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Original article

Mapping the Naked Neck (*NA*) and Polydactyly (*PO*) mutants of the chicken with microsatellite molecular markers

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Abstract – The bulked segregant analysis methodology has been used to map, with microsatellite markers, two morphological mutations in the chicken: polydactyly (*PO*) and naked neck (*NA*). These autosomal mutations show partial dominance for *NA*, and dominance with incomplete penetrance for *PO*. They were mapped previously to different linkage groups of the classical map, *PO* to the linkage group IV and *NA* being linked to the erythrocyte antigen *CPPP*. An informative family of 70 offspring was produced by mating a sire, heterozygous for each of the mutations, to 7 dams homozygous recessive for each locus. Three DNA pools were prepared, pool *PO* included 20 chicks exhibiting at least one extra-toe, pool *NA* included 20 non-polydactyly chicks showing the typical phenotype associated with heterozygosity for the naked neck mutation, and pool NP included 20 chicks exhibiting neither of the mutant phenotypes. Typings were done on an ABI-373 automatic sequencer with 147 microsatellite markers covering most of the genome. An unbalanced distribution of sire marker alleles were detected between pool *PO*, and pools *NA* and *NP*, for two markers of chromosome 2p, MCW0082 and MCW0247. A linkage analysis taking into account the incomplete penetrance of polydactyly (80%) was performed with additional markers of this region and showed that the closest marker to the *PO* locus was MCW0071 (5 cM, lod score = 9). MCW0071 lies within the engrailed gene *EN*2 in the chicken. In the mouse, the homologous gene maps on chromosome 5, close

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to the hemimelic extra-toes mutation Hx. In the case of the NA locus, markers of chromosome 3 were selected because *CPPP* was mapped on this chromosome. Analysis of individual typings showed a linkage of 5.7 cM (lod score = 13) between the NA locus and ADL0237 in the distal region of chromosome 3q. These results contribute to connecting the former classical map to the molecular genetic map of the chicken, and open the way to the identification of the molecular nature of two developmental mutations of the chicken that are known to occur in many breeds of chickens.

chicken / gene mapping / naked neck gene / polydactyly / molecular marker

Résumé – Cartographie génétique des mutations « Cou Nu » et « Polydactylie » du poulet à l'aide de marqueurs microsatellites. Un protocole de localisation de gènes, qui utilise des typages moléculaires sur échantillons de mélanges, a été appliqué à la localisation de deux mutations morphologiques chez le poulet, « Cou Nu » (NA) et « Polydactylie » (PO). Il s'agit de deux mutations autosomales, à dominance intermédiaire dans le cas de NA, à dominance avec pénétrance incomplète dans le cas de PO. Elles étaient précédemment localisées dans la carte classique du poulet, sur le groupe de liaison IV pour le locus PO, sur le groupe de l'antigène érythrocytaire *CPPP* pour le locus NA. Une famille informative de 70 descendants a été produite à partir d'un père double hétérozygote, accouplé à 7 mères homozygotes récessives pour chacun des locus PO et NA. Trois mélanges d'ADN ont été préparés en fonction du phénotype des descendants : le mélange PO comprenait 20 poulets portant au moins un doigt supplémentaire, le mélange NA comprenait 20 poulets non polydactyles montrant le phénotype cou nu attendu chez un hétérozygote, le mélange NP comprenait 20 poulets ne présentant aucun des deux phénotypes mutants. Les typages sur les mélanges et les parents ont été réalisés sur un séquenceur automatique ABI 373 pour 147 marqueurs microsatellites couvrant la plus grande partie du génome. Une distorsion de transmission des allèles paternels aux marqueurs a été détectée entre le mélange PO d'une part et les mélanges NA et NP d'autre part, pour deux marqueurs localisés sur le chromosome 2p, MCW0082 et MCW0247. Les typages individuels ont permis de confirmer une liaison de 20 cM entre le locus PO et MCW0082 (lod score = 5,75). L'utilisation de marqueurs supplémentaires de cette région a permis de localiser plus précisément le locus « Polydactylie » à une distance de 5 cM du marqueur MCW0071 (lod score = 9), en tenant compte d'un coefficient de 80 % de pénétrance du phénotype polydactyle. Le marqueur MCW0071 est situé dans le gène « engrailed » $EN2$, dont l'homologue murin est localisé sur le chromosome 5, très près du mutant Hx associé à une polydactylie chez la Souris. En ce qui concerne le locus NA, les typages individuels ont été réalisés pour des marqueurs du chromosome 3, en raison de la localisation du locus *CPPP* sur ce chromosome, et d'une transmission anormale, dans le mélange NA, des allèles paternels du marqueur ADL0237 de ce chromosome. L'analyse de liaison a permis de localiser le locus « Cou Nu » à 5,7 cM de ADL0237 (lod score = 13), en position distale du chromosome 3q. Ces résultats contribuent à connecter l'ancienne carte génétique classique du poulet avec la carte moléculaire actuelle, et ouvrent la voie vers l'identification de la nature moléculaire de deux mutations du développement chez le poulet, qui sont présentes dans les races actuelles.

