanned for single nucleotide polymorphisms (SNPs). Independent amplicons from exons 1-3 and the TATA box were generated, and SNP scanning was performed by sequencing these PCR amplicons. Allelic frequencies were obtained by individually genotyping the bulls using the Temperature Gel Gradient Electrophoresis (TGGE) system. The results from this study will be discussed in the context of using MCP-1 gene polymorphism(s) as a molecular marker in mastitis resistance breeding programs.

F 012

Genetic heterogeneity of a QTL with major effect on milk fat content at the *DGAT1* locus in cattle

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A prominent QTL affecting milk fat content has been mapped in the centromeric region of BTA14 and two independent studies have identified the non-conservative mutation *K232A* in the acylCoA-diacylglycerol-acyltransferase 1 (*DGAT1*) gene likely to be causal for the observed variation. Based on a large granddaughter design from the German Holstein population we provide evidence for genetic variability at the QTL beyond the *DGAT1 K232A* mutation by detecting grandsires with *DGAT1* genotypes *232A/232A* but still segregating at the QTL. In an association study including exclusively homozygote *232A/232A* animals we show that alleles of the *DGAT1* promoter defined by variable number of tandem repeats (VNTR) are associated with milk fat content. Due to the presence of a potential transcription factor binding site in the 18mer element of the VNTR, the variation in the number of tandem repeats might be causal for the variability in the transcription level of the *DGAT1* gene. The segregation of multiple alleles affecting milk production traits at the QTL on BTA14 has to be considered whenever marker-assisted selection programs are implemented in dairy cattle.

F 013

Analysis of polymorphisms in the Leptin gene associated with milk production using the GENERA bead system across Australian Dairy Cattle

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The C73T transition (Arg25Cys substitution) in the bovine Leptin gene has been associated with carcass fat content in beef cattle and milk and protein yield in dairy cattle. It was reported in Canadian Holstein Friesians that animals homozygous for the T allele produced more milk and had higher somatic cell count linear scores. We used a new microsphere based SNP genotyping technology for analysis by standard flow cytometers, developed at Genera Biosystems, to determine the Leptin C73T genotype for Holstein Friesian bulls. This new technology used a simple method of immobilization of individual PCR products to customised AmpaSand™ Beads (Genera's activated silica microspheres). These AmpaSand™ Beads were then probed with a hybridisation mix containing equimolar amounts of two fluorescent allele specific probes. Probed beads were analysed by flow cytometry on a Becton-Dickinson FACSArray and genotypes determined using Genera's ShowPlots software. These data were used to test for association with Australian Breeding Values (ABV) and Leptin genotype.

F 014

Association of the Protease Inhibitor Gene with Milk Production and Health Traits Holstein Dairy Cattle

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Positional comparative candidate gene analysis and previous QTL linkage mapping results were used to search for candidate genes affecting milk production and reproduction traits in dairy cattle. The protease inhibitor (*PI*) gene was chosen for examination and seven SNPs were detected in coding regions of the gene by direct sequencing of RT-PCR products from a wide range of cattle tissues. A total of 12 different intragenic haplotypes were identified in Holstein, Jersey, *Bos indicus*, and *Bison bison* samples with very strong differentiation between the species. Six Holstein haplotypes (three common haplotypes and three rare haplotypes) were examined for association with milk production traits in 30 half-sib, granddaughter-design families comprising 30 sires and 1,258 sons. One common haplotype, found only in *Bos taurus*, was associated with increased milk protein percentage, decreased somatic cell score, and increased productive life. Another common haplotype was associated with increased productive life and decreased somatic cell score. One rare haplotype was associated with increases milk fat percentage, and another rare haplotype was associated with increased milk protein percentage and decreased somatic cell score. The observation that the human *PI* gene is also associated with analogous traits demonstrates the effectiveness of the positional comparative candidate gene analysis that utilizes information about genes present in chromosomal regions with conserved synteny in other species.

F 015

SPP1 is a candidate gene for the QTL affecting milk protein concentration on BTA6 based on population-wide linkage disequilibrium, differential expression, and targeted inhibition

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We previously localized a quantitative trait locus (QTL) affecting milk protein concentration in the Israeli Holstein population to a confidence interval of 4 cM centered on microsatellite *BM143* on *BTA6* (Ron *et al*., 2001, *Genetics* 159; 727-735). The genes *IBSP, SPP1* and *LAP3* located adjacent to BM143 in the whole genome cattle-human comparative map were used as anchors for the human genome sequence and bovine BAC clones. Fifteen genes within 2 cM upstream of *BM143* were located in the orthologous syntenic groups on HSA4q22 and HSA4p15. Only a single gene, *SLIT2*, was located within 2 cM downstream of *BM143* due to a "gene desert" in the orthologous HSA4p15 region. The order of these genes, as derived from physical mapping of BAC end sequences, was identical to the order within the orthologous syntenic groups on HSA4: *FAM13A1, HERC3, CEB1, FLJ20637, PP2C-like, ABCG2, PKD2, SPP1, MEPE, IBSP, LAP3, EG1, KIAA1276, HCAPG, MLR1, BM143,* and *SLIT2*. Four hundred and twenty AI bulls with genetic evaluations were genotyped for 12 SNPs identified in 10 of the 16 genes, and for *BM143*. Seven SNPs displayed highly significant linkage disequilibrium effects on protein percentage ($P<0.0001$) with the greatest effect for *SPP1*. The causative mutation was not found in the SNP genotypes of two sires heterozygous for the QTL, and six sires homozygous for the QTL. The expression of *SPP1* and *ABCG2* in the mammary gland corresponded to the lactation curve, as determined by microarray and QPCR assays, but not in the liver. Furthermore, anti-sense *SPP1* transgenic mice displayed abnormal mammary gland differentiation and milk secretion (Nemir *et al*., 2000, *J Biol Chem*. 14; 969-976). Thus *SPP1* is a prime candidate gene for the QTL on BTA6 affecting protein concentration.

F 016

Comparative mapping of QTL affecting milk yield and protein percent in Holstein, Brown Swiss, and Simmental cattle

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Over 95.000 milk samples were sampled in a daughter design representing 47 half-sib families coming from five resource populations (RPs): Israel Holstein (8 families), Italian Holstein-Friesian (10), Italian-Austrian-German Brown Swiss (11), German-Austrian Simmental (10) and Simmental × Red Holstein Backcross (8). Pools for selective DNA pooling were prepared for milk yield (MY) and protein percentage (PP). Each family-trait combination was represented by 8 pools: (2 tails × 2 replicates per tail × 2 duplicates per replicate). The pools were genotyped for a genome wide marker set consisting of 150 to 200 markers in all RPs for both traits, for a total of 8.086 marker-family-trait tests. Using an approximate interval mapping procedure a QTL-map was constructed for each of the five RPs. All chromosomes had at least one chromosome-wide significant effect for at least one RP. The following chromosome fragments (p=proximal, c=central, d=distal) showed a significant effect for PP in 4 or 5 RPs: BTA , 1d, 5c, 6cd, 7d, 10p, 11p, 13c, 16cd, 20c, 26c, 28d; and the following for MY : 4pc, 6cd, 7p, 10d, 13c, 14p, 16d, 19c, 22d, 23c. Among these QTL regions we will select the candidates for fine mapping by comparative and IBD approach.

F 017

Mapping of quantitative trait loci for performance and health traits in Holstein dairy cattle

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A genome scan in a granddaughter design has been conducted to detect quantitative trait loci (QTL) for performance and health traits in the Danish and Swedish Holstein-Friesian population. Twenty-six half-sib families with a total of 1973 sons were genotyped for 233 microsatellite markers covering all 29 autosomes. The numbers of sons per sire range from 38 to 169 with an average of 75.9. Phenotypic data recorded in the granddaughters included conformation traits, fertility traits, calving traits, milk yield and composition, somatic cell count, and mastitis. QTL were detected using single QTL regression analysis using all markers simultaneously and chromosomewise significance thresholds were determined by permutation analysis with 1000 permutations per test, as well as multivariate multi-QTL variance component analyses for selected combinations of chromosomes and traits.

F 019

The BovMAS Consortium for QTL mapping in dairy and dual purpose cattle: Overview of data structure, experimental and methodological results

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The BovMAS consortium, funded by the EU FP5 program, represents the most ambitious genome scan for QTL implemented in any livestock. 95,000 individual milk samples were obtained from 47 large sire-half-sib families representing five cattle populations: Italian Holstein, Israeli Holstein, Italian/German/Austrian Brown Swiss, German/Austrian Simmental, and a Red Holstein × Simmental backcross (Simmental as recurrent parent). Recorded traits were milk-, protein- and fat yield; fat- and protein percent, MSCC, fertility, calving difficulty and stillbirth. Genome scans for milk yield and protein percent by selective DNA pooling have been completed. New statistical methods for selective DNA pooling were developed, including a permutation test, an approximate interval mapping procedure, and a method for high resolution mapping. The modified granddaughter design was adapted for retroactive mapping and estimation of QTL allele frequencies. A comprehensive simulation model was developed to evaluate the contribution of MAS in a herd with multiple breeding objectives.

F 020

The BovMAS Consortium: A permutation test for marker-QTL linkage analysis by daughter design in dairy cattle based on using selective DNA pooling

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Selective DNA pooling is an efficient means of implementing a total genome scan for marker-QTL linkage. In this procedure, marker allele frequencies are estimated in pools made up of DNA samples of individuals from the high and low tails of the population. A difference in marker allele frequency between the "high" and "low" pools (D-value) indicates linkage between marker and QTL. Currently, the statistical test for linkage involves testing a D-value against its standard error, SE(D). SE(D) can be estimated on a priori grounds, based on the binomial distribution and the technical error of allele frequency estimation. The comparison-wise error rate (P-value) corresponding to a given D-value is obtained from the t-distribution. It is not known to what extent P-values obtained in this manner correspond to the actual P-values. A permutation test will enable a more exact determination of comparison-wise and chromosome-wise error rates. A basis for such a test is provided by dividing the pools into two or more independent sub-pools, each consisting of a fraction of the individuals in the tail. Some problems that must be taken into account in designing a permutation test for selective DNA pooling in dairy sire half-sib families include effects of: marker-QTL linkage; differential distribution of maternal grandsires between the two tails; frequency of the alleles of the sires among the dams; the number of subpools per tail; and combining pools from different sires and markers. Solutions and illustrative examples will be presented.

F 021

The BovMAS Consortium: A Complete Genome Scan of Brown Swiss Cattle for Milk Yield and Protein Percent Using Selective DNA Pooling With Milk Samples

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A selective DNA pooling approach using milk samples was applied to map QTL affecting milk yield (MK) and milk protein percentage (PP). in 10 half sib daughter families of Brown Swiss sires with 1000 to 3600 individuals each. Three families were sampled in Germany, three in Italy, one in Austria and three jointly in Austria and Italy. For each sire-trait combination the 200 high and 200 low daughters, ranked by dam-corrected EBV, were chosen for selective DNA pooling. For each tail two independent pools, each of 100 daughters chosen at random, were constructed. Sire allele frequencies were obtained by densitometry at 139 evenly spaced genome-wide autosomal markers. Significance was at 5% FDR level with nominal value of about 0.04 at the marker level and 0.01 at the sire by marker level. 19 markers were significant for PP, 29 for MK, 80 for both traits, and 11 markers were not significant for either. Out of the 846 (PP) and 844 (MY) sire by marker combinations, 154 and 138 were significant for PP and MK, respectively, and 62 for both traits. From the combinations significant for at least one of the traits, MK and PP showed the same direction of the effect in 162 instances, and opposite effect in 183 instances.

F 022

The BovMAS Consortium: Mapping QTL for milk yield and protein percentage in Italian Holstein-Friesian dairy cattle by selective DNA pooling of milk samples

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A first genome scan for milk yield (MY) and protein percentage (PP) was applied in the Italian Holstein-Friesian dairy cattle breed using a selective milk DNA pooling strategy in a daughter design. Milk pools from about 200 daughters with high and 200 with low DYD values for MY and milk pools from about 200 daughters with high and 200 with low estimated breeding values for PP were prepared for each of ten sires with at least 3500 daughters each. The DNA extracted from these pools was genotyped for 146 dinucleotide microsatellites that covered all the bovine autosomes, and shadow corrected estimates of sire allele frequencies were compared between high and low pools. An adjusted false discovery rate was applied to calculate experimentwise significant levels and allele substitution effect was calculated for all significant sire by marker and trait combinations. Significant associations for MY and/or PP were identified for markers on most of the chromosomes. The most significant markers for MY were on BTA1, BTA3, BTA4, BTA5, BTA13, BTA14, BTA19 and BTA28; those for PP were on BTA2, BTA4, BTA5, BTA6, BTA13, BTA14 and BTA22.

