

Advances in DNA markers in cherry: From the S-locus to a genome-wide array

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DNA markers in sweet cherry

Self-incompatibility locus

- **34 *S*-alleles identified to date**
- **Within these *S*-alleles, 6 pollen-part mutants (***SFB: S*** haplotype-specific F-box protein)**

& 5 stylar part mutants (*S*-RNase) **were identified**

At the same time, polymorphic genome-wide markers for cherry were not available.



Advances in genome-wide DNA markers for cherry were possible due to the available peach genome sequence



NATURE GENETICS | ARTICLE OPEN

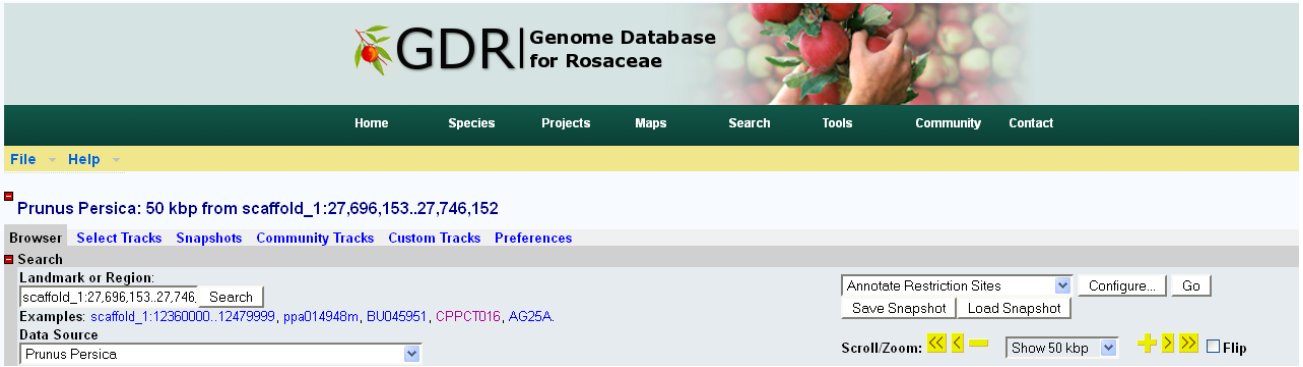
日本語要約

The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution

The International Peach Genome Initiative, Ignazio Verde, Albert G Abbott, Simone Scalabrin, Sook Jung, Shengqiang Shu, Fabio Marroni, Tatyana Zhebentyayeva, Maria Teresa Dettori, Jane Grimwood, Federica Cattonaro, Andrea Zuccolo, Laura Rossini, Jerry Jenkins, Elisa Vendramin, Lee A Meisel, Veronique Decroocq, Bryon Sosinski, Simon Prochnik, Therese Mitros, Alberto Policriti, Guido Cipriani, Luca Dondini, Stephen Ficklin, David M Goodstein + *et al.*

Outline

- **Rosaceae COS markers**
- **High Density SNP markers**
- **Application – Why are some cherry fruit large and some small?**



Cherry markers aligned to the peach genome sequence (www.rosaceae.org)

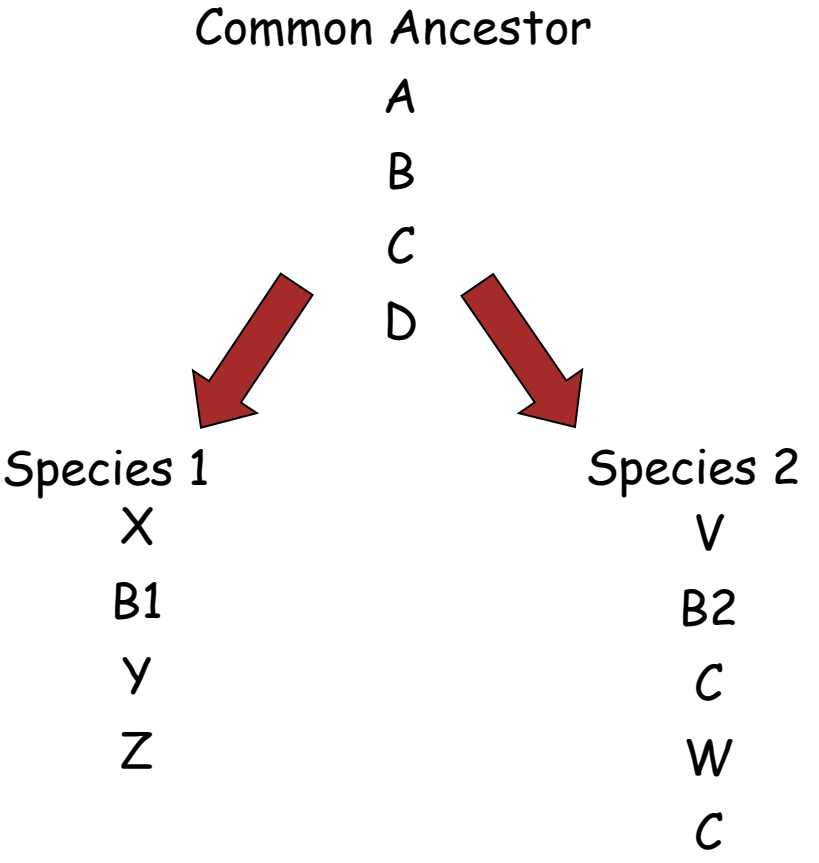


Conserved Ortholog Set (COS) markers

- Orthologous: common evolutionary origin, conservation in sequence
- Single copy

<u>Strategy used for the identification of the Rosaceae- COS</u>

The COS set was comprised of Rosaceae single copy genes (ESTs) that were orthologous to the single copy genes in the Arabidopsis genome.



627 RosCOS were identified

To identify RosCOS polymorphic in sweet cherry, all these RosCOS were sequenced from a diverse panel of sweet cherry cultivars.

Emperor Francis	Krupnoplodnaya
Lambert	Cristobalina
Regina	New York 54

282 polymorphic sweet cherry RosCOS were identified

BMC Genomics

BioMed Cent

Research article

Open Acce

Development and bin mapping of a Rosaceae Conserved Ortholog Set (COS) of markers

Antonio Cabrera[1], Alex Kozik[2], Werner Howad[3], Pere Arus[3], Amy F Iezzoni[4] and Esther van der Knaap*[1]

Illa et al. BMC Evolutionary Biology 2011, 11:9
http://www.biomedcentral.com/1471-2148/11/9

BMC Evolutionary Biology

RESEARCH ARTICLE Open Access

Comparative analysis of rosaceous genomes and the reconstruction of a putative ancestral genome for the family

Eudald Illa[1], Daniel J Sargent[2], Elena Lopez Girona[2], Jill Bushakra[3], Alessandro Cestaro[4], Ross Crowhurst[5], Massimo Pindo[4], Antonio Cabrera[6], Esther van der Knaap[6], Amy Iezzoni[7], Susan Gardiner[3], Riccardo Velasco[4], Pere Arús[1], David Chagné[3], Michela Troggio[4*]