poulet / gène cou nu / polydactylie / carte génétique / marqueur moléculaire

1. INTRODUCTION

The first "classical" genetic map of the chicken was established from the compilation of many linkage studies that were conducted with morphological mutations or biochemical polymorphisms [3]. The recent development of a genetic map based upon polymorphic molecular markers [9, 10, 12] makes it possible to establish a linkage between these mutations and anonymous markers, in order to connect both the former "classical" map and the molecular map, and to lead the way to the molecular identification of mutations with major phenotypic effects. Molecular mapping of a mutant can be done in the reference families set-up to establish the molecular map, provided that these families are segregating for the mutant type. This was the case for the sex-linked mutation *ID*, inhibitor of dermal melanin, which was mapped on the *Z* chromosome in the East Lansing reference family [12]. However, most often, specific families have to be designed to map one or several mutants, as long as the phenotypic expression of each mutant can be clearly identified. When there is no prior knowledge of the chromosomal position of a mutant, mapping has to be done by screening the whole genome with molecular markers. The cost and labour effort of this approach can be efficiently reduced by using the strategy of bulked segregant analysis, initially proposed in plants [24], and successfully applied in chickens to map the dominant white mutation, *I* [25]. The aim of the present study was to use bulked segregant analysis methodology with microsatellite markers to map the naked neck, *NA*, and the polydactyly, *PO*, mutants.

Polydactyly, a phenotype easily identified at hatching is characterised by the presence of a fifth toe on top of the normal first toe, on one foot or possibly on both feet. Inheritance of polydactyly was considered to be determined by an incompletely dominant autosomal gene, as reviewed by Somes [27]. Crosses between a homozygous polydactyly parent with a normal one yielded a 96% penetrance of the polydactyly condition, whereas crosses between a heterozygous male and normal females exhibited a lower penetrance of the polydactyly condition of 79% [28]. The *PO* mutant has been assigned to the linkage group IV of the "classical" map [20].

The naked neck mutation is characterised by a reduction of feathered areas, mainly of the neck, but also in other regions such as the ventral region, as reviewed by Somes [27]. The phenotype is easily observed at hatching, when it is possible to distinguish homozygous carriers from heterozygous carriers by looking at the presence of feathers on the neck and around the eye. The trait is inherited as an autosomal incomplete dominant [11]. The *NA* locus was shown to be linked to the erythrocyte antigen *P*, now renamed *CPPP* [5, 7], and was tentatively mapped to the linkage group of blue-egg shell, *O*, and pea-comb, *P*, on chromosome 1 by Hutt [19]. This position was not confirmed, however, by further linkage studies involving *NA* and other traits or chromosome rearrangements known to be on chromosome 1 [5]. Furthermore, the *CPPP* locus was mapped on chromosome 3, when microsatellite markers were used for gene mapping on the East Lansing reference population [12]. The current map position of *CPPP* has been updated on the chicken genome database available on the web (http://poultry.mph.msu.edu/ or http://www.ri.bbsrc.ac.uk/chickmap). Thus the map position of the NA locus still awaits confirmation.

2. MATERIAL AND METHODS

2.1. Animals

An informative family was produced at INRA, Jouy-en-Josas, by mating a sire heterozygous for the mutant allele at each locus, to 7 dams homozygous for the recessive wild-type allele at each locus. According to the nomenclature rules adopted for the chicken [13], the sire genotype can be written $NA*NA/NA*N;PO*PO/PO*N$, where $*N$ is the wild-type allele and $*NA$ the mutant allele at the NA locus, and $*PO$ the mutant allele at the PO locus. The sire was derived from a cross between two experimental brown-egg laying strains kept at INRA, Jouy-en-Josas, and the dams were obtained from line WG, a White Leghorn inbred line free of *ALVE* insertions [16] that was imported from Ottawa, Canada. A total of 70 progeny was scored for the presence of $*NA$ or $*PO$ mutant sire alleles. In order to overcome the difficulty of variable expressivity of the polydactyly phenotype, each chick which showed at least one extra toe was considered to have received the mutant sire allele $*PO$.

2.2. DNA extraction and pool preparation

Blood was sampled from paternal grand-parents, sire, dams and all progeny. Individual extraction of high molecular weight DNA was performed according to standard procedures. The concentration of each DNA sample was assessed by U.V. spectrophotometry and found to vary from 100 to 200 ng·μL^{-1}. The volume corresponding to 2 μg DNA was calculated for each of the 7 dams. Individual aliquots of 10 to 20 μL were prepared and mixed to obtain a pooled sample for the dams. Following this procedure, the mixed sample represents an equal contribution of each individual. Finally, the concentration of the mixed DNA sample was adjusted to 10 ng·μL^{-1} by an appropriate dilution. Pooled samples for the progeny were prepared differently: aliquots of 4 μL of whole blood of each chick were taken in order to prepare a mixed blood sample, from which DNA was extracted with the same procedure as individual samples. The extraction of DNA from blood mixtures was used previously and found to be reliable for the study of DNA fingerprints mixtures in chickens [18]. The concentration of the DNA solution obtained from blood mixes was also adjusted to 10 ng/μl prior to the typing procedure. Three mixed samples were made according to chick phenotypes: pool NP included 20 chicks of normal phenotype that did not receive any of the sire mutant alleles ($^*NA$ or $^*PO$), pool NA included 20 naked neck chicks that had received the sire $^*NA$ allele but not the sire $^*PO$ allele, pool PO included 20 chicks showing polydactyly and having received the sire $^*PO$ allele; 5 of these also received the sire $^*NA$ allele. Pool NP was a control sample for both pools PO and NA, in addition, NA also represented a control sample for pool PO but pool PO was not as good as a control because it included 5 naked neck chicks among the 20. Pools were not prepared in duplicate because each pool was used already in two independent comparisons (PO versus NP, PO versus NA, NA versus NP). Individual DNA of the progeny were extracted later on, in order to be used for individual typings.