F 023

The BovMAS Consortium: Effects of QTL for Milk Protein Percent on Milk Somatic Cell Count

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Selective DNA pooling was used to investigate the effect of QTL affecting milk protein percent (PP) on milk somatic cell counts (MSCC). A total of 33 markers were used, representing regions shown to harbour QTL affecting PP. The mapping population consisted of daughters of six Israel Holstein sires; each with at least 2000 daughters. For each sire and trait the daughters were ranked by dam-corrected EBV, and the 200 high and 200 low daughters were selected. Within each tail, two replicate pools were constructed in duplicate, each with 100 randomly chosen daughters. Significance was set at 5% false discovery rate (FDR) level, representing a nominal significance level of about 0.02 at the marker level and 0.003 at the sire by marker level. Twenty markers were significant for PP only, 1 for MSCC only, 6 for both traits, and 6 for neither. At the individual sire by marker level, 46 combinations were significant for PP only, 12 for MSCC only, 7 for both, and 85 for neither of the traits. Of the 65 sire x marker combinations that were significant for one or both of the traits, PP and MSCC showed the same direction of effect in 32 instances, and the opposite direction in 33 instances. These results are consistent with an absence of a correlation between effects on PP and effects on MSCC at the QTL level.

F 026

Genome Screen for Twinning Rate QTL in North American Holstein Families

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The objectives of this study were to identify putative locations of twinning rate QTL by typing pooled samples in a preliminary screening followed by interval mapping to test QTL effects. Four elite North American Holstein half-sib sire families with high twinning predicted transmitting abilities (PTA) were used in this study. Chromosomes 5, 7, 19, and 23 were not genotyped because these chromosomes were scanned for QTL in these families in a previous study. PTA values for twinning rate were estimated from calving data including granddaughter and great granddaughter pedigree information. DNA was extracted from phenotypically extreme individuals in each sire family. Two pools were prepared from the sons of sires in each phenotypic tail with respect to high and low PTA levels for twinning rates. Each pool contained DNA from 3% to 15% of extreme animals depending on the families. A total of 268 fluorescently labeled microsattelite markers were tested for heterozygosity in sires. Between 135–170 informative markers per family were genotyped using pooled DNA samples. In preliminary analysis, evidence for putative twinning rate QTL was found on BTA 8, 10, 11, 16, 27, and 29 with nominal p-value < 0.001. Interval mapping will be performed to estimate and test QTL effects for particular chromosomal regions.

F 027

Assessment of selective DNA pooling on cow fertility in the New Zealand dairy population

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We present an ongoing report of a study, which is currently being conducted to identify genetic markers and genes involved in cow fertility. The genome scan is based on a selective DNA pooling experimental design. Daughters from a Holstein sire and a Jersey sire were selected from the New Zealand dairy population. Within each sire, cows were selected for three groups: two high fertility groups and one control group. Overlapping of animals among high groups was allowed. Two high fertility groups were identified; one based on high phenotype i.e. consistently getting in calf to artificial breeding (usually the first 5 weeks of the mating season) for over 5 lactations. The other high fertility group consisted of daughters that have high fertility breeding values. Fertility breeding values are calculated from the first 3 lactations of a cows' life and are based on their ability to get in calf to an artificial insemination mating. Only 15% of animals were selected for both the high breeding value and high phenotypic performance groups. The control group consisted of cows exhibiting low fertility breeding values. The control cows have survived at least 6 lactations and thus are representative of an average group of cows rather than a "low group". In total, 500 animals were selected for each group from a population of approximately 35,000 cows for each sire. The two sires were screened for 320 microsatellite markers for heterozygosity across the genome and were informative for 169 and 132 markers. DNA pools within each sire are being constructed and will be screened for each informative marker. Differences in sire allele frequency among high and low pools will be evaluated.

F 028

Quantitative trait loci for male reproductive traits in beef cattle

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The objective of the present study was to detect quantitative trait loci (QTL) for male reproductive traits. A Brahman × Hereford sire was mated to MARC III (1/4 Hereford, 1/4 Angus, 1/4 Red Poll, and 1/4 Pinzgauer) cows. Male offspring born in 1996 (n = 126) were castrated at 8.5 months of age and testicular traits were measured. Traits analyzed were concentration of FSH in blood at castration (FSH), paired testicular weight adjusted for age of dam (PTW), paired testicular volume adjusted for age of dam (PTV), calculated age at puberty (AGE), and body weight at castration (BYW). A putative QTL (expected number of false-positives < 1) was observed for FSH on chromosome (Chr) 5. The support interval for this QTL spans cM 47 to 82, where the maximum *F*-statistic was detected at 70 cM from the beginning of the linkage group. Animals inheriting the Hereford allele had a 2.47 ng/ml higher concentration of FSH in peripheral blood at castration than those inheriting the Brahman allele. Evidence also suggests the existence of a putative QTL on Chr 29 for PTW, PTV, AGE, and BYW. The maximum *F*-statistic was detected at cM 44 from the beginning of the linkage group for PTW, PTV, and AGE, and at cM 52 for BYW. The support intervals for PTW, PTV, AGE, and BYW spanned from cM 38 to cM 46, from cM 37 to cM 46, from cM 34 to cM 57, and from cM 37 to cM 59, respectively. Those animals that inherited the Brahman allele at this chromosomal region had a 45 g heavier PTW, a 42 cm^3 greater PTV, a 39 day younger AGE, and a 22.8 kg heavier BYW, compared to those inheriting the Hereford allele. This is the first report of QTL for male reproductive traits in cattle.

F 029

Identifying genes controlling net feed efficiency in cattle

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Net feed efficiency (NFE) of an animal depends on the weight gained per unit of feed consumed. More efficient livestock require less feed to attain the same level of production as their counterparts. Consequently, the goal of the present study is to identify the genes involved in NFE to expedite selection of efficient animals. To identify these genes, a double back-cross design of two extremely divergent *Bos taurus* breeds [Jersey dairy breed and Limousin beef breed] was used in a QTL mapping experiment. A full genome scan revealed 6 QTL for feed intake on 6 different cattle chromosomes. One of these QTL was related to growth (that is, larger animals ate more food). However, the other QTL mapped to regions of the genome that controlled the level of feed intake independent of growth or weight (that is, the QTL regulate net feed efficiency). To confirm these mapped QTL in cattle, another QTL mapping experiment was conducted in mouse. Two mouse lines selected for high and low NFE were crossed to create 4 full-sib F2 families. After analysing the mapping data, 6 QTL for NFE were found in the mouse genome. Of these, 4 of the mouse QTL were homeologous to 4 of the cattle QTL. Based on comparative homology studies with the human genome, NFE candidate genes have been identified for these QTL. Currently, single nucleotide polymorphisms within these genes are being used for fine mapping and association studies. Once verified, the genes can be utilised for selection and to delineate the metabolic pathways involved in feed intake and efficiency.

F 030

QTL mapping of resistance to Theileria sergenti in backcross calves

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Bovine theileriosis is one of the most harmful anemic diseases for grazing calves in Japan, which is caused by a tick-transmitting protozoan parasite *Theileria sergenti* (TS). To identify QTL controlling resistance to TS, we constructed four half-sib backcross families using Japanese Black (resistant) and Hereford (susceptible). One hundred eighteen backcross (BC) calves were inoculated intravenously with TS-infected blood at the rate of 10^8 parasitized erythrocyte per kg of body-weight. We also used 9 purebred Japanese Black(B), 17 purebred Hereford(H) and 8 F1 (B × H) calves. The parasitemia and anemia (PCV; packed red blood cell volume) in calves were monitored after TS infection for 3 months. Three half-sib familes (3 F1 sires, 72 BC calves) were genotyped for 440 markers and linkage analysis was performed. The maximum parasitemia were 3.93 ± 2.27 %, 1.30 ± 0.96 % and 1.20 ± 0.65 %, for H, B and F1 calves, respectively. The minimum PCV were 22.5 ± 2.3 %, 24.8 ± 1.8 % and 25.0 ± 1.1 %, for H, B and F1 calves, respectively. The maximum parasitemia and minimum PCV for BC calves were 1.98 ± 1.45 % (0.30 % - 8.00 %) and 24.0 ± 2.0 % (18.0 % - 28.4 %) , respectively. The range of maximum parasitemia and minimum PCV indicated the segregation of resistance to TS in backcross calves. QTL for maximum parasitemia and minimum PCV were detected on chromosomes 8, 12 and 18 at 5% chromosome-wise significance level.

F 031

Genetic markers for behavioral traits in German Angus and German Simmental beef cattle– An association study

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Importance of beef cattle is continuously growing in Europe. Reduced and often adverse animal-human contact may lead to serious problems and injuries during necessary manipulations requiring handling and fixation. Selection for docility is therefore requested, which requires phenotypical testing and selection procedures. Consequently implementation of molecular markers for temperamental traits is a following aim. In this study different traits of behavior were recorded in German Angus and German Simmental suckler calves from four consecutive calving years (from mating of 30 cows each to 5 bulls per breed) at 3 weeks of age (n = 944), during a weighting process at an age of 5 month (WT; n = 933) and in a combined separation and restraint test after weaning at 7 month of age (ST; n = 947), respectively.

Heritabilities for the behavior score in the scale and for the score for the ST were estimated 0.27 (±0.05) and 0.31 (±0.06), respectively. Genotyping of microsatellites (*BMS574, RM103, ILSTS036, BMC1207, MB085*) at five potential QTL locations was performed (n = 470) and marker effects were tested using ANOVA. High significant effects were found for *MB085* (and significant effects for *BMC1207*) on the behavior score in the scale and running activities during the separation process. Effects of marker *BMS574* were detected for different parameters of the WT only, for *ILSTS036* for behavior in ST only. No significant effects were detected for *RM103*.

F 032

Marker-Assisted Selection in the Korean Cattle (Hanwoo) for Economic Traits Using Microsatellite DNAs

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Among a total of 331 cattle from the progeny-tested population group in Korea, thirty animals displaying extreme economic traits from the normal distribution of marbling score were selected for the test of microsatellite length polymorphism. Twenty of $(CA)_n$-type dinucleotide microsatellites, obtained from the BAC end sequences of Korean cattle (Hanwoo) were employed for this primary screening and further utilized for confirming test using the whole population. The results revealed that 208 and 210 alleles of HW-YU-MS#3 were closely related to economic traits such as marbling score, daily gain, backfat thickness and *M. longissimus dorsi* area in Hanwoo. Interestingly, HW-YU-MS#3 microsatellite was localized in bovine chromosome 6 on which QTLs related to regulation of the body fat content and muscle hypertrophy loci are previously known to exist. In addition, HW-YU-MS#10 microsatellite appears to show significant difference in marbling score, daily gain and backfat thickness. While HW-YU-MS#12 microsatellite showed a significant negative correlationship with marbling score. In conclusion, the results from the present study suggest the possible use of the two alleles as a DNA marker related to economic trait to select the Hanwoo in the future.

F 033

Mapping of Quantitative Trait Loci for marbling score and daily gain in Chromosome 2 of Korean Cattle (Hanwoo)

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The Korean cattle (Hanwoo) have been sustained in Korea peninsula for several thousand years as a unique genetic source. Lately, Hanwoo meat has become the most favorable beef to Korean consumers who prefer the taste of high quality of fresh meat produced domestically as compared with that of imported beef. Due to this fact, high beef quality and growth rate became targets for the Korean cattle farmer as the most essential traits to compete with the imported beef. Therefore, this study was focused on constructing a genetic linkage map of microsatellite loci shown to be associated with meat quality in cattle and identify the specific alleles related with marbling score for beef quality and daily gain through QTL (quantitative trait loci) analysis. Fifty-five microsatellites at BTA2 were adopted to map, using CRI-MAP program, 305 heads of 36 paternal half-sib families which were slaughtered at 24 months. Two point linkage analysis was accepted, if greater than 3.0 in LOD score between microsatellite loci. Five alleles of 3 microsatellite loci were detected using bootstrap analysis as the useful criteria for marker assisted-selection (MAS) to Hanwoo beef quality and growth.