Based on the position of 129 orthologous markers

Prunus

Fragaria

Hypothetical ancestral Rosaceae genome : 9 chromosomes

Malus

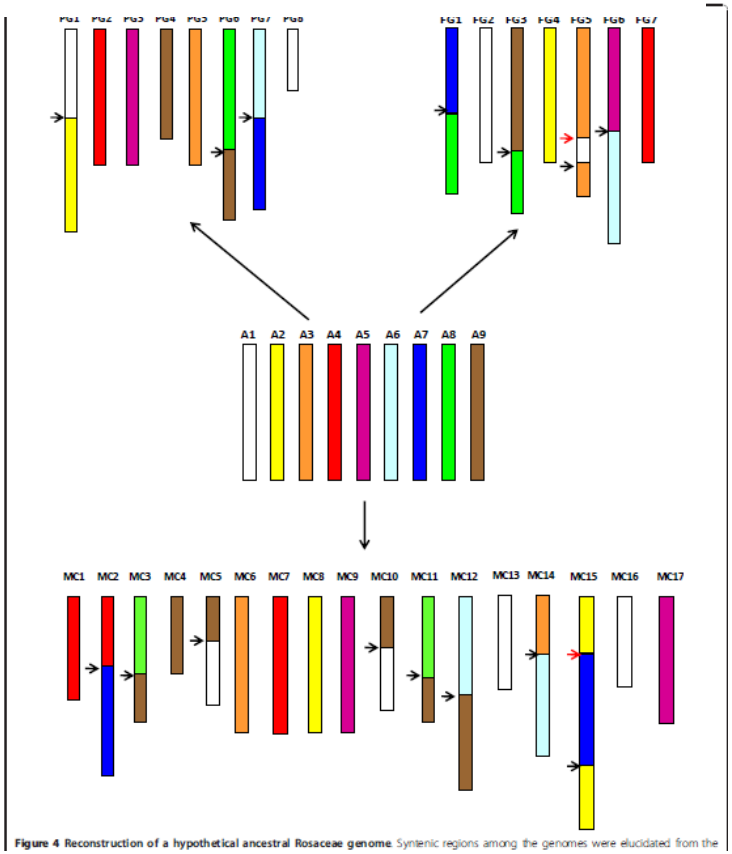
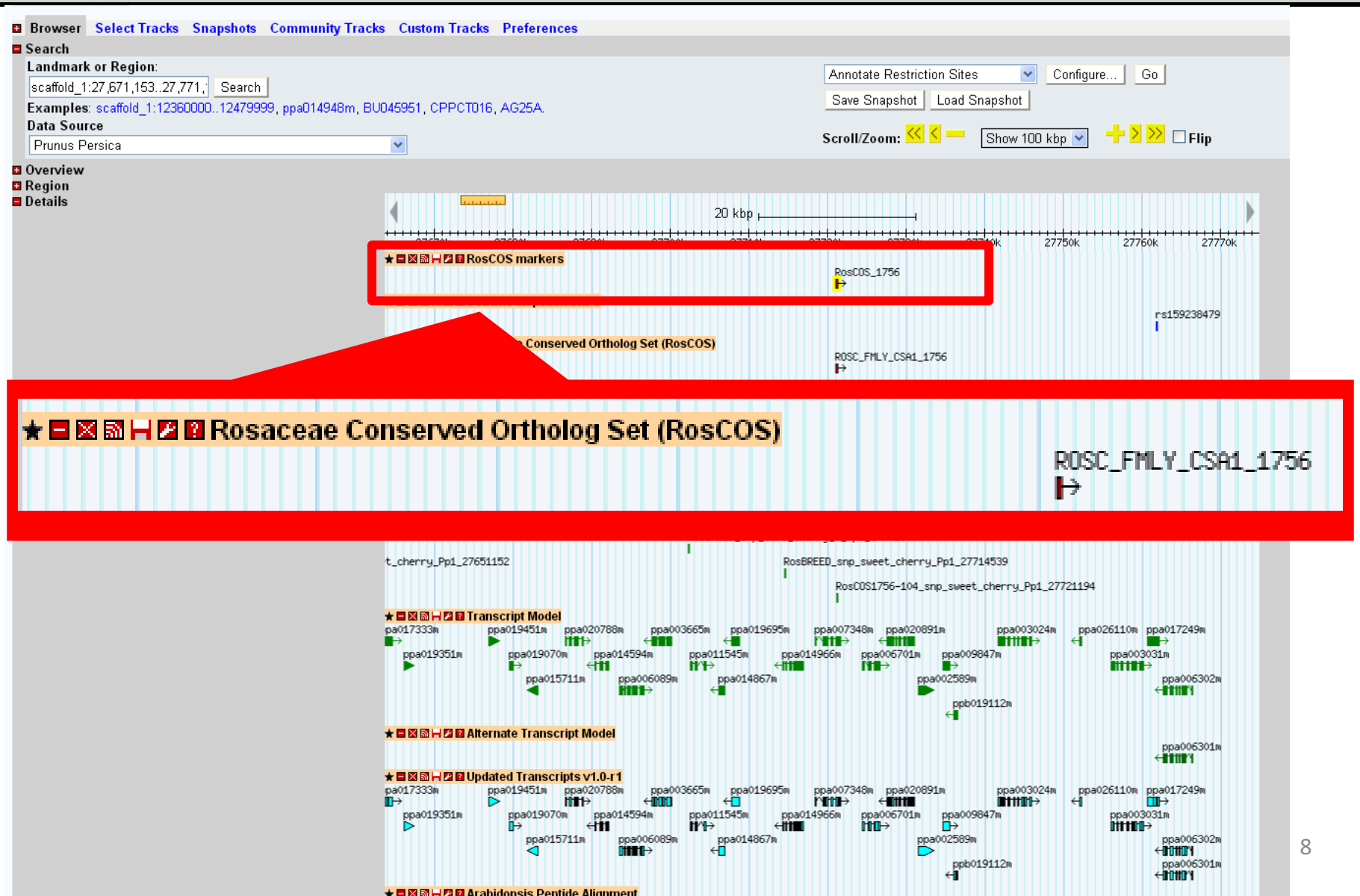


Figure 4 Reconstruction of a hypothetical ancestral Rosaceae genome. Syntenic regions among the genomes were elucidated from the

Gbrowse view of the peach sequence with RosCOS highlighted



Outline

- Rosaceae COS markers
- High Density SNP markers
- Application – Why some cherry fruit are large and some fruit are small.



RosBREED

Enabling marker-assisted breeding in Rosaceae



MSU
Amy Iezzoni (PD)
Jim Hancock
Dechun Wang
Cholani Weebadde

Univ. of Arkansas
John Clark

WSU
Cameron Peace
Dorrie Main
Kate Evans
Karina Gallardo
Raymond Jussaume
Vicki McCracken
Nnadozie Oraguzie
Mykel Taylor

Univ. of Minnesota
Jim Luby
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Alexandra Stone

USDA-ARS
Nahla Bassil
Gennaro Fazio
Chad Finn

Plant Research Intl, Netherlands
Eric van de Weg
Marco Bink

Cornell
Susan Brown
Kenong Xu

Clemson
Ksenija Gasic
Gregory Reighard

Texas A&M
Dave Byrne

Univ. of CA-Davis
Tom Gradziel
Carlos Crisosto

Univ. of New Hamp.
Tom Davis

Genome Wide SNP arrays

- Genome-scanning SNP arrays developed and utilized for apple (8K), peach (9K) and cherry (6K) by international RosBREED-led efforts



OPEN ACCESS Freely available online — PLoS one

Genome-Wide SNP Detection, Validation, and Development of an 8K SNP Array for Apple

David Chagné[1], Ross N. Crowhurst[2], Michela Troggio[3], Mark W. Davey[4], Barbara Gilmore[5], Cindy Lawley[6], Stijn Vanderzande[4], Roger P. Hellens[2], Satish Kumar[7], Alessandro Cestaro[3], Riccardo Velasco[3], Dorrie Main[8], Jasper D. Rees[9], Amy Iezzoni[10], Todd Mockler[11], Larry Wilhelm[12], Eric Van de Weg[13], Susan E. Gardiner[1], Nahla Bassil[5], Cameron Peace[8]*



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Development and Evaluation of a 9K SNP Array for Peach by Internationally Coordinated SNP Detection and Validation in Breeding Germplasm

Ignazio Verde[1]*[9], Nahla Bassil[2]*[9], Simone Scalabrin[3], Barbara Gilmore[2], Cynthia T. Lawley[4], Ksenija Gasic[5], Diego Micheletti[6], Umesh R. Rosyara[7], Federica Cattonaro[3], Elisa Vendramin[1], Dorrie Main[8], Valeria Aramini[1], Andrea L. Blas[6], Todd C. Mockler[9,10], Douglas W. Bryant[9,11], Larry Wilhelm[12], Michela Troggio[13], Bryon Sosinski[14], Maria José Aranzana[6], Pere Arús[6], Amy Iezzoni[7], Michele Morgante[3,15], Cameron Peace[8]*



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Development and Evaluation of a Genome-Wide 6K SNP Array for Diploid Sweet Cherry and Tetraploid Sour Cherry

Cameron Peace[1]*, Nahla Bassil[2], Dorrie Main[1], Stephen Ficklin[1], Umesh R. Rosyara[3], Travis Stegmeir[3], Audrey Sebolt[3], Barbara Gilmore[2], Cindy Lawley[4], Todd C. Mockler[5], Douglas W. Bryant[5,6], Larry Wilhelm[7], Amy Iezzoni[3]