F 034

Associations of calpastatin and μ calpain markers with meat tenderness in cattle

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Tenderness is the number one meat quality concern of beef customers. Marker Assisted Selection (MAS) offers great potential to improve tenderness as it can not be measured in the live animal. Previous research has linked sequence variability in the Calpastatin (CAST) and μ Calpain (CAPN1) genes with variation in Warner-Bratzler shear force (WBS), an index of beef tenderness. We investigated the effect of two polymorphisms in CAPN1 (CAPN316, CAPN530) and a single polymorphism in CAST on WBS in 330 Santa Gertrudis and 753 Angus cattle. Significantly lower WBS values were observed for CAST genotype AA in Angus ($p > .001$) and Santa Gertrudis ($p > .03$). The difference between homozygotes was 0.49 kg and 0.20 kg, resp. CAPN316 was more strongly correlated to tenderness in Angus ($p > .01$) and Santa Gertrudis ($p > 0.02$) than CAPN530. CAPN530 did not account for additional variation when CAPN316 was already in the statistical model ($p > .29$ and $> .43$, resp.). Furthermore, the effect of CAPN530 genotypes was opposite in direction for Angus and Santa Gertrudis. No interaction between CAST and CAPN316 was found. In both breeds CAST and CAPN316 accounted for ~ 3% of the phenotypic variance. There was no statistically significant effect of all three markers on other carcass traits. It was concluded that the combination of the CAST and the CAPN316 markers has great utility in MAS programs in beef cattle.

F 035

A mutation in the bovine leptin promoter associated with serum leptin concentration, growth rate, body weight, feed intake and body fat content

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Leptin is the hormone product of the obese gene synthesized and secreted predominantly by adipocytes and functions as a lipostatic signal regulating body weight, food intake, energy expenditure, reproduction, and certain immune system functions. Circulating leptin and adipose tissue mRNA levels are correlated with body weight, food intake, nutritional status and adipose tissue mass in humans and animals. Previous studies have identified polymorphisms in the coding regions of the leptin gene in cattle that show potentially significant associations with feed intake, milk quality and quantity, and carcass fatness. The present study detected a cytosine/thymine (C/T) substitution at position 528 in the bovine leptin promoter (GenBank accession number AB070368) that shows significant associations with serum leptin concentration, growth rate, body weight, feed intake, and body fat content in an experimental population of hybrid cattle. Frequencies of the T allele were 21% and 20%, respectively, in an experimental ($n = 180$) and commercial ($n = 160$) population of cattle. Serum leptin concentration was 49% and 39% higher ($P < 0.0001$) for TT animals compared to CC or CT animals, respectively. Daily dry matter intake was positively correlated ($r = 0.26$) with serum leptin concentration and was 8.5% and 6.0% lower ($P = 0.001$) in CC animals compared to CT or TT animals, respectively. Postweaning average daily gain was 10% higher for CT and TT animals compared to CC animals. Liveweight was 7% and 3% higher for TT and CT animals, respectively, compared to CC animals. Ultrasound backfat thickness was correlated ($r = 0.54$) with serum leptin concentration and was 45% and 32% higher ($P < 0.0001$) for TT animals than for CC or CT animals, respectively. Marbling score was also correlated ($r = 0.38$) with serum leptin concentration and was 12% and 9% higher ($P = 0.001$) for TT animals than for CC or CT animals, respectively. These results represent the initial associations of the polymorphism with these traits. Further efforts are underway to validate these findings in other populations.

F 036

Relation between growth hormone gene and fat content in the muscle and growth rate of beef cattle

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Growth hormone (GH) is one of the peptide hormones and it enhances the release of fatty acids from fat tissue. GH seems to have an impact on the fat metabolism of cattle. The GH gene resides on chromosome 19 of cattle and two mutations were found in the fifth exon of the GH gene of Japanese Cattle. The mutated points correspond to the 127th and the 172nd amino acid of the GH hormone and these mutations change the amino acids of those corresponding parts, from leucine (Leu) to valine (Val) at 127th and from threonine (Thr) to methionine (Met) at 172nd. The genotype which makes Val at 127th and Met at 172nd has been found only in the Japanese breed of cattle at this time. Around 190 head of Japanese Black steers grown on the same plane of nutrition were tested at the same age for elucidating the relationship between GH genotype and both intramuscular fat (marbling) and growth rate. The intramuscular fat levels of steers which had the genotype of val/val at 127th was significantly ($p<0.05$) higher than those steers that had the val/leu or leu/leu genotype. Daily gain of the steers which had Thr/Thr at 172nd was significantly ($p<0.05$) higher than those that had Thr/Met or Met/Met.

F 039

Association between the deletion mutant allele of MCSU deficiency and carcass traits in Japanese Black cattle

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MCSU (molybdenum cofactor sulfurase) deficiency is a xanthine metabolic disorder controlled by autosomal recessive gene in the Japanese Black cattle. The association among the mutant allele (*m*) of *MCSU* deficiency and carcass traits was investigated. The *MCSU* genotypes were diagnosed in 317 fattened steers whose either father, grandfather, or great-grandfather was the career sire. The steers were composed of 240 normals (*MM*) and 77 careers (*Mm*). The association analysis was carried out by two different approaches, i.e., using adjusted carcass records based on BLUE for non-genetic factors of shipping year, fattening farm, fattening period and slaughter age, and using non-adjusted carcass records. In the former approach the BLUE were preliminarily obtained by using 48,045 carcass records. On the other hand, the non-genetic factors were included in the model in the latter approach. The significance testing for the *MCSU* genotype was based on the GLMTEST program that handles the hypothesis tests for fixed effects under animal models. While the association among the *MCSU* genotype and carcass traits was not significant in carcass weight, daily gain, longissimus muscle area, rib thickness and subcutaneous fat thickness, only BMS number showed the significant association ($p<0.05$) in both approaches.

F 040

Meta analysis confirms associations of the TG5 thyroglobulin polymorphism with marbling in beef cattle

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The TG5 polymorphism (C/T) occurs in the 5' promoter region of the thyroglobulin gene and is widely used in Marker Assisted Selection (MAS) programs to improve the predictability of marbling level and eating quality in beef cattle. A number of studies have been conducted in recent years to evaluate the association in different production systems. Meta-analysis is an increasingly popular method of pooling results from separate association studies to quantify effects. It was applied to 14 different estimates of the TG5 effect measured in nine separate studies using four different grading systems for marbling. To account for the data structure, three different models, all based on a standard random effects model were fitted, viz a multi-level classical random effects model, a Bayesian hierarchical model and a Bayesian model with study-effect and trait-effects. The TG5 effect was significant for all models at the 90 to 99% level of significance. The TT genotype was associated with higher levels of marbling relative to the CC genotype with the CT heterozygote being intermediate. The results confirm the association of the TG5 marker with marbling and the utility of TG5 for MAS programs which target eating quality in beef cattle.

F 041

Relationships of the genotypes of the growth hormone and carcass traits in Japanese Black cattle

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The relationships of the genotypes (the genetic variant in exon 5 codon 127/codon 172) of 5 types (A/A, A/B, A/C, B/B, B/C, C/C) of the growth hormone (GH) and carcass traits in Japanese Black cattle were investigated. Subjects were the offspring (2370 samples) used for progeny testing (from 1997 to 2002) carried out at the Livestock Improvement Association of Japan. The gene frequency of the A gene was the highest, followed by the frequencies of the B and C genes respectively. The relation between the genotypes of the GH and the 5 carcass traits (body weight, daily gain, carcass weight, carcass yield, rib eye area, and beef marbling scores) were analyzed using a multiple comparison test. Type A/A showed significantly higher values than almost all other types. Types A/B and A/C showed significantly higher values than types B/C, C/C in terms of body weight, daily gain and carcass weight. This implies that the A gene may be responsible for the effects influencing weight increases. No significant relationship was discovered with regard to the genotypes of the GH and carcass yield. Although values for subjects with the type A gene were higher than other types in the rib eye area, only type A/B showed significantly higher values than type B/C. Type A/A showed significantly lower values than types A/B, B/B and B/C in beef marbling scores.

F 042

A comprehensive QTL map of Japanese Black cattle (Wagyu)

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To identify economically important quantitative trait loci (QTL) of Wagyu, fifteen half-sib families composed of 110 ~ 524 offspring collected at commercial markets were subjected to genotyping of 160 ~ 250 markers spanning 29 bovine autosomes. *F*-statistic values and significance levels were calculated by linear regression method and permutation test, respectively. We have identified 12 loci for body weight, 21 loci for carcass weight, 44 loci for beef marbling score, 36 loci for rib eye area, 16 loci for rib thickness, and 16 loci for subcutaneous fat thickness at 5% chromosome-wise significance level. Sixty-three QTL regions at 1% chromosome-wise significance level were comprehensively illustrated on an autosome genetic map.

F 043

Mapping of QTL for carcass traits in Japanese Black cattle using data from half-sib families

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The objective of this study was to identify candidate regions including quantitative trait loci (QTL) for carcass traits in Japanese Black cattle using data from half-sib families. Two paternal half-sib families derived from sire KG and sire FS were analyzed by using interval mapping. One hundred eighty-three microsatellite markers distributed among all 29 bovine autosomes were genotyped in 364 male offspring sired by KG. Samples and carcass records were collected from three carcass markets. Two hundred forty-one microsatellite markers were genotyped in 240 female offspring of sired by FS and collected from one carcass market. The analyses for the half-sib family of sire KG were performed using the standardized value of phenotype data for each market. In sire KG's family analyses, two significant QTL regions at the 5% chromosome-wise significance level were detected for beef marbling score. Results of analyses using alternative genotyping of additional markers and samples within those two QTL regions revealed a lod score of 7.1 in one region (Chr. 21) and were significant at the 0.1% chromosome-wise significance level. In sire FS's family analyses, 3 significant QTL regions for rib eye areas (Chr. 7, 10, 13) and one significant QTL region for beef marbling scores (Chr. 12) were detected at the 5% chromosome-wise significance level. A study of these 4 regions by analyses using alternative genotyping of additional markers and samples is currently being performed.

F 044

A 1.4 Mb-critical region for carcass weight QTL on BTA 14 (*CW-1*) identified in Japanese black cattle population using IBD-based analysis and association study

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A quantitative trait locus (QTL) for carcass weight in Japanese black cattle (Wagyu), designated *CW-1*, has been fine-mapped in the central region of chromosome 14 (Mizoshita *et al*., 2004, in submission). The *Q* to *q* substitution effect accounted for 16.6% of genetic variance, corresponding 37 kg difference, indicating importance of *CW-1*. We detected the same *CW-1* region as QTL for carcass weight in another pedigree of purebred Wagyu population, and found an apparent IBD spanning 8.1 cM. Using 5 human homologus genes and 18 MS markers developed from a BTA 14-specific genomic library we generated a BAC contig composed of 60 BAC clones corresponding human 6.8 Mb. Fifty-four MS markers were developed from these BAC clones. Using the high-density MS marker map, we reconstructed haplotypes of five bulls probably harboring the *Q* of *CW-1*, and detected IBD spanning 1.4 Mb on human genome. This 1.4 Mb-region was confirmed by association study using CW-high and -low populations in Wagyu. SNPs discovery in the 1.4Mb-region is now underway. Association study using SNPs will be useful to narrow down the region and for positional cloning of the *CW-1* gene.