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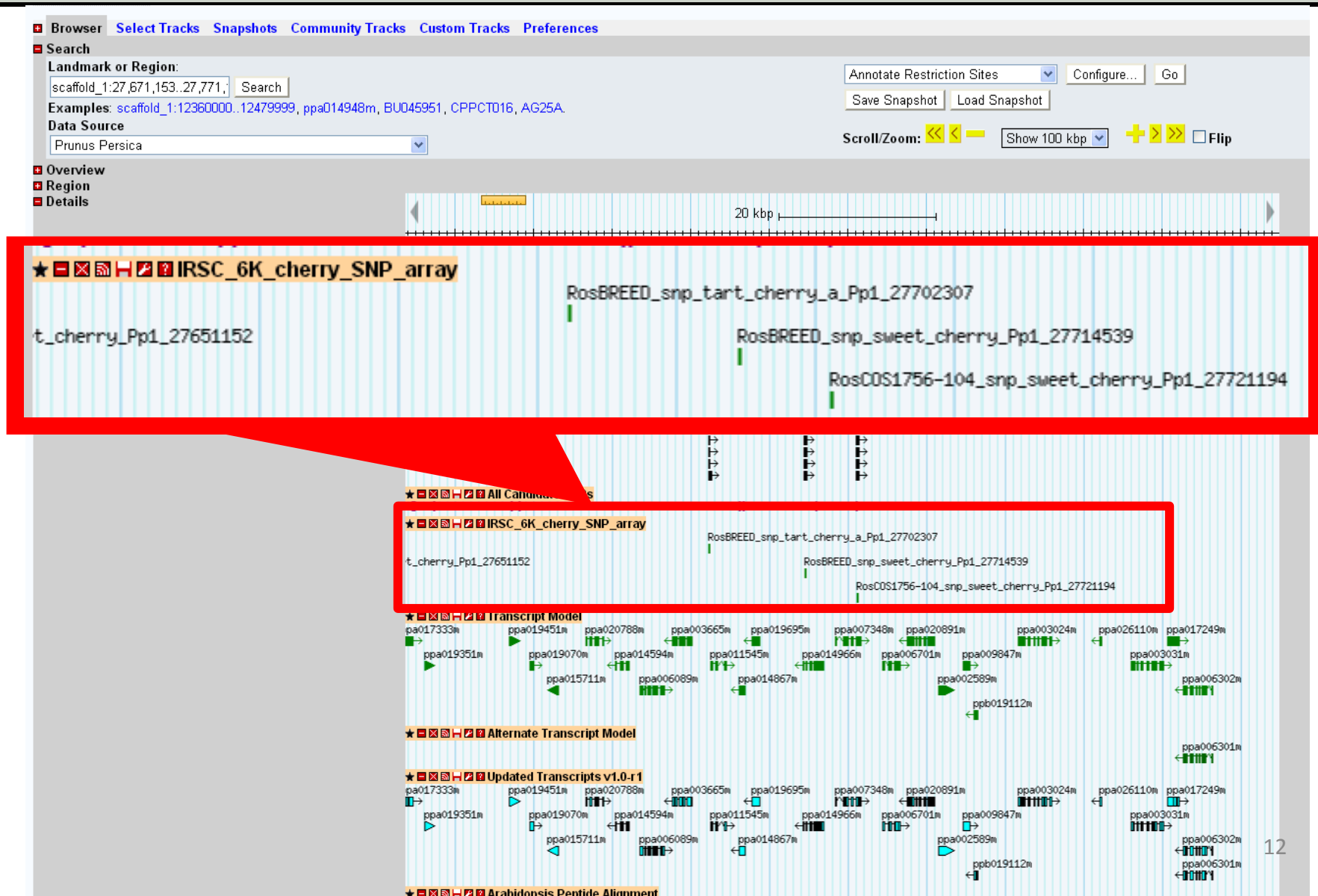


NIFA USDA
United States Department of Agriculture
National Institute of Food and Agriculture

RosBREED
Enabling marker-assisted breeding in Rosaceae
www.rosbreed.org

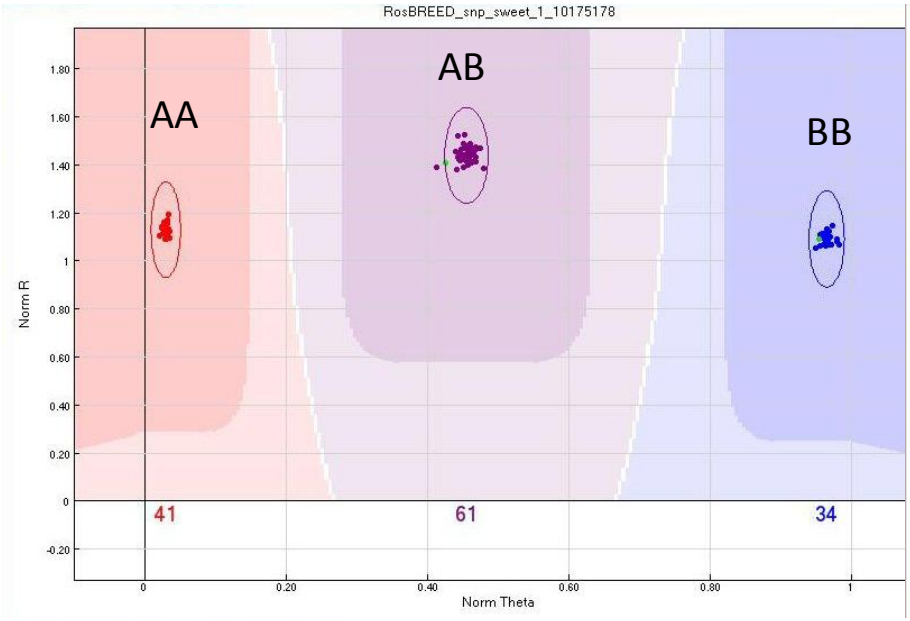
illumina®

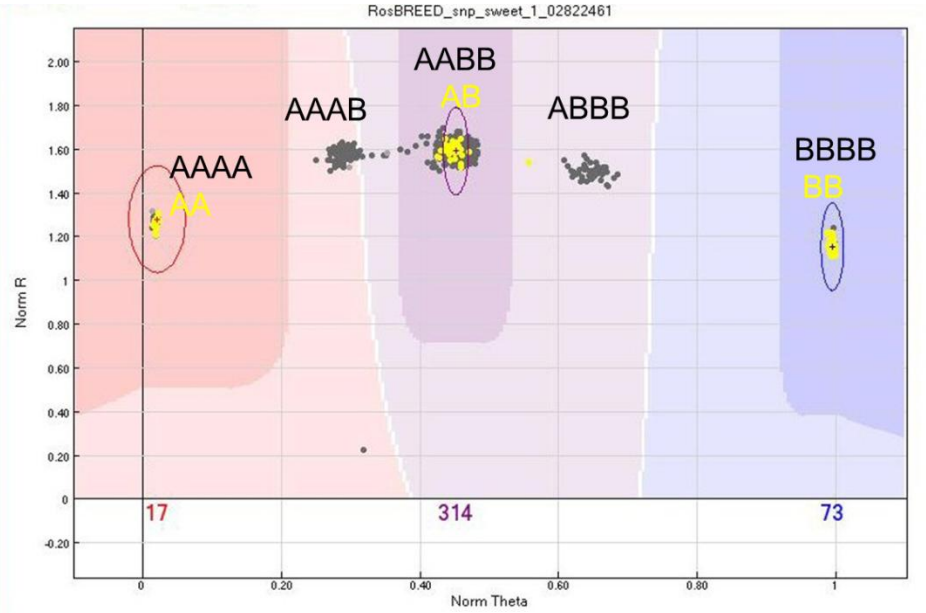
Gbrowse view of the peach sequence with the RosBREED SNPs highlighted



RosBREED Cherry 6K SNP Array v1
Quality-controlled genotypic data

Michigan State University cherry: genetics and breeding team





United States Department of Agriculture
National Institute of Food and Agriculture

NIFA

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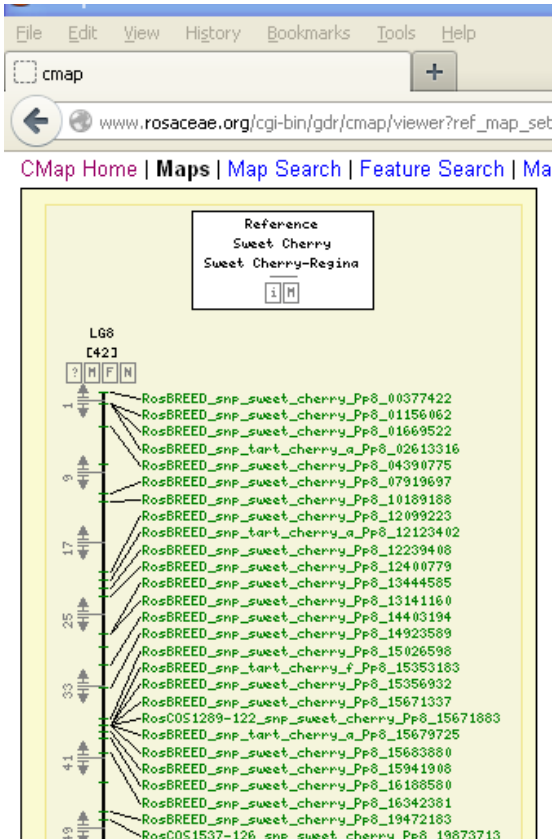
PLOS ONE