F 045

QTL mapping for carcass traits on BTA 2 and BTA 24 in a paternal half-sib family comprising purebred Japanese Black cattle (Wagyu) population

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To detect QTL affecting economically important traits in purebred Wagyu population, we performed a genome-wide scan across a paternal half-sib family composed of 190 offspring with 254 markers covering autosomes as primary screening. Two significant loci for marbling were detected on BTA 2 and BTA 24 ($P < 0.05$). Beef color standard (BCS) was on the BTA 2 region ($P < 0.05$), while carcass weight (CW) was on the BTA 24 region ($P < 0.05$). To confirm the presence of the three QTL on BTA 2 and 24, 52 microsatellite markers and 488 offspring were employed for the second screening. QTL for marbling and BCS ($P < 0.05$) were confirmed at 92 cM on BTA 2 whereas BTA 24 at 50 cM harbored QTL for marbling ($P < 0.05$) and CW ($P < 0.01$). Average marbling score (488 offspring) were 6.98±2.21, 6.68±2.26, 6.50±2.03, 6.02±2.02 in Q_2Q_{24}, Q_2q_{24}, q_2Q_{24}, and q_2q_{24}, respectively. Significant differences ($P < 0.01$) were observed between Q_2Q_{24}, q_2q_{24} and q_2Q_{24}, suggesting that Q_2 and Q_{24} additively affect marbling score. The Q_2 decreased BCS by 0.15, and the Q_{24} increased CW by 14.9 kg. The haplotype information on BTA 2 and BTA 24 obtained here would accelerate to improve marbling as well as BCS and CW.

F 046

Identification of a major gene locus for marbling spanning 450 kb at the telomeric region of BTA 21 using Japanese Black cattle population

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We have previously mapped a major gene locus for marbling on BTA 21 using a half-sib family comprising 872 offspring. To identify the responsible gene, we constructed a high-resolution physical map using BAC clones and performed association study in the region. We present here that the association study successfully narrowed the region to 450 kb. The contig map was constructed with 65 bovine BAC clones (RPCI-42 and Wagyu BAC libraries) using PCR screening systems, and 74 novel microsatellite markers were developed from every BAC clone. *In silico* human draft sequence information (http://genome.ucsc.edu) suggested that the region spanned 6.5 Mb containing 35 known and 27 predicted genes. To narrow down the critical region, we performed association study with 47 microsatellite markers using 378 highly marbled and 430 poorly marbled steers randomly selected among 3,747 Japanese Black cattle population. We significantly identified the locus spanning an approximately 450 kb region ($p < 0.001$) including six human orthologue genes. The 450 kb region is being shotgun-sequenced, followed by development of SNP and analysis of gene expression profile.

F 047

QTL for beef marbling mapped on BTA 9 and BTA 14 in a paternal half-sib family from purebred Japanese Black cattle (Hiroshima-gyu) population

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The aim of this study is to establish a DNA-based breeding system for beef marbling in local Japanese Black cattle population called Hiroshima-gyu, relying on marbling QTL information. To detect QTL, a paternal half-sib family was employed for analysis. Marbling trait was scored from one to 12 with a standard model panel, in which higher scores correspond to more marbling. In genome scan of 228 individuals (169 males and 59 females) with 247 informative microsatellites covering 29 autosomes, we detected QTL for marbling in the centromeric regions of BTA 9 and 14. Interestingly, QTL for marbling and growth traits were repeatedly detected in the same region of BTA 14 in Japanese Black cattle population (see Watanabe *et al*., ISAG 2004). *Q* to *q* allele substitution effect of BTA 9 on marbling was 0.8 ($p < 0.01$) while that of BTA 14 was 0.6 ($p < 0.05$). We examined effect of possible combinations of marbling-associated alleles, and observed significant differences ($p < 0.01$) in average marbling score of Q_9Q_{14} and $Q_{14}q_9$, and Q_9Q_{14} and q_9q_{14}, respectively. These results suggest additive effects of these marbling QTL and can provide a primary platform for marker-assisted selection system for marbling in Hiroshima-gyu population.

F 048

Fine-mapping of a bovine QTL for marbling on BTA 4 using association study

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Although BMS (beef marbling score) is one of the most important economical traits of beef cattle, any master regulatory gene controlling BMS has not been identified yet. We have identified two QTL for BMS overlapped on BTA 4 (35-85 cM) in two historically different pedigrees of Japanese Black cattle population, exhibiting major effects ranging 11.5-15.7 % of genetic variance. Since no apparent identical by descent (IBD) was observed between the *Q* alleles, it is possible to narrow down a putative IBD region through association study using markers from our up-dated high-density linkage map. We performed association study using BMS – high and – low groups of cattle with totally 56 microsatellite markers in the 40-80 cM region, and observed five regions significantly associated with BMS ($p < 0.05$). The most associated region ($p < 0.012$) included an apparent *Q* IBD composed of three consecutive microsatellites spanning 2 cM. To confirm whether it is a real IBD, we are building a BAC contig covering the possible IBD, and developing microsatellites from the contig for further analysis.

F 049

Characterization of marbling *Q*-specific haplotype in Japanese Black cattle (Wagyu) population through BAC contig construction

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Identification of QTLs for economically important traits provides the more efficient breeding system used marker-assisted selection. We performed a genome-wide scan for carcass traits in a Wagyu paternal half-sib family comprising 327 offsping, and mapped a locus for marbling on BTA 7. This locus did not exhibit the significant association with marbling trait in other two closely related half-sib families. By comparison of mean phenotypic data of marbling in the these families and haplotypes of three sires, we speculated that marbling *Q* was present in only one of the three sires, resulting that the region was narrowed in less than 6 cM. To precisely characterize the *Q* region, we constructed a BAC contig spanning the region using the RPCI-42 library, and developed new microsatellite markers and SNPs from the region. BAC library was initially screened with two microstellites and 24 known genes in the 6-cM region. End sequences of BACs isolated were also used to extend a contig.

The resulting contig was composed of 60 BACs, corresponding 7.1-Mb of human genome. By comparative analysis with human genome, it was suggested that there would be 148-genes located in the region. We developed 68 polymorphic microsatellites and 45 SNPs and used to determine haplotypes of the three sires. We finally identified the *Q*-specific haplotype spanning 50-kb covered by a single BAC.

F 050

Selective genotyping for marker assisted selection in Japanese Black cattle

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Using amplified fragment length polymorphism (AFLP) fingerprinting, selective genotyping was performed to determine if this method was effective for selecting superior breeding stock. Twenty cows with extreme genetic merit for beef marbling score (BMS) were selected from 46 progeny of sire-Y, including 10 with the highest for predicted breeding value (PBV) and 10 with the lowest. 24 AFLP fragments were selected for further analysis based on fragment frequency differences between the high and low groups. A linear discriminant analysis using these AFLP fragments was applied in order to derive a discriminant function that classified the cows into high and low groups. Six of 24 fragments were included in the resulting function and the discriminant scores (general genetic values, GGV) of the 20 cows were calculated using the function. The same function was then applied to 26 additional cows that were randomly selected from the original sire-Y family. A significant correlation coefficient of GGV on BMS-PBV ($r = 0.70$) was obtained, which indicates that the GGV can be used as a selection criterion for BMS in a sire-Y family. These results suggest that AFLP fingerprinting can be used for animal breeding without identifying the underlying genes affecting the trait of interest.

F 051

QTL mapping in experimental backcross populations of sheep: some statistical considerations

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Two major studies are underway to map QTL for production and disease traits in sheep. The first involves a backcross between an Awassi × Merino F_1 with a Merino line, and the second is a multi-sire experiment being a backcross between an Indonesian Thin Tail (ITT) × Merino F_1 with a Merino line. Initial work on QTL mapping was conducted using the half-sib module of QTL Express, a web-based program using a regression technique. However, this technique does not address the backcross design (where linkage phase will be known), and nor does it utilise all the genotypic information available. Consequently, a maximum likelihood technique implanted using the E-M algorithm has been developed for these experiments to address these issues. In particular, a probability-based method was devised to make use of an extended set of flanking markers around a putative QTL, the set containing unambiguous (informative) genotypes at the end of the interval, with intervening ambiguous (partially informative) genotypes between. This required estimates of the marker allele frequencies in the Merino population, so an additional likelihood-based technique was developed for this. The method has been implemented using S-PLUS, allowing easy modification and extension to include QTL × fixed effect interactions, multi-sire analysis, with possible extensions to multi-trait models and non-normal (e.g. survival time, count) traits, etc.

F 052

Searching for QTL underlying udder morphological traits on chromosome 4 in dairy sheep

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A study to detect quantitative trait loci (QTL) on ovine chromosome 4 (OAR4) affecting udder morphological traits was undertaken. Six microsatellites (BMS1788, BMS1172, MCM144, ILSTS062, OARHH35, and OARHH64) were genotyped in a daughter design comprising 1421 ewes belonging to eleven half sib families from the Spanish Churra dairy breed. Phenotypic measurements were linear scores of five udder morphological traits (udder shape, udder depth, udder attachment, teat placement, and teat size). A male linkage map was estimated using CRIMAP software. The resulting map extended over 104 cM (Haldane) with an average distance within two markers of 20.8 cM and the information content map exceeded 57% along the entire chromosome. QTL detection was carried out by multi-marker regression analysis. The across-family analysis revealed a putative QTL in the telomeric region of chromosome 4 (chromosomewise signification *P=0.055*) affecting udder shape trait. Within-family analyses revealed evidences for two segregating families. The estimated effect in the two segregating families showed a different sign and reached around 0.6 SD. Taking into account that P-value reached only the "suggestive level" a confirmation of this putative effect in a new set of daughters for the segregating rams should be suggested before encountering fine mapping strategies or maker assisted selection.

F 053

Scanning OAR15 for QTL affecting milk production traits

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A daughter design was carried out with families belonging to the selection scheme of the Spanish Churra dairy sheep looking for QTLs affecting milk production and udder traits. Eleven half-sib families comprising a total of 1421 ewes were investigated for marker-phenotype associations. Five microsatellites located on OAR15 were chosen from the ovine linkage map. Yield deviations of production traits (milk yield, fat percentage and protein percentage) were estimated and used as quantitative measurements. Multipoint linkage analysis was performed across families using CRIMAP program and information content (IC) along the map was measured. QTL mapping was performed by multimarker regression of trait data by applying an across-family analysis to each trait separately. Chromosomewise critical values were determined through 10,000 permutations of the phenotypic data. The linkage map obtained for the microsatellites analysed extended along 117 cM (Haldane) and the IC average along the chromosome was 63.15%. The QTL analysis across-family suggested that a QTL may be segregating in the Churra population at the proximal end of the chromosome OAR15 for protein percentage at map position 30 cM. The chromosomewise signification reached the 5% level with a *P-value of 0.045*. In the within-family analysis two families presented evidences for a segregating QTL in the centromeric region of ovine chromosome 15.

F 057

Association of polymorphism in alpha (1,2) fucosyltransferase gene with growth performance of pig population in Taiwan

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The mutation at 307bp (M307) of alpha (1,2) fucosyltransferase gene has been suggested as a favorable candidate marker for selection of *E. coli* F18 adhesion resistant pigs. Nonetheless, the effect of this mutation on pig growth performance remains unclear. This study investigates the genotypic frequencies and the effect of M307 on the growth performance of pig population in Taiwan. A total of 375 boars (230 Duroc, 109 Landrace and 36 Yorkshire) were performance tested using segregated early weaning entrance. The genotype of M307 was determined by PCR-RFLP. The performance traits included average daily gain, feed efficiency, backfat thickness and age at 110 kg body weight. The statistical model included starting age, test season, genotype of M307, and the interaction between the genotype and test season. Consequently, the genotypic frequencies of AA genotype were 6.9%, 6.4% and 0.0%, and of GG genotype were 47.0%, 73.4% and 61.1% in Duroc, Landrace and Yorkshire. Boars with AA genotype in Duroc and AG genotype in Landrace and Yorkshire significantly revealed higher backfat thickness than those with GG genotype (P<0.05). The M307 did not affect other growth performance traits. The results suggest that selection for resistance to *E. coli* F18 adhesion resistance may not affect most of the growth performance traits; however, it may result in adverse effect on backfat thickness.

F 058

Association of IGF1 gene polymorphism with circulating insulin-like growth factor 1 concentration in swine

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The polymorphism of the pig *IGF1* gene has been analysed in three Landrace lines, in which individuals were measured for several growth and carcass-related traits. Plasmatic IGF1 concentrations were determined at 160 days by using an enzyme-linked immunoassay. The *IGF1* polymorphism was typed in the three lines (n = 127) by using a polymorphic cytosine-adenosine (CA) repeat located in the first intron of the *IGF1* gene. A quantitative reverse RT-PCR protocol was used to determine *IGF1* mRNA in the liver (n = 16). Positive significant associations were found between the length of the CA repeat and circulating IGF1 concentration ($p < 0.05$) and with liveweight and backfat thickness ($p < 0.10$) at 160 days. The association between the length of the CA repeat and circulating IGF1 was simultaneously found in the three lines, although only was significant in males. No association was observed between the CA sequence repeat and *IGF1* mRNA expression in the liver, which displayed a very high variability. However, the results showed that expression of IGF1 in the liver was correlated to IGF1 levels in plasma.