Construction and Comparative Analyses of Highly Dense Linkage Maps of Two Sweet Cherry Intra-Specific Progenies of Commercial Cultivars

Carolina Klagges[1☯], José Antonio Campoy[2☯], José Quero-García[2], Alejandra Guzmán[3], Levi Mansur[3], Eduardo Gratacós[3], Herman Silva[4], Umesh R. Rosyara[5], Amy Iezzoni[5], Lee A. Meisel[1,6*], Elisabeth Dirlewanger[2*]

Regina x Lapins

(687 SNPs mapped)



Black Tartarian x Kordia

(723 SNPs mapped)

RNA based cherry SNPs

Koepke *et al. BMC Genomics* 2012, **13**:18
http://www.biomedcentral.com/1471-2164/13/18

BMC Genomics

METHODOLOGY ARTICLE **Open Access**

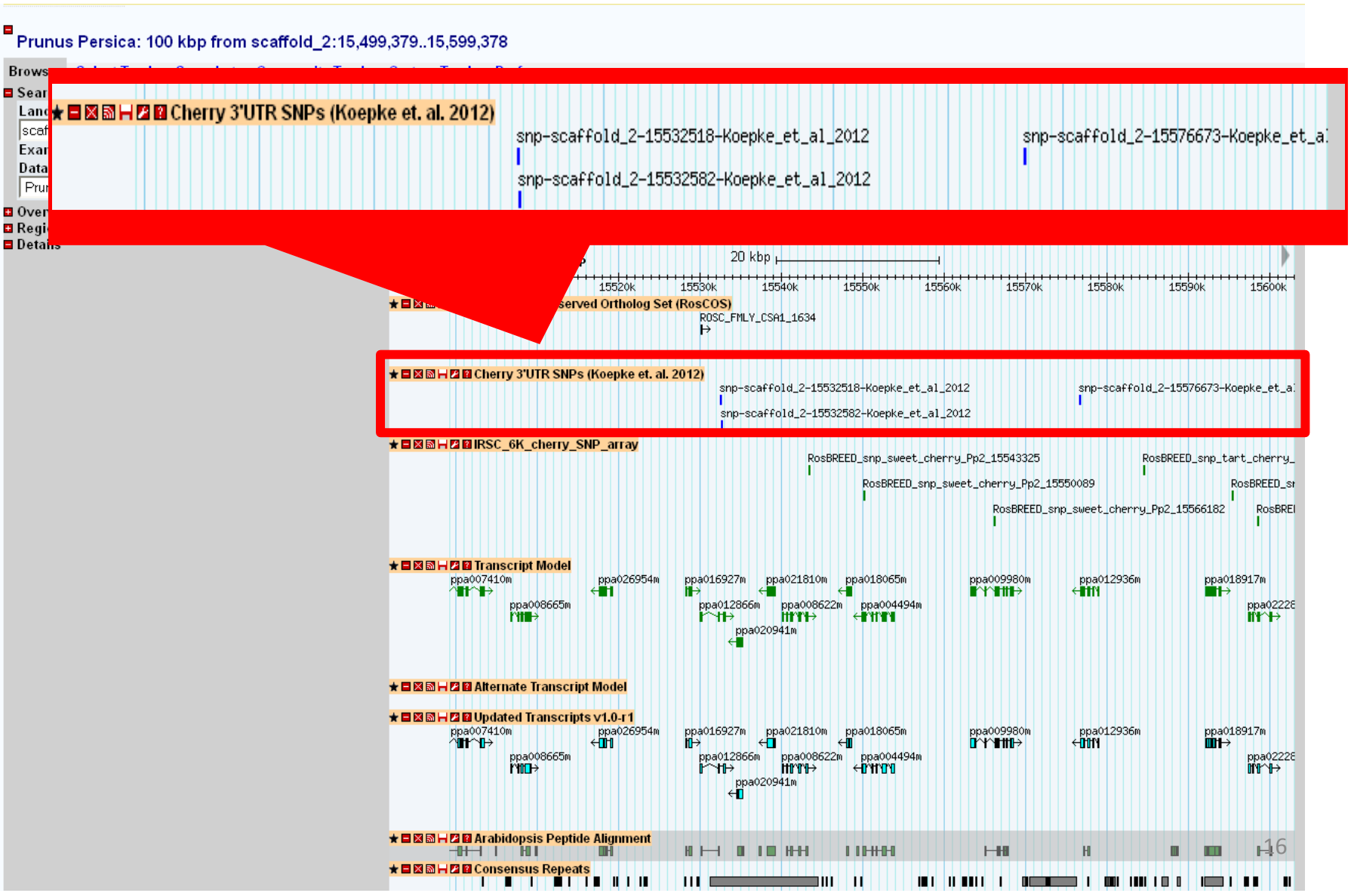
Rapid gene-based SNP and haplotype marker development in non-model eukaryotes using 3'UTR sequencing

Tyson Koepke[1], Scott Schaeffer[1], Vandhana Krishnan[2], Derick Jiwan[1], Artemus Harper[1], Matthew Whiting[3], Nnadozie Oraguzie[3] and Amit Dhingra[1*]