F 059

Novel SNPs and microsatellite polymorphisms in chosen candidate genes (*MyoD, GHRHR, IGF1* and *IGF2*) and analysis of their associations with carcass quality traits in pigs

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Carcass quality traits, as all quantitative traits, are polygenic and affected by non-genetic environmental factors. Several candidate genes, affecting carcass quality, may be selected on the basis of their participation in the processes of muscle development and growth. The myogenic bHLH family of transcription factors (MyoD) plays an important regulatory role in development of skeletal muscle. There are four members of this family in vertebrates, MyoD1, myogenin, myf-5 and myf-6. Altogether 12 novel SNPs were identified in the coding and 5' flanking regions of genes encoding these factors in pigs. A significant associations between genotypes at these SNPs and several carcass quality traits (weight of loin, muscle eye area, meat content of carcass) were identified in pigs of three different breeds (Polish Landrace, Polish Large White, commercial line 990). Possible binding sites for pituitary tissue-specific transcription factors Pit-1 was found at 8645 bp upstream from transcription starting site of *GHRHR* gene where microsatellite sequence is located. Two polymorphic microsatellite sequences located in the porcine insulin-like growth factor genes (*IGF1* and *IGF2*) and this located relatively near to *GHRHR* gene were used in analysis of their association with growth rate and carcass performance traits.
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F 061

The effect of porcine LEPR-derived microsatellites on estimation of genetic parameters for growth characteristics, backfat depth and body fat content using animal model

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Two genetic evaluation models were studied to investigate the genotypic effect of porcine LEPR-derived microsatellites on estimation of genetic parameters. The data used were growth characteristics, backfat depth and body fat content in F_2 population produced by inter-crossing F_1 from Korean native boars and Landrace sows. Variance components were estimated using the MTDFREML computer programs with single trait animal model. The fixed part of the model, contemporary group and sex was constant across Model[1] and Model[2]. Two models were compared using likelihood ratio tests on a chi-square distribution. Likelihood ratio tests indicate that for all the traits the Model[2] including genotypic effect as covariate provides better fit than Model[1] ($p<.05$). When genotypic effects are included as covariate in the model, heritability estimates from Model[2] were 0.08, 0.26, 0.73, and 0.67. for mature weight, maturing rate, backfat depth, and body fat content, respectively, and moderately increased by 14.0% for backfat depth and 8.1% for body fat content compared to those from Model[1]. This increase in heritability estimate would be expected to create a wider distribution among EPDs, resulting in more desirable numbers for trait leaders.

F 060

QTL and Leptin Receptor candidate gene analysis in pigs

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The leptin receptor gene (*LEPR*) is a candidate for traits related to growth and body composition, and it is located on SSC6 in a region where several QTL have been detected. The aims of this work were: *a*) to verify these QTL on a larger sample of animals and generations and *b*) to examine the effects of the *LEPR* variants on fatness and body composition traits, to evaluate the causality of the polymorphisms analysed respect to the QTL. Three missense polymorphisms, located on exons 4 (T69M), 9 (D382A) and 14 (L663F), have been genotyped by pyrosequencing in a cross between Landrace and Iberian pigs comprising 33 F_0, 70 F_1, 418 F_2, 86 F_3 and 128 individuals coming from the backcross of four F_2 males with 24 Landrace females. Thirteen microsatellites and one SNP on *MC1R* gene were also genotyped. Traits analysed were: backfat thickness (BFT), intramuscular fat percentage (IMF) and eye muscle area (MA). Four statistical models were used. For the QTL study, a QTL detection model with sex and batch as fixed effects and a two-QTL model were applied. For the candidate gene study, an animal model with the effect of *LEPR* alleles, and a QTL model including the effect of *LEPR* haplotypes as fixed effects were evaluated. The results support the presence of two QTL on SSC6, one affecting BFT (position 60cM), and the other and more significant one affecting BFT, IMF and MA (position 130cM). Results also confirm the implication of *LEPR* gene variants on the variability of these traits. The Iberian *LEPR* haplotype, increases BFT and reduces MA. Functional studies are required to explain the effects observed.

F 062

Molecular markers for fresh and dry-cured ham processing quality traits: effect of three cathepsin genes

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In recent years, an increase in consumer demand for high quality fresh and processed pork products has occurred. Understanding the genetic factors controlling pork meat quality variation is essential. Several genes are good biological candidates to explain the genetic variability observed for these traits and the use of polymorphisms identified in these genes can accelerate genetic improvement. Cathepsins are lysosomal enzymes involved in the proteolysis process observed during the dry curing process of hams. We investigated the effect of three cathepsin genes (B, F and Z) on several processing characteristics of fresh and dry-cured hams. The genotypes for all genes were obtained using procedures developed in our group (cathepsins B and Z) or published in the literature (cathepsin F). The results obtained revealed associations between cathepsin Z (CTSZ) genotypes and several fresh and dry-cured ham traits. CTSZ genotype 22 was found to be associated not only with higher fresh and cured ham weight but also with higher yield. A similar effect was also detected at the cathepsin F (CTSF) locus, where CTSF variants significantly influenced fresh and dry-cured ham weight, average marbling and intramuscular fat. No associations were found between cathepsin B (CTSB) genotypes with any of the fresh ham traits analyzed. However, CTSB genotypes had a significant effect on several dry-cured ham traits, including cured weight, yield, weight loss and moisture content. This study suggests that variations in the cathepsin genes analyzed can be used by the swine industry as tools to select pigs with characteristics more suitable for dry-cured ham production.

F 063

Novel mutations in the *FABP3* promoter region associated with intramuscular fat content in pigs

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Intramuscular fat content (IMF) is considered as one of major economic traits in the pig breeding and industry. In general, high IMF results in better meat quality. Several approaches to detect quantitative trait loci (QTL) for IMF indicated a strong possibility of the existence of a QTL related to IMF between MS marker Sw71 and Sw1881 on SSC6. Porcine *FABP3* has been considered as a candidate gene affecting IMF due to its physiological roles and position on the pig genome. Two novel mutations in the promoter region of porcine *FABP3* were detected by resequencing of the promoter and coding region of porcine *FABP3* using 4 breeds (Korean native, Landrace, Yorkshire and Duroc). The mutation was used for testing relationship with growth and fat depot traits. The *GG* genotype of the g.-158T>G polymorphism showed a highly negative effect ($P<0.01$) on body weight at 3 and 12 weeks of age, and a positive effect ($P<0.05$) on IMF. However, backfat thickness and carcass fat content were not significantly associated with the genotype. The result indicates that the novel mutation could be utilized as a genetic marker in a marker-assisted selection program to improve IMF independent of BF in pigs.

F 064

Association of heart fatty acid-binding protein (H-FABP) genotypes and meat quality traits of commercial black coated pigs in Taiwan

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Intramuscular fat is an important component of meat quality. Heart fatty acid-binding protein (H-FABP) has been shown associated with IMF content in Duroc pigs. The commercial black coated pigs in Taiwan are usually the hybrids of local breed with Duroc as terminal sire. Whether the H-FABP affect the meat quality traits of the commercial black coated pigs in Taiwan still remains unclear. The purpose of this study was to investigate the influence of the H-FABP genotypes on meat quality traits of the commercial black coated pigs in Taiwan. A total of 37 commercial black coated pigs were performance tested using segregated early weaning entrance. The meat quality traits surveyed included intra muscular fat (marbling score), meat color (Hunter's L, a and b value), pH, shear force, water holding capacity and loin eye area. The H-FABP genotypes were determined by PCR-RFLP. The results were as follows: The genotype of Hinf I site significantly affect meat color and marbling score ($P<0.05$). The Hae Ⅲ site significantly associate with meat color and loin eye area. The polymorphism in Msp I site did not have significant effect on the meat quality traits. The results suggest that the polymorphisms in Hinf I and Hae Ⅲ sites of H-FABP may influence the meat quality traits of the commercial black coated pigs in Taiwan.

F 065

Characterization of the polymorphism in swine *EGF, RBP4* and *PRL* genes and their association with reproduction traits

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Efficiency of production of swine is highly influenced by reproductive success, especially litter size. One of the approaches to gather genomes information that might be used in genetic improvement of litter size is the candidate gene approach. The current investigations cover several genes, the product of which is associated with different aspects of reproduction. The effect on litter size was observed between other for Epidermal Growth Factor (*EGF*), The Retinol Binding Protein4 (*RBP4*) and Prolactin (*PRL*) genes. In our experiment we examined the polymorphism in this genes for 300 sows from polish herds: pbz, wbp breed and commercial line 990. The polymorphism of the genes was determined by the PCR/RFLP method. The frequency of individual alleles amounted to 0.7 and 0.3 for B and A allele in EGF locus and 0.73 and 0.27 for 1 and 2 allele in RBP4 locus. We identified also a novel single-nucleotide polymorphism (SNP) in the promoter region of PRL at position 499 (T/C). The SSCP method and direct sequencing performed the SNP detection in this gene. The relationship between candidate gene genotypes and reproductive traits will be evaluated with the Least Squares Method. The fixed effects of the season of the farrow, year of the farrow and boars will include into linear model. The data will be analyzed separately for the first and later parities. Additional regression coefficients will be obtained for additive and dominance effects of RYR 1 genotypes (HAL^A and HAL^D). The research is still continued and more data will be collected soon.

F 066

Mucin 4: A candidate gene for susceptibility towards E. coli F4ab/ac diarrhea in the pig

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Enterotoxigenic Escherichia coli (ETEC) that express the F4ab or F4ac fimbriae (formerly known as K88ab/ac) are major causes of diarrhea and death in neonatal and young pigs. A locus controlling susceptibility towards ETEC F4ab/ac has previously been mapped to pig chromosome 13q41. A number of studies indicate that candidate genes for this trait could either be transferrin-like-genes or mucin-like sialoglycoproteins. Deductions based on linkage and comparative mapping data point to mucin 4 (MUC4) as a likely candidate. Incidentally, the gene lies in one of the candidate regions on Hsap3 (viz., Hsap3q29). We isolated pig BAC clones containing MUC4, and mapped the BACs by FISH to SSC13q41. FISH mapping on interphase nuclei positioned MUC4 between microsatellite markers Sw207 and S0283 while radiation hybrid mapping positioned it between SST and SWR2189. Initial screening for polymorphism in the gene revealed a SNP in intron 7 that shows complete co-segregation with ETEC F4ab/ac susceptibility in a Wild boar/Large White intercross. We report on the genomic characterization of the porcine mucin 4 gene spanning 25 exons and more than 31 kb. Comparison between the porcine mucin 4 gene and the human ortholog shows conservation between 58% and 89% on the nucleotide level of the different exons. Highly conserved non-protein coding sequences are also evident from the comparison. Alignment between porcine and murine MUC4 shows less conservation than the pig-human comparison. In addition to the genomic sequence of pig mucin 4 we also report on more than 15 SNPs discovered in the porcine MUC4 sequence.

F 067
QTL for traits related to humoral immune defence in pigs

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Disease resistance and immune competence are heritable but difficult to determine phenotypically. This study aimed to detect QTL for traits related to humoral immune defence. F2-piglets (n=457) of a resource population based on the reciprocal cross of Berlin Miniature Pig and Duroc (DUMI) were vaccinated against Mycoplasma hyopneumoniae, Aujeszky's disease virus and porcine reproductive and respiratory syndrome virus at 6, 14 and 16 weeks of age, respectively. The haemolytic activity in the alternative and classical complement pathway, serum levels of C3c and haptoglobin (HP) and antibody titers were determined in samples taken before and after vaccinations. Animals were genotyped at 76 type II and 9 type I markers distribute over autosomes. QTL analysis was performed using interval mapping by regression under the line cross and half sib model. Phenotypic data were adjusted for systematic effects by mixed models with and without repeated measure statement as appropriate. In total, 49 and 22 QTL with genome wide significance at the .05 and .01 level were detected. QTL for antibody titers are on Sscr 1, 2, 6, and 7 supporting and supplementing previous results. For the first time QTL for haemolytic complement activity and acute phase response (C3c, HP) are shown, with the first located on Sscr 2, 3, 4, 6, 7, 11, and 17 and the others distribute over all autosomes except Sscr 13. Effects of the QTL are as low as 2.8 and as high as 25.5. The results show that with regard to number and effect QTL for immune traits behave like those for other quantitative traits and facilitate the identification of candidate genes for disease resistance and immune competence.