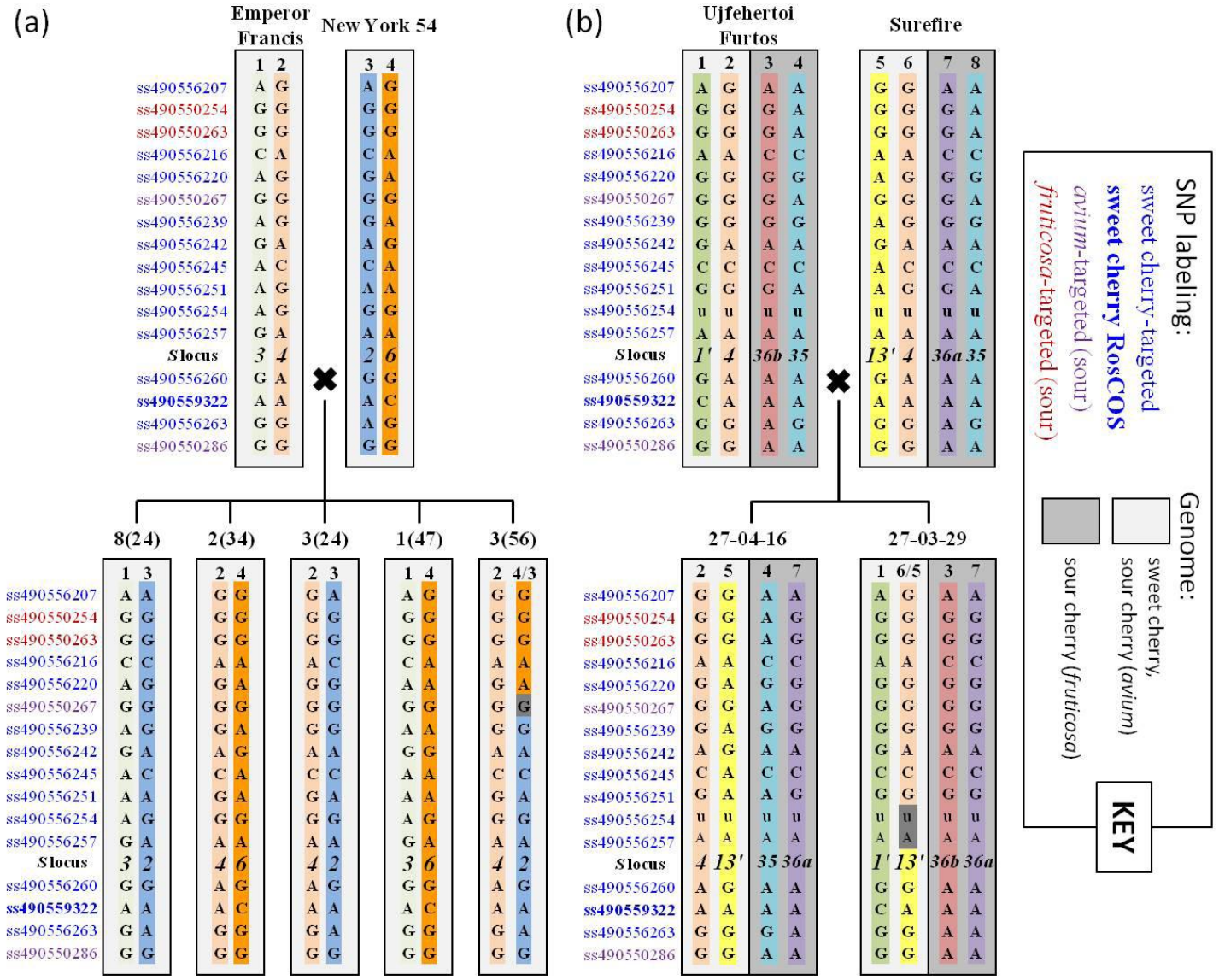
RNAseq Bing / Rainier



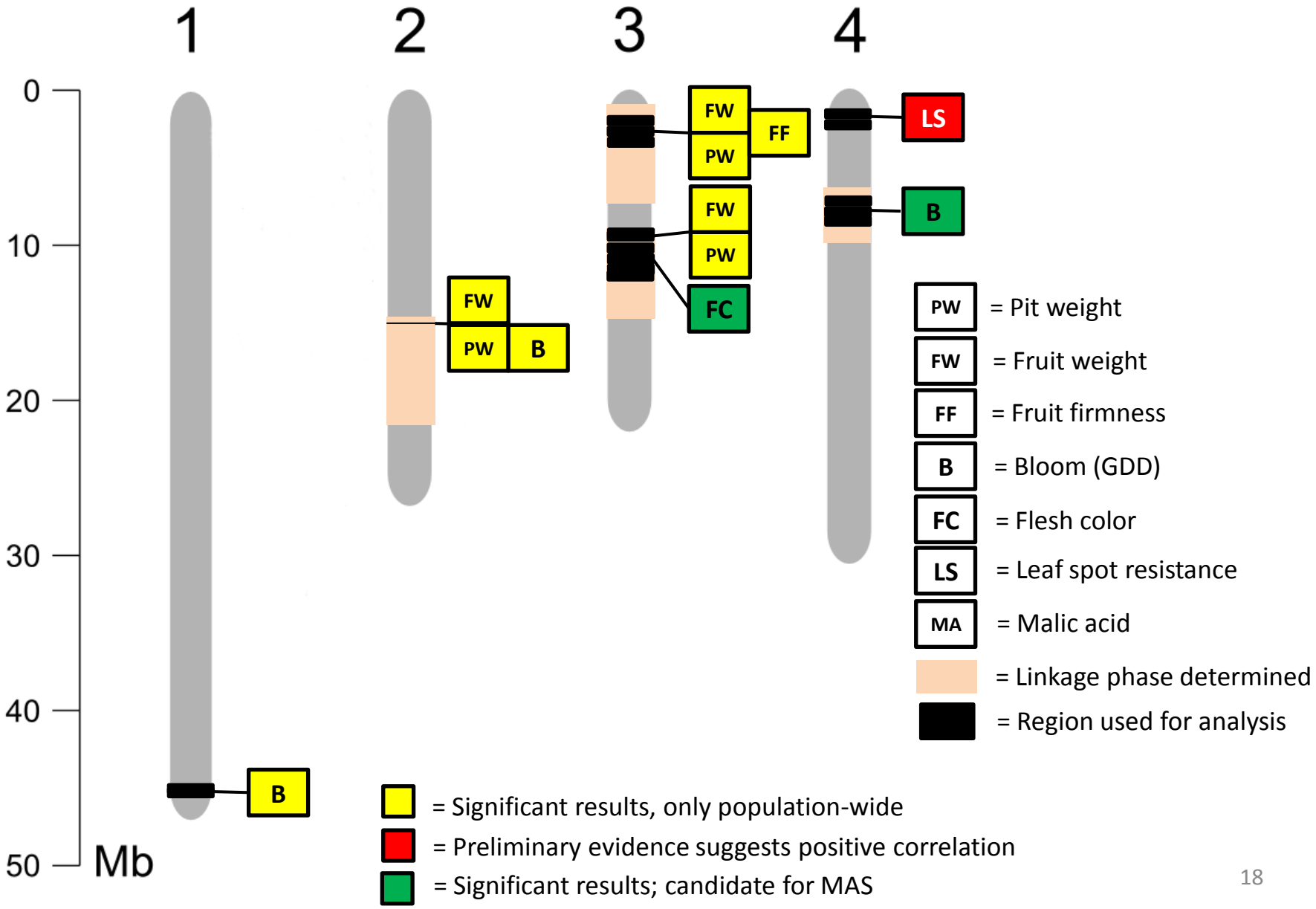
Gbrowse view of the peach sequence with the 3’ UTR SNPs highlighted



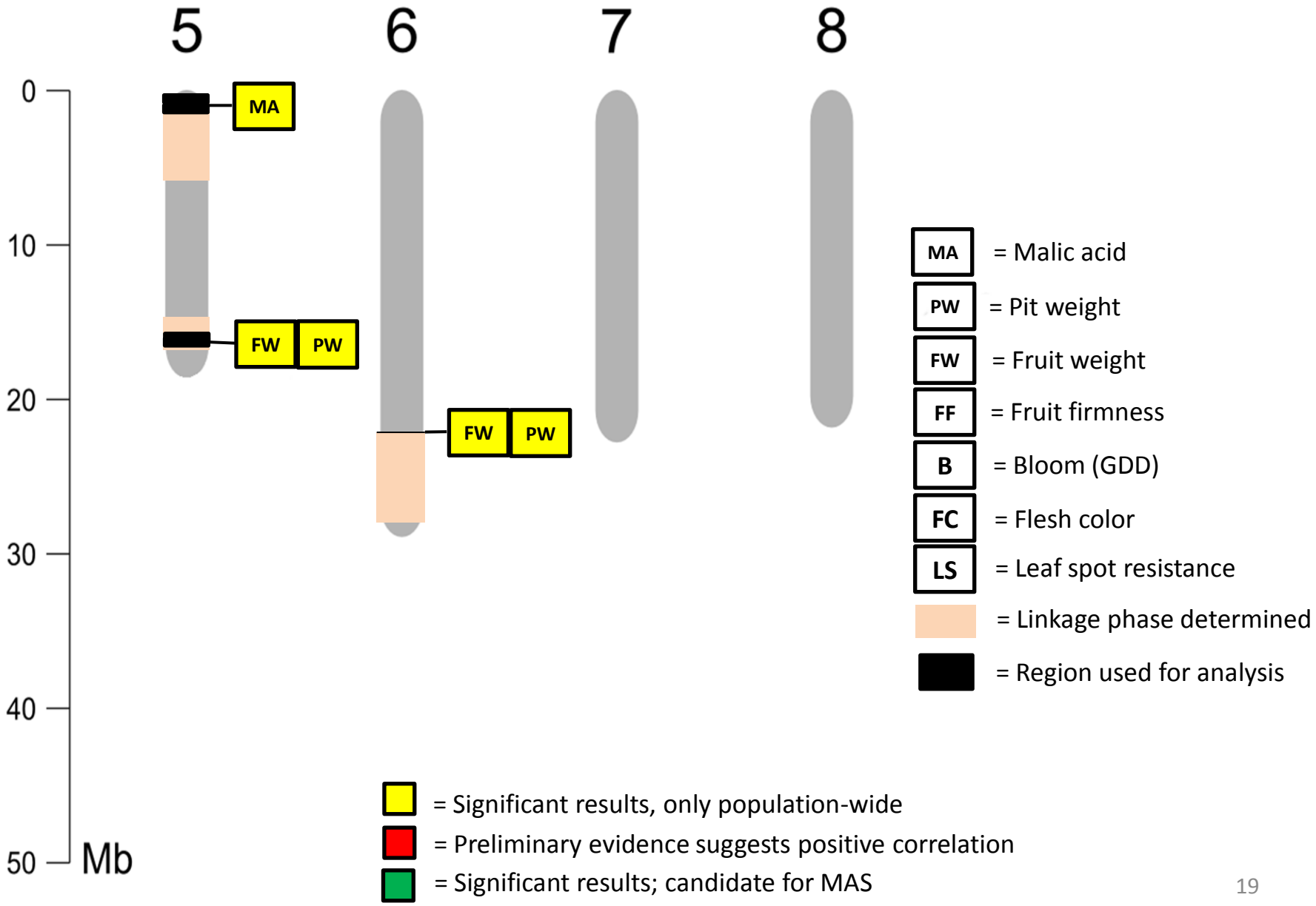
Progress in sour cherry genetics



QTL verified in sour cherry: LG 1-4



QTL verified in sour cherry: LG 5-8



Outline

- Rosaceae COS markers
- High Density SNP markers
- <u>Application</u> – Why some cherry fruit are large and some fruit are small?