F 068
Discovery and mapping of a QTL affecting scrotal hernia incidence on chromosome 2 in domestic pigs

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Scrotal hernia (SH) is a congenital defect that results from protrusion of part of the intestine through the abdominal opening of the inguinal canal into the scrotum. Incidence of SH is affected by genetic as well as ill-defined environmental factors and is reported to be in the range of 1-6% of all male pigs. Incomplete penetrance of the SH phenotype in animals with a genetic predisposition to display this defect complicates attempts to identify genetic determinants. However, a whole genome scan (using microsatellite markers) of 34 independent SH-affected boar families identified, with a high degree of probability, a region on chromosome 2 (approximately 20-70 cM) that contains genes affecting SH incidence. Over 135 SNP markers were developed for this region. Sequence tagged sites (STS) selected from public domain genetic and physical maps were used to screen a BAC library. Subsequently identified BACs were subcloned, sequenced, and primers designed for locus specific re-sequencing and SNP discovery. In addition, SNPs were also identified for a number of positional candidate genes for SH. Approximately 2,000 DNA samples (from 143 sire families with ≥1 affected progeny and 72 sire families with ≥80 progeny with no recorded SH incidence), from a Pietrain-based line of pigs, were genotyped for all SNP markers. Linkage disequilibrium analysis identified three, apparently independent loci, at approximately 3, 42 and 65 cM, on SSC2. Interestingly, none of the locus groups located near selected positional candidate genes had the strongest SH associations.

F 069
Association of Candidate Genes for Inverted Teat Defect in Pig

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The inverted teat defect results in non-functional teats that cannot be suckled by the piglets. As a consequence the number of piglets raised is reduced for sows suffering from inverted teats. This disorder has a considerable negative impact in pig production. It occurs in many commercial pig breeds with frequencies between 8 to 30%. The transforming growth factor beta 1 gene (TGFB1) and relaxin gene (RLN) were proposed to be tested for association in a positional candidate gene approach which combines linkage information for inverted teat defect and mapping information of a candidate gene. Both genes map in QTL regions for inverted teats discovered in our resource family and are involved in proliferation and differentiation processes of mammary gland. We applied a general purpose family-based test for allelic association between inverted teat defect and genotype. We found highly significant evidence for association of RLN and the inverted teat defect affection in the Bonn-Berlin DUMI resource population. No significant effects of TGFB1 on affection status were detected. In addition, parathyroid hormone like hormone gene (PTHLH) was proposed as a functional candidate gene that regulates epithelial mesenchymal interactions during the formation of mammary gland. It could also been shown to be highly significantly associated with affection status. High significantly association was also found between TGFB1, RLN and PTHLH loci and number of teats and number of inverted teats. Further confirmation of these results in independent samples of other populations will be conducted. -patent pending-

F 070
Genomescan for major genes responsible for a hereditary defect of the Mammary gland in pigs

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Mammary gland abnormalities reduce the number of weaned piglets and limit selection intensity in pig breeding programs. With a prevalence of up to 30 % in commercial populations and an average estimated heritability of about 0.2, inverted teats are one of the most important hereditary abnormalities of the mammary gland. The objective of this study was to localize genome regions, which have relevant influence on the number of inverted teats. Based on a reciprocal cross of Berlin miniature pig and Duroc, a F_2-population was established. Besides other traits, data on total number of teats and number of inverted teats was collected from 912 F_2-animals at the age of 120 days. The observed prevalence of inverted teats in this population was 42 %. On the 18 autosomes, 68 microsatellite markers were typed and linkage maps were constructed with the CRIMAP software. After splitting the pedigree into full sib families with a maximum of 12 nonfounders and including grandparents as founder, multipoint linkage analysis of the affection status was performed with GENEHUNTER. Three genome regions with a NPL-score above 3.0 could be identified. Alternatively, the dataset was analyzed with a mixed model, comprising random additive genetic effect and random QTL-effect. In contrast to GENEHUNTER no restriction on pedigree structure was necessary. Essentially, the previous detected significant regions could be confirmed. However, some additional regions with influence on the portion of inverted teats could be localized.

F 071

Bayesian analysis of Quantitative Trait Loci for boar taint in a Landrace outbred population

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The genetic basis of main components of boar taint in intact male pigs has been investigated in a commercial population. We have analyzed data of fat androstenone and skatole levels from 217 males of a Landrace outbred population. Records were normalized using a logarithm transformation, and tested for normality using a Wilk-Shapiro test. Afterwards, we performed a Bayesian analysis to map Quantitative Trait Loci (QTL) in ten candidate regions previously selected on chromosomes 1, 2, 3, 4, 6, 7, 8, 9, 10 and 13. The criterion for QTL detection was the Bayes Factor (BF) between polygenic models with and without QTL effects. Both traits presented a huge genetic determination, with posterior means of total heritabilities ranging from 0.59 to 0.73 for androstenone, and from 0.74 to 0.89 for skatole. Strong evidence for a fat skatole QTL was detected on SSC6 (BF=5.16), and two suggestive QTL in SSC1 and SCC10 (BF=1.523 and 1.602 respectively). However, no QTL for androstenone have been found in any of ten chromosomal regions analyzed. The detection of a QTL for the fat skatole level segregating in this population involves the possibility of applying Marker Assisted Selection or even Gene Assisted Selection after the identification of the causal mutation of the QTL.

F 072

Candidate gene detection of QTL for the number of vertebrae on SSC7

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We had detected two QTL affecting the number of vertebrae on SSC (*Sus scrofa* Chromosome) 1 and 7 using pig F2 experimental families. For the QTL on SSC1, alleles of European breeds have effects increasing the number of vertebrae by 0.55 in average compared with alleles of Asian breeds. The QTL on SSC7 was suggested not to be fixed in European breeds and some of European alleles (Q) had effects increasing the number of vertebrae by 0.65 in average compared with the others (q) and Asian alleles. In a commercial Large White population, we detected a variation of the QTL on SSC7 by a half-sib analysis of sires with microsatellite markers, SW147, SW252 and S0115, in an approximately 10 cM region. In the QTL region on SSC7, PSEN1 and NUMB were located as candidate genes, both are thought to be involved in NOTCH signal transduction system, which controls the formation of somite in embryonic development. We cloned and analyzed swine PSEN1 gene but could not detect any polymorphisms associated with the QTL variation in its transcripts and promoter region (1.5 kb). Then we cloned swine NUMB gene and cDNA, and analyzed its gene structure and polymorphisms. Now we are searching polymorphisms associated with the QTL variation in NUMB transcripts and its promoter region. [This work was supported by the Grant-in-Aid of the Japan Racing Association.]

F 073

Identification of a highly significant QTL for carcass length on porcine chromosome 17

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We have performed a genome scan on a combined F2 backcross family of 8 Swedish Landrace boars and 105 Hampshire sows. One of the most interesting findings so far has been a highly significant QTL for carcass length on SSC17. In a multiple linear regression analysis fitting herd, sex and breed of F1 father as fixed effects, body weight as covariate and estimating additive and dominance effect we obtained an F-value of 42.8 ($p<0.01$) corresponding to a LOD-score of 16.2. The origin of the effect is primarily derived from one particular F0 boar and may therefore be the result of a recent mutation rather than a fixed Landrace QTL effect. Mapping of 20 EST markers within the 95 % confidence interval for the QTL position demonstrates homology to human chromosome 20. Presently, we have covered the QTL-region with three microsatellite markers and 20 SNP markers. We are currently characterizing the region and possible candidate genes.

F 074

Detailed mapping of the porcine chromosome X region harbouring QTL for fat deposition

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The QTL affecting fat deposition on SSCX is well documented in Meishan × Western breed pedigrees. In two German Meishan derived families the QTL has been more pronounced in Wild Boar × Meishan ($W \times M$, $n = 335$) than in Meishan × Piétrain ($M \times P$, $n = 316$) pedigree and mapped between microsatellites *SW259* and *SW1943*. To align human and porcine gene maps for this orthologous region, 10 genes from HSAXq13.1-q24 (70-113 Mb) have been PCR cloned and sequenced. SNPs suitable for linkage analysis and porcine specific primers for RH mapping have been detected within 8 and 9 genes, respectively. Linkage mapping conducted on the USDA-MARC backcross and Hohenheim $W \times M$ and $M \times P$ pedigrees as well as RH mapping on IMpRH panel showed that *RPS4X, XIST, POU3F4, NOX1, FSHPRH1, SERPINA7, ACSL4. PAK3, CAPN6* and *HTR2C* were located within the chromosome region. Though the gene orders on three linkage maps were in agreement with gene order on HSAX, the RH mapping showed that minor rearrangement could not be excluded at the level of resolution used. Association analyses performed with detected SNPs showed that in $W \times M$ the highest association was found between fat cuts (%) and *RPS4X* ($VF_2 = 14.7\%$, $F = 23.6$, $P < 0.0001$) while in $M \times P$ surprisingly between lean cuts (%) and *ACSL4* ($VF_2 = 16.6\%$, $F = 26.9$). (Supported by GA CR 523/04/0106).

F 075

Identification of polar overdominant QTL for growth and body composition traits in pigs

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The Polar Overdominance (POD) model of inheritance was proposed to explain the non-Mendelian expression of the callipyge muscular hypertrophy in sheep. The term "polar" implies an imprinting effect in a cross and "overdominance" implies that the effect is seen only in the heterozygote. The POD mode of inheritance might be involved in other complex genetic phenotypes in mammals. The objectives of this study were to detect POD QTL in a cross of pig breeds and to characterize candidate imprinted genes for the POD phenotypes. The POD candidate gene analysis of the *DLK1* locus in an F2 cross of the Berkshire and Yorkshire breeds of pigs confirmed that the inheritance mechanism of callipyge sheep has a conserved role for growth and fat deposition phenotypes in pigs. Additionally, novel POD QTL were detected on pig chromosomes 6, 10 and 12 for longissimus muscle total lipid, loin eye area, and 10th rib backfat traits respectively ($P<0.05$ at genome-wise level). Based on the comparative gene map between pigs and humans, these porcine POD QTL regions correspond to human chromosomal regions 1p, 10p and 17q respectively. Several candidate-imprinted genes in the homologous mouse and human chromosomal regions are available for further characterization of the identified porcine POD QTL (http://fantom2.gsc.riken.go.jp/imprinting/). Further analysis of POD QTL will be important to understand the complex genetic mechanisms in mammals and to develop optimum mating programs for more desirable phenotypes in commercial livestock breeding programs.

F 076

Genome scan for loci affecting pork quality in a Duroc-Landrace F_2 population

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A genome scan was conducted on 371 F_2 Duroc-Landrace pigs with 182 microsatellite markers. Breed of origin of chromosomal segments inherited in the F_2 progeny were predicted using GenoProb. Statistical tests for QTL were conducted on 43 phenotypes with SAS. The probability of inheriting a Duroc allele from the sire and the probability of inheriting a Duroc allele from then dam were used to develop covariates for additive and dominance effects or maternal and paternal imprinting effects. Fixed effects fitted were sex and slaughter age. Carcass weight, RYR1 genotype and/or PRKAG3 genotypes also were included in some analyses. Subjective and objective measures of composition and pork quality were recorded. The two regions with the greatest evidence of containing QTL were located on chromosomes 2 and 17. Chromosome 2 (60-66 cM) had two significant QTL detected for taste panel tenderness scores along with suggestive QTL for pH at 1 hr postmortem, intramuscular moisture content, slice shear force at 7 days postmortem and taste panel amount of connective tissue. Chromosome 17 harbored a significant QTL affecting loineye area along with suggestive QTL for intramuscular fat content determined by chemical analysis and subjective marbling score at position 32-39 cM. While the Duroc allele increased marbling and muscling at the chromosome 17 region, the Landrace allele was more desirable for chromosome 2. Follow-up studies are necessary to validate these QTL and determine their utility to the commercial industry.

F 077

Quantitative trait loci mapping for fatty acid contents on porcine autosomes

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A genome scan using microsatellite markers was conducted to detect quantitative trait loci (QTLs) for 10 fatty acid contents of backfat in a Landrace * Yorkshire F2 pig population. The fatty acids investigated in this study were myristic, palmitic, palmitoleic, stearic, oleic, octadecenoic, linoleic, linolenic, eicosenoic, and eicosadienoic acids. Two QTLs were found in SSC7 and SSC18. In SSC7, we found that the QTL influenced both stearic acid and linoleic acid, and their likelihood ratio values were both larger than a genomewide significance threshold generated by permutation algorithm with 20,000 replicates ($P<0.05$). The other QTL on SSC18 was significant ($P<0.05$) only for myristic acid, which concurred with a previous finding. The 24% and 29% of phenotypic variation was explained respectively for stearic acid and linoleic acid by the QTL on the SSC7, and the 20% for myristic acid by the QTL on the SSC18. Three suggestive QTLs were also found in SSC1, SSC4, and SSC6. Further studies on fine mapping and positional comparative candidate gene analyses will be the next step toward better understanding the genetic architecture of fatty acid contents.

F 078

From meat quality QTL to positional candidate genes

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The objective of this study was to map QTL for meat quality traits in a commercial porcine pedigree. The design of the project is based on a five line cross. 19 hybrid boars (Piétrain+ x (Hampshire × Piétrain)) were mated to 52 hybrid sows (Leicoma x (Large White × Landrace). Their 333 progenies were fattened on our experimental farm and slaughtered at 115 kg live weight. For these animals meat quality traits as for example pH-value, conductivity and reflectance values were recorded. A genome scan covering all chromosomes with 159 microsatellite markers and 3 class-I-markers was performed. Marker linkage maps were computed with CriMap and used for interval mapping using a least-squares regression model in parental half-sib families. Effects for pH-values were detected on chromosome 4, 6, 7, 9, 10, and 15. Using the comparative human-porcine map and physiological information two calcium channel genes, *CACNA1S* and *CACNA2D1*, were identified as positional candidate genes. In the next step the IMpRH panel was used to map the *CACNA2D1*gene. It was mapped on SSC9 close to the QTL-region and therefore our hypothesis was supported that this gene could be identical with the QTL. Furthermore a confirmation study with additional resource families is under way and will give more precise information about the location of the QTL.

F 079

QTLs affecting egg production rate in Chickens

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The genome-wide QTL analysis of egg production rate at 400 days of age was performed on 267 F_2 individuals of the chicken resource family made from a cross of Oh-Shamo (Japanese Large Game) and White Leghorn breeds in Hiroshima University. The Oh-Shamo is a large Japanese native chicken for cockfighting. The primer pairs of 50 microsatellite DNA markers were obtained from Dr. H.H. Cheng, USA. We are grateful to Dr. Cheng for his kind supply. A linkage map of the markers was constructed with the software Map Manager QTX using the Kosambi map function. Simple interval mapping was performed with the software QTL Cartographer (windows version 1.17e). According to van Ooijen (1999), genome-wide significant threshold levels were calculated: 4.4 at 5% and 5.2 at 1%. We identified two highly significant QTLs affecting egg production rate on chicken chromosomes 3 and 4. LOD scores for the chromosomes 3 and 4 QTLs were 7.8 and 6.6, respectively. Because of sparse marker spacing around the two QTLs, their parameter estimates may not be correct. Thus, we are now genotyping additional markers to characterize the QTLs more precisely and to map additional new QTLs, the results of which will be presented in the conference.

F 080

Quantitative trait loci affecting egg shell strength using an F2 population derived from a strong egg shell line X weak egg shell line of White Leghorn

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Broken and cracked eggshells cause major economic losses for egg production. An F2 population of 262 hens, obtained by crossing a strong egg shell line with a weak egg shell line of White Leghorn, was used for detecting Quantitative Trait Loci (QTL) affecting egg character. The two lines were developed from the same line, D2 (Okazaki Station, National Livestock Breeding Center of Japan), by two-way selection for egg shell strength with nondestructive deformation (Nirasawa *et al.*, J. Anim. Breed. Genet. 115, 1998). 1,014 microsatellite markers including 696 ABR markers were tested. Of the markers, 35 markers were informative and mapped on 10 autosomal linkage groups. There was no informative marker on chromosome Z. The QTL for 7 traits (i.e. body weight, egg weight, short length of egg, long length of egg, shell strength (SS), shell thickness (ST) and shell weight (SW)) were identified. Highly significant ($p<0.01$) QTL for SS, ST and SW were identified a region flanking with ABR362 and ABR545. These QTL are good candidates for the reduction of broken and cracked eggs in commercial layers by marker assisted selection.

F 081

Identification of quantitative trait loci for carcass composition in chicken using genomic scanning

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A genomic scanning to detect quantitative trait loci (QTL) affecting carcass composition was pursued. The F2 resource population was established from a reciprocally cross of the White Plymouth Rock and the Silky. Over 2,700 F2 offspring from six hatches were reared to slaughter at 12 week of age. 369 individuals from 15 families were genotyped for 134 microsatellite markers. Interval mapping QTL analyses were carried out. For carcass weight, a QTL was identified on GGA4. For eviscerated yield with giblet weight, a QTL was identified on GGA4. For eviscerated yield weight, two QTL were identified on GGA1 and GGA4. For breast muscle weight, a QTL was identified on GGA4. For leg muscle weight, a QTL was identified on GGA4. For shank and claw weight, five QTL were identified on GGA1, GGA4, GGA7, GGA11 and GGA14. For wing weight, a QTL was identified on GGA4. For heart weight, a QTL was identified on GGA3. For liver weight, a QTL was identified on GGA1. For muscular stomach weight, two QTL were identified on GGA1 and GGA2. For glandular stomach weight, two QTL were identified on GGA3 and GGA17. For small intestine length, two QTL were identified on GGA4 and GGA8. The most interesting area was on GGA4, where highly significant QTL effects were detected for carcass weight, eviscerated yield with giblet weight, eviscerated yield weight, breast muscle weight, leg muscle weight, shank and claw weight and small intestine length in the same area.

F 082

Specific DNA microsatellite marker allele affecting reduction of abdominal fat deposition in chickens

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One QTL affecting abdominal fat deposition in chickens was mapped at 147 cM on chromosome 7, using a F2 population from a cross between a Japanese native breed and a broiler breed. The LOD score and the variance explained of this QTL was 11.3 and 36.7%, respectively. The closest loci to the QTL were MCW0316 and ADL0169. In MCW0316, there were 2 alleles (A:165bp, B:179bp) and 3 genotypes (AA:60birds, AB:114, BB:48). The ratio of the F2 abdominal fat weight to live body weight at 16 weeks of age (abdominal fat %) was higher in AA (6.88%) than in AB (5.49%) and BB (5.50%) ($P<0.0005$). The abdominal fat % in AA was 25% higher than that of AB and BB. In ADL0169, there were 2 alleles (A:101bp, B:107bp) and 3 genotypes (AA:77birds, AB:95, BB:48). The abdominal fat % was higher in AA (6.82%) than in AB (5.27%) and BB (5.41%) ($P<0.0001$). The abdominal fat % in AA was 30% higher than that of AB and BB. In both markers, when the number of A alleles was 4 (38 birds), 3 (44 birds), 2 (85 birds), 1 (26 birds) or 0 (27 birds), the abdominal fat % was 7.60%, 6.10%, 5.37%, 5.06% and 5.40%, respectively. The abdominal fat % of birds with 4 A alleles was 50% higher than that of birds with 1 A allele. It seems that the presence of the B allele in MCW0316 and ADL0169 was associated with a decrease in abdominal fat deposition in chickens. The B allele is inherited dominantly. It suggests the possibility of marker-assisted selection for abdominal fat deposition in chickens by testing for the presence of this allele.

F 083

Genetic mapping of quantitative trait loci affecting carcass traits in F2 intercross chickens

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We constructed a chicken F2 resource population to facilitate the genetic improvement of economically important meat production. An F2 population comprising 265 chickens obtained by crossing a Shamo (lean, light-weight Japanese native breed) male and White Plymouth Rock breed (fat, heavy-weight broiler) females was recorded carcass weight (CW), abdominal fat weight (AFW), and percentage abdominal fat (PAF). These traits were adjusted for sex. Genotypes for 250 microsatellite markers to detect quantitative trait loci (QTL) were determined. Two hundred thirty four markers were mapped on 25 autosomal linkage groups, and 5 markers on 2 Z chromosomal linkage groups. For CW, two significant QTLs were detected on chromosomes (Chrs) 1 and 3. For AFW, two significant QTLs were detected on Chrs 1 and 5. For PAF, two suggestive QTLs were detected on Chrs 1 and 5. The QTLs detected for carcass traits on Chrs 1 and 5 were in the same regions. Interestingly, the region on Chr 3 detected highly QTL effect has been characteristic for CW but not AFW and PAF. These QTL regions need to be further narrowed to find candidate genes for carcass traits.

F 084

Chicken resource populations used for QTL mapping of growth and meat quality traits

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Using F_2 design of outbreed in the present study, the parental lines that included Xinghua Chicken (A), Recessive White Rock Cock (B) and Silkie Chicken (C) were used for reciprocal cross. Four cross combinations of A♂×B♀, B♂×A♀, A♂×C♀, C♂×A♀ were performed. F_2 populations were produced, in which two groups of full-sib individual and half-sib individuals were included. As a result, four chicken resource populations for mapping QTL of growth and meat quality trait were built-up. The analysis of phenotypic value of varied traits for F_2 populations showed that the variations of growth and meat quality traits were very great in divergent populations. Meanwhile, the chicken resource populations had enough individuals and groups (Table 1).

Table 1 Individual numbers and family numbers in F_2 populations

	Group 1	Group 2	Group 3	Group 4
Individual number	497	984	539	815
Family number	17	11	19	10

F 085

Genome-wide survey of chicken genomic regions involved in genetic resistance to Rous sarcoma virus

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Rous sarcoma virus (RSV) is an avian retrovirus that causes chicken sarcoma and leukemia. Two inbred chicken lines (#01 and #11) which have the different resistibility to RSV are kept at the National Livestock Breeding Center in Japan. RSV injection into the wing webs does not result in tumor-formation in the #01 line, but does in the #11 line, although the tumor is reduced with time. The present study investigated the genomic regions responsible for the resistance to RSV using a two-dimensional genomic differential cloning technique with two inbred resistant and a susceptible chicken lines. The two-dimensional electrophoretic pattern comprised about 850 separate spots in each line, and 60 spots displayed genomic differences detected as an appearance/disappearance change or altered intensity of a spot among the three lines. Of these spots, a #11 line-specific spot (spot 18) was cloned and sequenced. DNA sequence analysis and homology search against DNA databases revealed that spot 18 is identical to a portion of a certain gene which is homologous to a human homeotic gene involved in differentiation of hepatocytes and thyroid tumorigenesis. In addition, five nucleotide substitutions in the genomic region surrounding spot 18 were examined using PCR-RFLP genotyping method. Results of PCR-RFLP analyses for these sites allowed clear differentiation of the three chicken lines by haplotype.

F 086

Identification of candidate genes for exploratory behaviour in chickens: a preliminary SNP analysis guided by bioinformatic methods

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Feather pecking and cannibalism are important problems in free-range housing of chickens. In attempting to uncover the genetic predisposition for feather pecking, we consider it as a redirected exploratory behaviour, whereby the lack of opportunity for normal exploration might represent the trigger leading to aggressive feather pecking and cannibalism. Our research merges bioinformatics, molecular genetics, and behavioural observation experiments. Because robust QTL for feather pecking or cannibalism have yet to be found in chickens, we used bioinformatics to generate a ranked list of over 400 potential candidate genes based on correlations between terms describing the behaviours and a diverse range of known functional genomic information. To search for promising single nucleotide polymorphisms (SNPs), we assembled a panel of DNA samples (mostly collected by the AVIANDIV project) from 12 genetically diverse chicken breeds including Red Jungle Fowl. The most likely candidate genes have been cloned and physically mapped by FISH, providing full genetic characterization of the locus in question. Initial analysis of these genes revealed high levels of polymorphism. Continued study will include quantitative behavioural observation experiments to further integrate functional information with the molecular data.