Relative Contribution of Fruit Size Components (Olmstead et al. 2007)

Selah



14 grams

2.5 grams

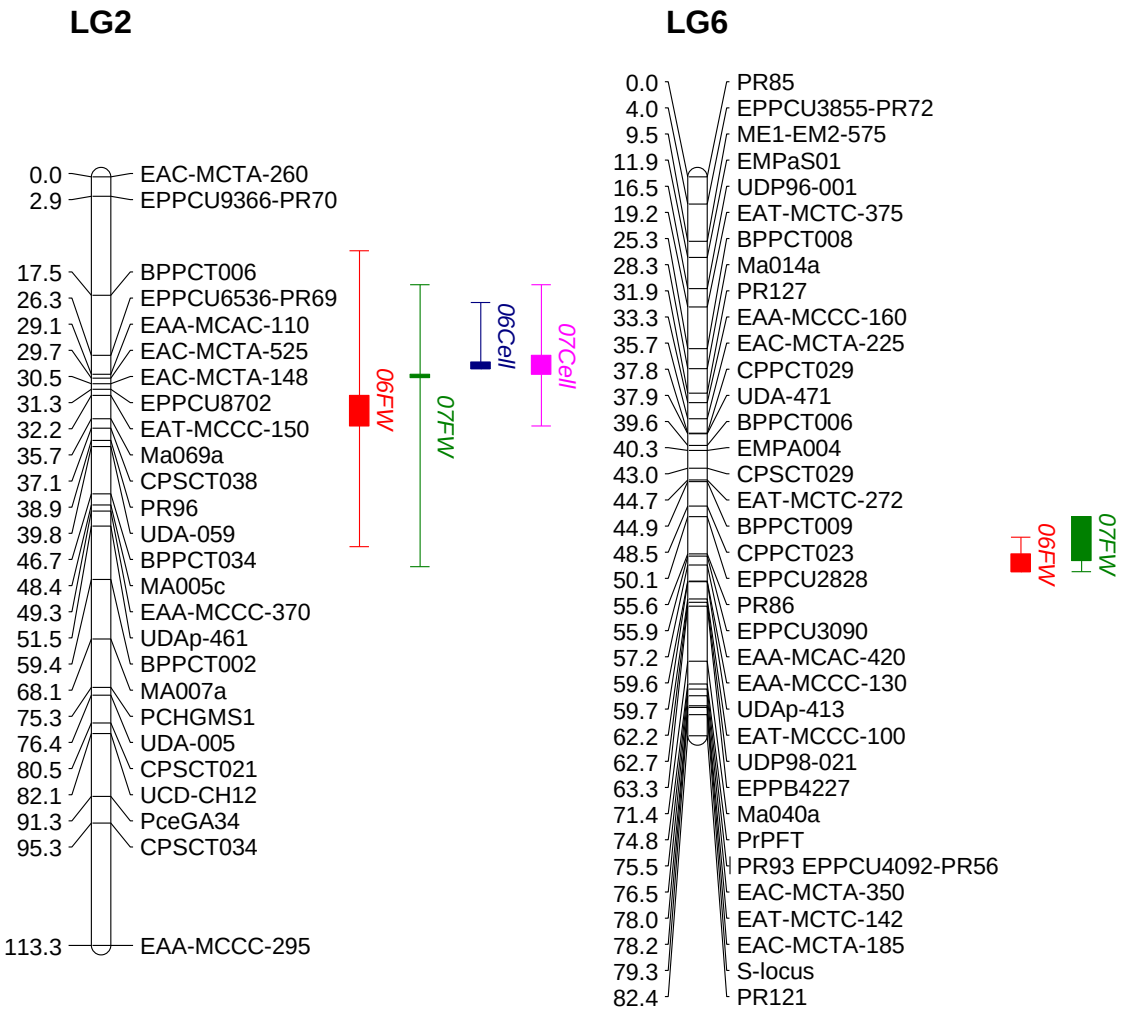


NY54

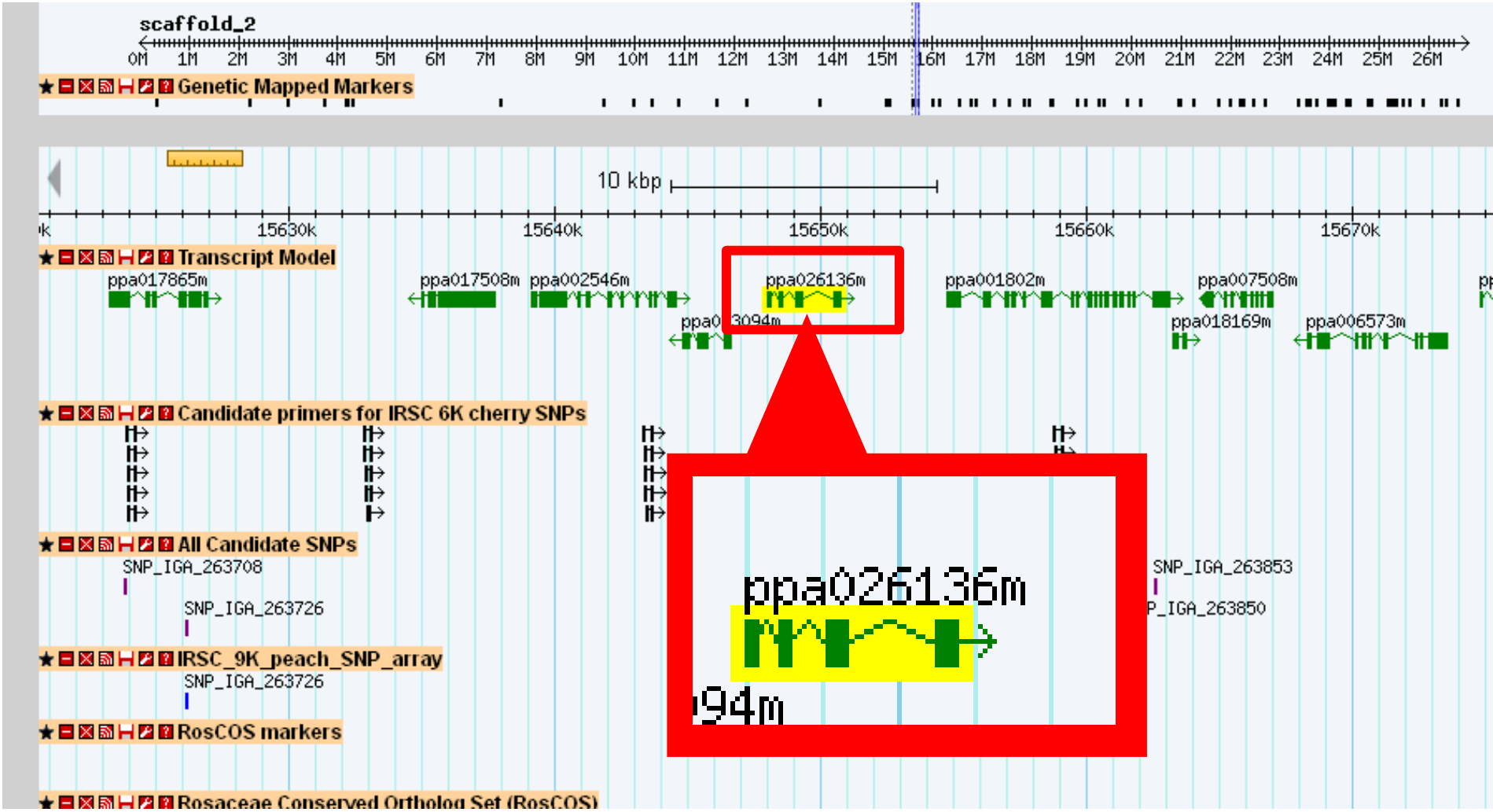
74% increase in cell number

26% increase in cell size

Two fruit size QTLs were identified: LG2 and LG6



Gbrowse view of the peach sequence for the G2 fruit size QTL region



Tomato: *fw2.2*

fw2.2: A Quantitative Trait Locus Key to the Evolution of Tomato Fruit Size

Anne Frary,[1*] T. Clint Nesbitt,[1*] Amy Frary,[1†] Silvana Grandillo,[1‡] Esther van der Knaap,[1] Bin Cong,[1] Jiping Liu,[1] Jaroslaw Meller,[2] Ron Elber,[2] Kevin B. Alpert,[1] Steven D. Tanksley[1§]

Domestication of many plants has correlated with dramatic increases in fruit size. In tomato, one quantitative trait locus (QTL), *fw2.2*, was responsible for a large step in this process. When transformed into large-fruited cultivars, a cosmid derived from the *fw2.2* region of a small-fruited wild species reduced fruit size by the predicted amount and had the gene action expected for *fw2.2*. The cause of the QTL effect is a single gene, *ORFX*, that is expressed early in floral development, controls carpel cell number, and has a sequence suggesting structural similarity to the human oncogene c-H-*ras* p21. Alterations in fruit size, imparted by *fw2.2* alleles, are most likely due to changes in regulation rather than in the sequence and structure of the encoded protein.

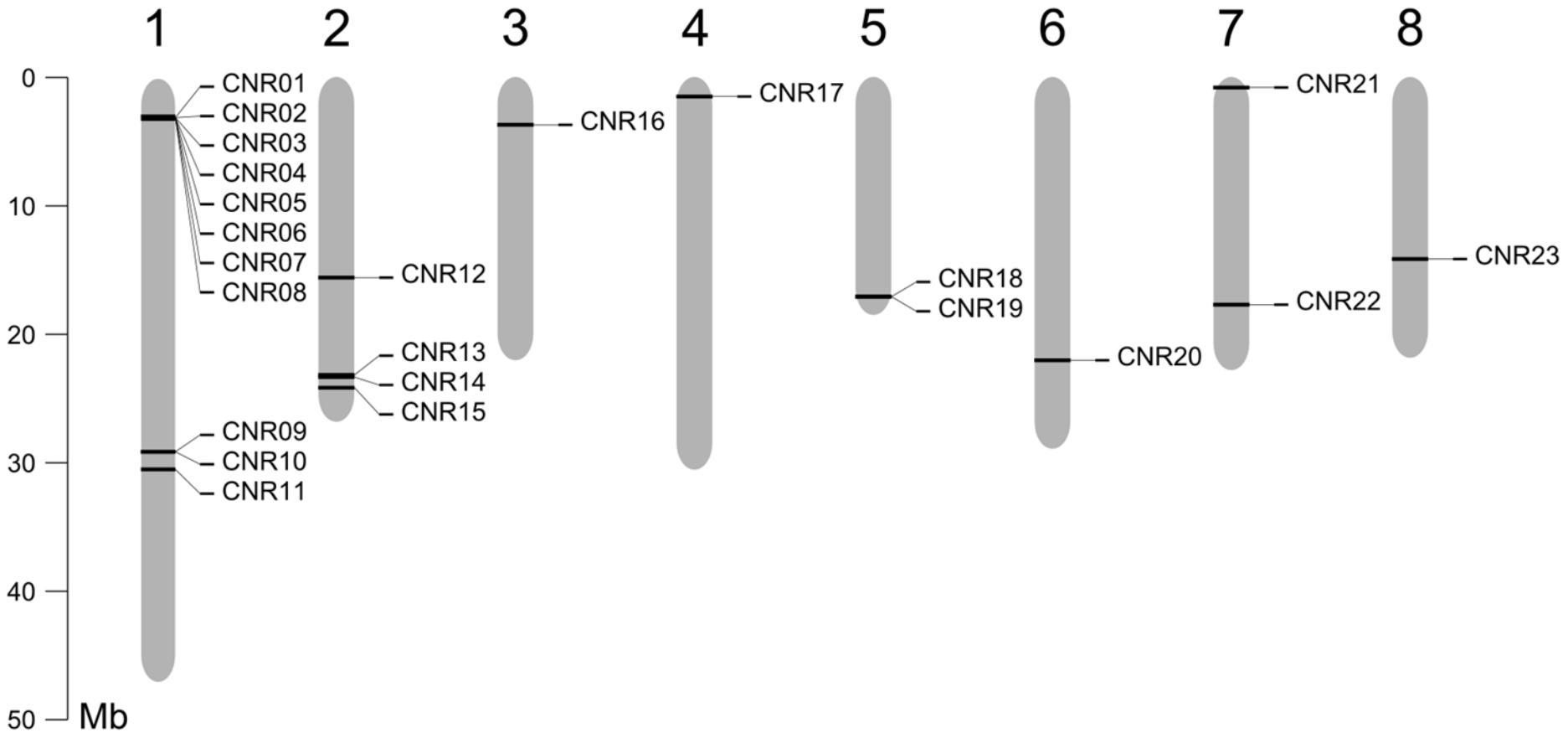
A fruit size gene was discovered in tomato that is a regulator of cell division



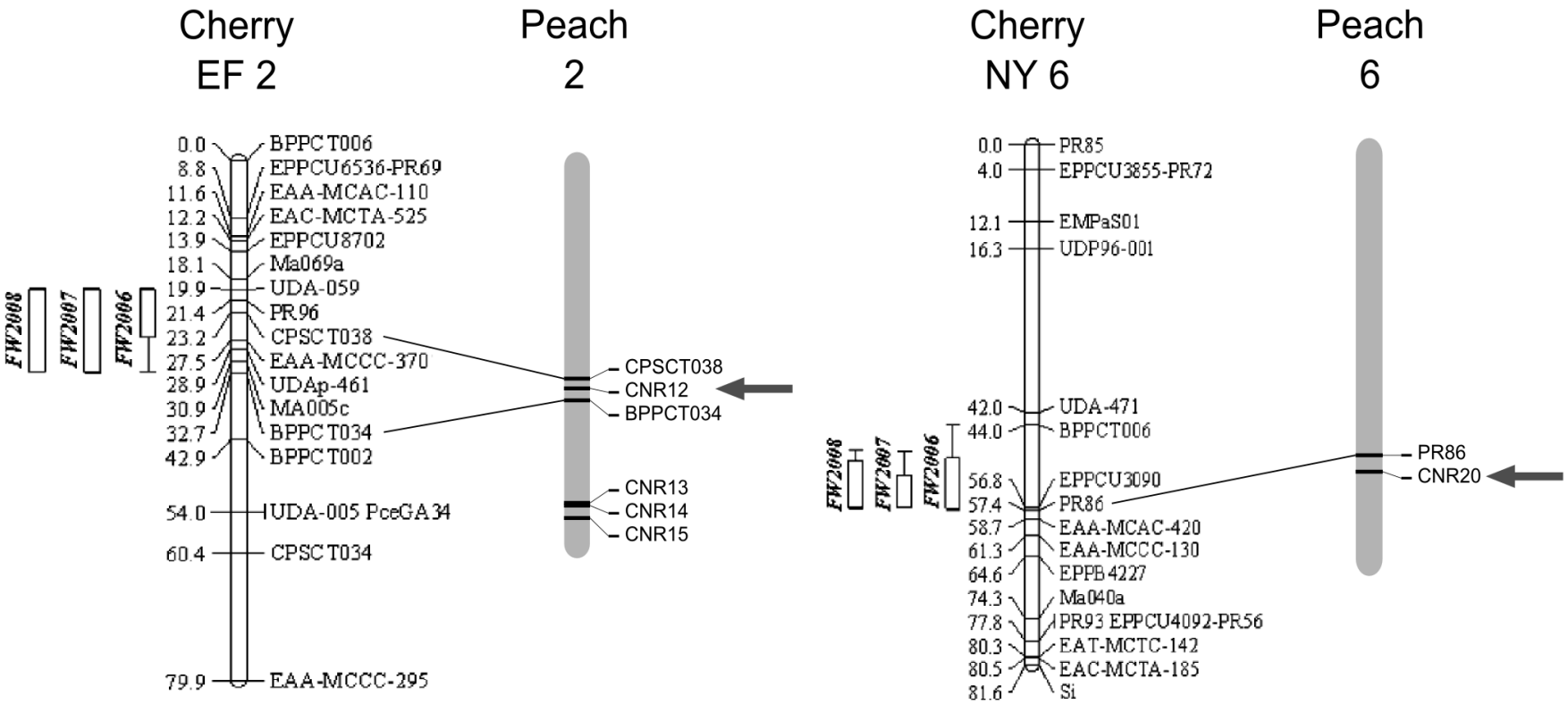
Tomato and cherry fruit are both enlarged ovaries



23 cell number regulator (CNR) genes were identified in the peach genome sequence