F 087

Detection of QTL for production traits in Japanese quail

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The Mendelian and quantitative genetics of Japanese quail have been extensively studied, but the use of modern markers is only at its beginning in this species. In the present QTL study, 438 F2 quail were obtained from 10 F1 males and 30 F1 females which were born from 10 single pair reciprocal matings between a high emotivity line and an experimental egg line. Individual body weight, feed intake, egg production and egg quality traits, rectal temperature and tonic immobility were recorded in the F2. A partial genome scan was carried out using 58 microsatellite loci assigned to 13 linkage groups for a total length of 576 cM. Interval mapping was performed using a half-sib approach with a single QTL model. QTLs with a "suggestive" level of significance were obtained for feed intake, 5-wk bodyweight and yolk weight on the Q01 linkage group, for clutch length, egg number and egg weight on Q02, for egg number, rectal 5-wk temperature and tonic immobility on Q04, for shell weight on Q05, for 5-wk body weight on Q08, for feed intake, ratio yolk/albumen, yolk weight, shell weight and egg weight on Q10, for age at first egg on Q11, for 5-wk body weight and age at first egg on Q12, and for body weight, feed intake, egg weight and yolk weight on QZ. "Genome-wide" significant QTL for body weight were found on Q01 and QZ.

F 088

Analysis of FADS1 as candidate gene for egg yolk fatty acid profiles and ω3 fatty acid contents in Japanese quails

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A lower ratio of ω6:ω3 fatty acids with increased docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) content in human nutrition improves health. Poultry is able to synthesize EPA and DHA and to deposit it in the yolk. There is minor knowledge on the genetic variation of fatty acid profiles in egg yolk and whether and to which extent the ω6:ω3-ratio could be reduced by breeding. The aim of this study is to elucidate the genetic basis of variation of the ω3 and ω6 fatty acid contents of egg yolk by estimation of genetic parameters, identification and characterisation of functional candidate genes for traits related to fatty acid profiles of egg yolk. Therefore mass selection was performed to establish 8 divergently selected lines of Japanese quails with high and low ω6:ω3-ratio differing by 2.4 units in the ω6:ω3-ratio, i.e. 1.6 phenotypic and 4 genetic standard deviations. Subsequently, three more generations of pedigree selection were done. Moderate heritability of ω6:ω3-ratio and ω3 contents were achieved (h^2~.3). Fatty acid desaturases (FADS) regulate unsaturation of fatty acids through the introduction of double bonds between defined carbons. Quail cDNA sequence of FADS1 was obtained and SNPs were detected by comparative sequencing animals of divergent lines. Validation of possible association of FADS1 with yolk fatty acid profiles will be conducted in all 8 selection lines. Liver expression profiling will be done to identify other candidate genes.

F 089

Multitrait QTL mapping of traits related to body weight and growth in a cross between NMRI8 and DBA/2 mice using least squares

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Multitrait QTL analyses have been shown to increase the power to detect pleiotropic QTL and the precision of location estimates. They also enable tests to be performed to determine whether the same or different genes are influencing the traits being analysed. Data were available for body and organ weights in 275 F2 mice generated from an intercross between the high body weight selected mouse line NMRI8 and the inbred line DBA/2. The original lines differed 2.5 fold in body weight at the age of 6 weeks. Body weights were recorded at weekly intervals from 2 to 6 weeks of age and organ weights at 6 weeks. Within the F2 population, the correlations between body weight and weights of abdominal fat weight, muscle, liver, and kidney were about 0.8 for both sexes. A least squares multitrait QTL analysis was performed on these data to investigate the underlying mechanism for the genetic control of these traits. In particular, to understand the cause of the genetic correlation between the body weight traits and whether this is due to pleiotropic QTL affecting body weight throughout life or whether different, linked QTL, are responsible for weights at different life stages. An additional objective was to explore whether the same genes controlling body weight also affect organ weight. Models were considered with one or two pleiotropic QTL or with linked QTL affecting the traits.

F 090

A genetic map of quantitative trait loci for postnatal growth in an intersubspecific backcross of C57BL/6J and Philippine wild mice

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A genome-wide search for quantitative trait loci (QTLs) affecting postnatal mouse growth was carried out in an intersubspecific backcross population (n=352) derived from a cross between the common inbred strain C57BL/6J and wild mice captured in the Philippines. Single-trait QTL analyses of 8 body weights recorded weekly from 3 to 10 weeks of age and 2 weight gains recorded between 3 and 6 weeks and between 6 and 10 weeks identified 17 main-effect QTLs on 11 chromosomes at the genome-wide 5% level. Interestingly, 4 of these QTLs had epistatic interaction effects. In addition, 6 new QTLs having only interaction effects were found on different chromosomal regions at the genome-wide 5% level. Of the 23 identified QTLs (including main effects and/or epistatic interaction effects), several showed sex-specific effects on postnatal growth. Most of the identified QTLs exerted their effects during either the early or late growth phases. The wild mice showed about 60% lower adult body weight than C57BL/6J; wild-derived alleles enhanced growth at 2 QTLs but reduced growth at all other identified QTLs. Individual QTLs explained 3-12% of the total phenotypic variance. These results suggest that the identified QTLs may provide new insights into the complex genetic networks of postnatal growth, and also that wild mouse populations may be used as important new sources of variable QTLs.

F 091

Quantitative trait loci for proteinuria and glomerulosclerosis in the FGS mouse model

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FGS (Focal Glomerular Sclerosis) is one of the most common and nonspecific patterns of glomerular injury in humans, and is associated with proteinuria, steroid resistance, hypertension and progressive loss of renal function. The FGS mouse strain, FGS/Kist, was reported as a new model for renal disease. This mouse develops both FGS and proteinuria at 6 weeks after birth, and kidney failure occurs within one year. In the present study, to identify quantitative trait loci for FGS- related traits, proteinuria and glomerulosclerosis were selected as the representative characterization on FGS. The backcross population of 368 animals was generated from a cross between FGS/Kist and the standard normal strain C57BL/6J. By QTL analyses, we detected four proteinuria QTLs (*Ptnu1-4*) and one glomerular sclerosis index QTLs (*Gsi1*) at the genome-wide 5% levels. *Ptnu1* and *Gsi1* were located on Chromosome (Chr) 8, and *Ptnu2* on Chr 17. Of these QTLs, *Ptnu1* and *Ptnu2* interacted epistatically with each other. *Ptnu3* on Chr 1 and *Ptnu4* on Chr 9 without main effect both interacted with *Ptnu2*. At most of the identified QTLs, homozygotes for alleles derived from FGS/Kist increased proteinuria and GSI values. Uniquely at *Ptnu3*, heterozygotes increased the proteinruia level. These results suggest that complications of various FGS are caused by combinations of susceptibility and resistant genes.

F 092

Genetic investigations of exocrine pancreatic insufficiency in the Eurasian dog breed

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A hereditary disease of the pancreas has been observed in the Eurasian dog breed. Affected dogs show polyphagia, weight loss and voluminous faeces of light colour due to the lack of pancreatic enzymes. The disease shows great similarity to the pancreatic acinar atrophy of the German shepherd breed in which an autosomal recessive mode of inheritance has been demonstrated. Cholecystokinin (*CCK*) is a human brain/gut peptide. In the gut, it induces the release of pancreatic enzymes making it a possible candidate gene for pancreatic insufficiency. The *CCK* gene has been mapped to HSA 3pter-p21, a region orthologous to CFA20 11-15.2 and CFA23 12.1-24. In the present study we performed a complex segregation analysis in two families of Eurasian dogs segregating for pancreatic insufficiency. The segregation analysis as well as our pedigree observations supported the autosomal recessive mode of inheritance. To evaluate the *CCK* candidate gene, we performed a linkage analysis with five microsatellite markers (PEZ19, C20.610, FH2625, CPH16, FH2148) spanning the relevant region of CFA20. No linkage was observed between exocrine pancreatic insufficiency and the microsatellite markers. To finally include or exclude *CCK* as a candidate gene, markers on CFA23 remain to be evaluated.

F 093

Genetic tests for domestic cats

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Domestic cats, both random bred and fancy breed, are the most popular pet in the USA and in many regions of the world. Although fancy breeds represent 20% of the cat population or less, Persian cats constitute about 80% of fancy bred cats worldwide. Several genetic tests have been developed for cats, particularly ones that are useful for the Persian breed. Approximately 40% of Persians, worldwide, are afflicted with autosomal dominant polycystic kidney disease (PKD). This suggests that approximately 6% of the world's domestic cat population suffers from PKD, making it one of the most prominent inherited diseases in most any species. The mutation that causes feline PKD has been identified in the gene that causes 85% of human PKD, *PKD1*. The identified mutation causes a stop codon, disrupting that last 30% of the protein. Additionally, Persian cats have a variety of coat colors, many which have recessive alleles that are highly desirable. Mutations have been identified in the gene for tyrosinase, TYR, that control the "pointing" coloration of Persians (Himilayan). The Burmese and complete albino alleles have also been identified. The albino allele could be used to replace dominant white, which is associated with deafness in cats. A variety of techniques have been explored, including RFLPs, sequencing, pyrosequencing, and dHPLC, to evaluate the best methods for testing. Each test includes use of a feline DNA fingerprinting panel that is undergoing Comparison Testing for the 2004 ISAG meeting.

F 094

Construction of a microsatellite linkage map and identification of two QTL associated with spawning date in Ayu (*Plecoglossus altivelis*)

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We developed 175 microsatellite markers to construct a genetic linkage map and search for quantitative trait loci (QTL) associated with spawning date in Ayu (*Plecoglossus altivelis*). We used five backcross families (A, B, C, D and E) produced by crossing between two outbred strains that spawn in different periods for this study. We constructed a genetic map using 163 microsatellite markers in backcross family C. The parental male linkege map consisted of 33 linkage groups while the female map consisted of 28 linkage groups, with the an average resolution of 4.5 cM and 6.5 cM respectively. We have mapped fifteen QTL markers (PalAyu25TUF, PalAyu60TUF, PalAyu103TUF, PalAyu110TUF, PalAyu125TUF, PalAyu161TUF, PalAyu182TUF, PalAyu232TUF, PalAyu244TUF, PalAyu248TUF, PalAyu284TUF, PalAyu311TUF, PalAyu493TUF, PalAyu525TUF and PalAyu526TUF) for spawning date representing six linkage groups (1, 3, 11, 21, 24, 29) in backcross family C. Four of these QTL markers (PalAyu125TUF, PalAyu161TUF, PalAyu232TUF and PalAyu311TUF) located on two linkage groups (11, 24) show significant value in another backcross families. These results suggest this trait is polygenic, and these QTL markers could be possible to use in marker-assisted selection for other genetic backgrounds.

F 095

A genetic linkage map of amago salmon (*Oncorhynchus masou ishikawae*) and mapping of two QTL associated with smoltification

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Amago salmon (*Oncorhynchus masou ishikawae*) is Japanese endemic species in salmonids. We constructed a genetic linkage map using 110 microsaterllite markers, and identified two quantitative trait loci (QTL) associated with smoltification in amago salmon. We searched for linkage among 110 microsatellite markers used to construct the male genetic linkage map in backcross family (F_1; sire) of amago salmon, produced by crossing high-smoltification (C3) and low-smoltification (G4) strains. Four QTL markers (Omi127/iTUF, Omi174/iTUF and OmiFGT8/iTUF on linkage group 3 and Omi65TUF on linkage group 21) were mapped by detecting an association between smoltification and alleles in the sire. Among three QTL markers on linkage group 3, no recombination was observed. Recombination rates in male salmonids are repressed relative to females in centromeric regions of the chromosome. Block segregation of large chromosomal regions is thus common in male salmonids for most of the intertelomeric regions of a linkage group. Therefore in other backcross family (F_1; dam), we searched for linkage among 3 QTL markers on linkage group 3 to analyzed marker-trait associations in detail. We found Omi174/iTUF closely linked QTL associated with smoltification on linkage group3.

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