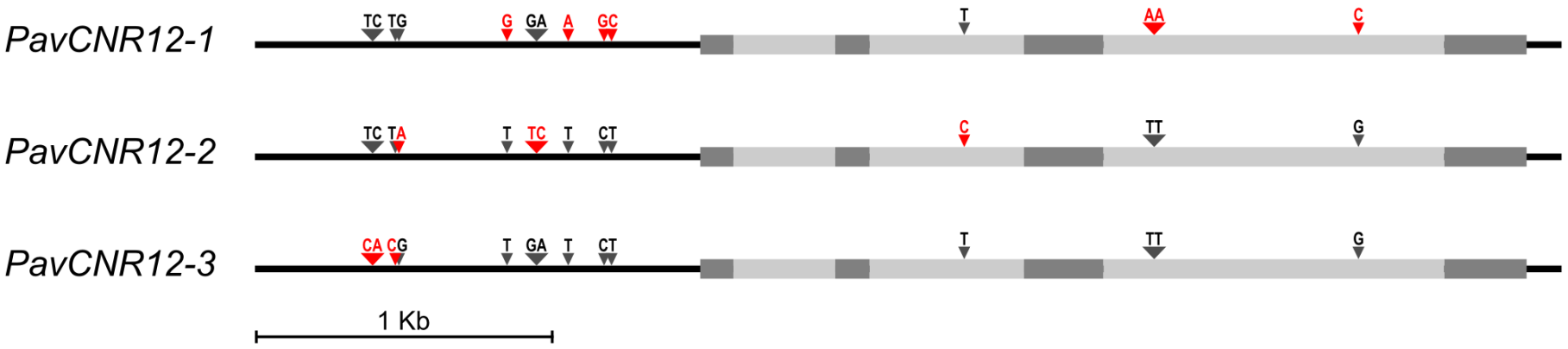


Three sweet cherry Pav*CNR12* alleles were identified that were differentiated by 14 SNPs





Three sweet cherry Pav*CNR12* alleles were identified that were differentiated by 14 SNPs





Mean fruit weight and cell number based on Group 2 *PavCNR12* allelic variants



PavCNR12

Regina

1 2

X

Lapins



1 2

2 2 | 1 2 | 1 1

Fruit weight (g)	6.28 a	7.43 b	8.29 c

Cell number regulator genes in *Prunus* provide candidate genes for the control of fruit size in sweet and sour cherry

P. De Franceschi · T. Stegmeir · A. Cabrera · E. van der Knaap · U. R. Rosyara · A. M. Sebolt · L. Dondini · E. Dirlewanger · J. Quero-Garcia · J. A. Campoy · A. F. Iezzoni

Mol Breeding 2013



Frary et al. 2000. Science

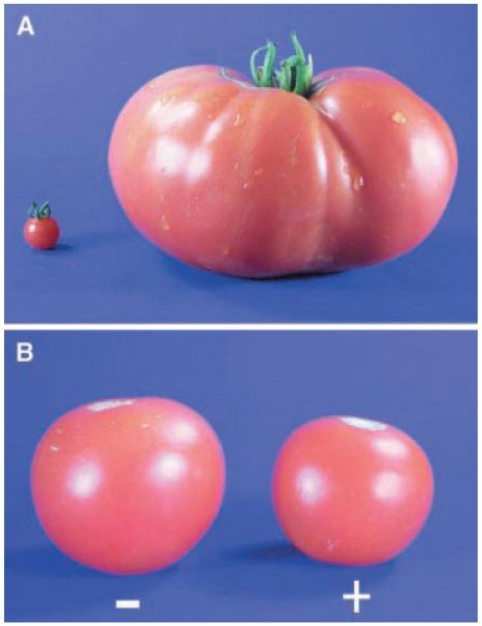


Fig. 1. (**A**) Fruit size extremes in the genus *Lycopersicon*. On the left is a fruit from the wild tomato species *L. pimpinellifolium*, which like all other wild tomato species, bears very small fruit. On the right is a fruit from *L. esculentum* cv Giant Red, bred to produce extremely large tomatoes. (**B**) Phenotypic effect of the *fw2.2* transgene in the cultivar Mogeor. Fruit are from R1 progeny of fw107 segregating for the presence (+) or absence (−) of cos50 containing the small-fruit allele.

Fw2.2 – Prunus avium CNR12 & 16 have a common genetic basis, and possibly a common role in fleshy fruit growth & development

Conclusions

- Genome-wide polymorphic DNA markers are available for cherry
- The peach physical map locations for all these markers are known
- The genetic linkage map locations for many of these markers are known
- This information is publically available and “just a click away” on the Genome Database Rosaceae (www.rosaceae.org)
- This genetic knowledge will enable researchers to determine the genetic basis of trait variation in cherry





Acknowledgements



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Audrey Sebolt
Umesh Rosyara

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Antonio Cabrera

Paolo De Franceschi
Luca Dondini
University of Bologna, Italy

Jose Antonio Campoy
Elisabeth Dirlewanger
Jose Quero-Garcia

www.rosaceae.org
Dorrie Main & Sook Jung

Knowledge For Tomorrow's Solutions

RosBREED
Enabling marker-assisted breeding in Rosaceae
www.rosbreed.org

United States Department of Agriculture
National Institute of Food and Agriculture

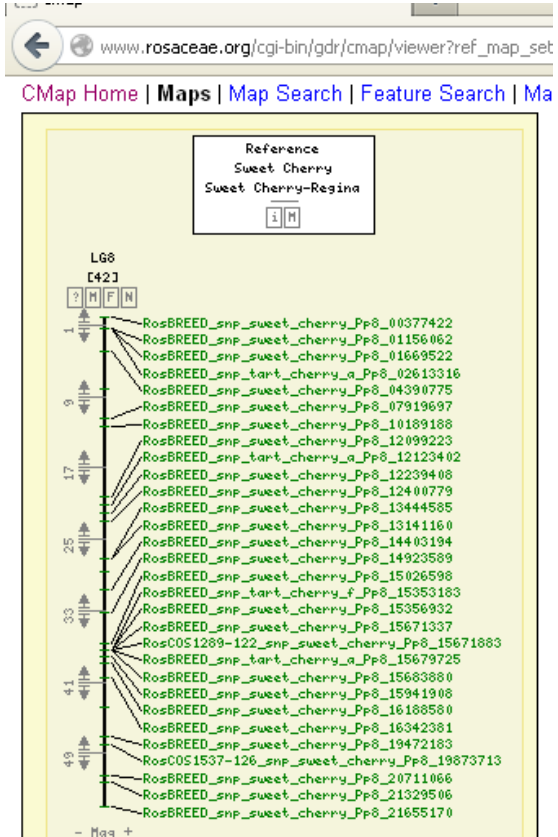
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PLOS ONE

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Regina x Lapins



Number of markers	BT	384
	K	275
	BT×K	**723**
	R	335
	L	247
	R×L	**687**
Size (cM)	BT	719
	K	788
	BT×K	**753**
	R	619
	L	610
	R×L	**640**
Average marker distance (cM)	BT	1,9
	K	3,0
	BT×K	1,1
	R	1,8
	L	2,5
	R×L	0,9

Black Tartarian x Attika

Table 1. Numbers of RosCOS and RosBREED Illumina SNP markers polymorphic in sweet cherry and average numbers of polymorphic RosBREED SNP markers based on the peach physical map (Mb) and sweet cherry genetic maps (cM). Values are presented for each of the eight *Prunus* linkage groups.

Linkage Group	Physical length (Mb)[a]	Genetic length (cM)[b]	Number of polymorphic RosCOS[c]	Number of polymorphic RosBREED SNPs[d]	Average physical distance between RosBREED SNPs (kb)	Average genetic distance between RosBREED SNPs (cM)
1	46.88	189	71	359 (42)	130	0.53
2	26.81	90	19	204 (17)	131	0.44
3	22.02	102	29	203 (14)	108	0.50
4	30.53	78	27	222 (26)	138	0.35
5	18.50	68	29	184 (33)	101	0.37
6	28.90	120	43	228 (30)	127	0.53
7	22.79	72	24	199 (22)	114	0.36
8	21.83	91	26	226 (19)	97	0.40
TOTAL	218.26	788	268	1825 (203)	120	0.43

[a] From Verde et al. (2013)

[b] Sweet cherry genetic map length from Klagges et al. (2013). The longest cM length reported is presented.

[c] From Cabrara et al. (2012)

[d] From Peace et al. (2012). Numbers in parentheses are totals from RosCOS SNPs derived from sweet cherry and included in the first number.