2.3. Typing of microsatellite markers

Typings on the sire DNA and the 4 DNA pools (dams + 3 progeny pools) were done with 147 microsatellite markers covering 22 autosomal linkage groups, and 9 unlinked markers [14, 15]. Although these markers were not regularly spaced, the length of the genome covered can be approximated by considering an average spacing of 20 cM between markers. Thus, about 3 000 cM were covered, which represents a major part of the genome, whose size is currently estimated to be 3 800 cM. The Z and W chromosomes were excluded from the analysis because NA and PO loci are not sex-linked. Then, individual typings with a small subset of markers were performed on grandparents, sires, dams and progeny, in order to confirm putative linkage suggested by the analysis of the pools. A few microsatellite markers in the vicinity of the erythrocyte antigen P ($CPPP$) on chromosome 3 were also chosen for individual typings, namely MCW0040, MCW0048 and MCW0207.

Polymerase Chain Reaction (PCR) amplifications of microsatellite markers were performed on pooled samples for each marker separately as described previously [25]. For individual typings, amplifications were carried out for each marker separately in 25 μL reactions containing 25 ng genomic DNA, 0.2 μM of each primer, 0.5 U of Taq polymerase (Life Technologies-GIBCO), 2 mM Tris-HCl pH 8.4, 5 mM KCl, 0.05% W-1 detergent, 1.5 mM MgCl2, 0.2 mM dNTP. A single protocol using a temperature of 55 °C for primer annealing was performed on an Omnigene thermocycler (HYBAID). The markers were previously optimised in 18 sets for simultaneous typing of 4 to 10 markers per lane. Thus, amplified products were multi-loaded onto a 6% denaturing polyacrylamide gel, Sequagel-6 (National Diagnostics), and electrophoresis was performed with 24 cm gels on an ABI-373 automatic sequencer. The results from the pooled samples and the sire were analysed with Genotyper software (ABI).

Markers that were homozygous in the sire or which showed the same alleles in the sire and the pool of dams were not considered for further analysis. The problem of stutter bands could be overcome because the individual sire sample was always run in parallel to the pooled samples on the same gel. Because the size of the sire-specific allele(s) was known, only DNA fragments of the same size were considered for the analysis of the pattern obtained on the progeny pooled samples. When a marker was heterozygous in the sire and showed at least one sire specific allele, the ratio between the peak heights obtained for each allele with the sire DNA sample was calculated in order to obtain a correction factor for differential amplification. The peak heights read for the same alleles on the pools of progeny were adjusted by this factor, assuming that differential amplification takes place to the same extent for the sire DNA sample and the pooled DNA samples. In the absence of any shared allele between the sire and the dams, the expected ratio between the adjusted peak heights of each sire allele in the progeny pools was 1 for a marker unlinked to PO or NA. For a marker linked to one mutant allele with r recombination units, the sire marker allele linked to the mutant allele is expected to be more frequent by a factor of $((1-r)/r)$ in the pool carrying the mutant allele than in the pool not carrying it. For instance, if $r = 20\%$, then the linked marker allele will be 4 times more frequent in the pool carrying the mutant allele. Markers that showed a

marked difference in allelic frequencies between the pools were thus selected for individual typings.

2.4. SSCP typing

The non-polymorphic microsatellite MCW0071 was localised through the SSCP (Single Strand Conformation Polymorphism) technique [2]. The amplified fragment was denatured by heating at 95 °C and loaded onto a non-denaturing Acrylamide/Bisacrylamide (49/1) gel containing 5% glycerol, to visualise a SSCP through silver staining [8].

2.5. Linkage analysis

The results from individual typings of both parents and progeny were read with GeneScan 2 and transferred to the GEMMA database [21] for genotype interpretation. The genotype derived from the phenotypic scoring was also introduced and linkage mapping was done with CRIMAP software. A LOD score ($\log^{10}$ of odds) higher than 3 was considered as indicative of significant linkage, and distances were calculated with the Kosambi function. A LOD score curve was established using LINKAGE software. In the case of the *PO* locus, a penetrance of 80% was considered for the linkage analysis, in order to take into account previous observations on the inheritance of this trait [28].

3. RESULTS

3.1. Phenotypic scoring

Among the 70 progeny, 35 exhibited the phenotype expected for a chicken heterozygous for the naked neck mutant allele. Only 28 showed the polydactyly phenotype (Tab. I), which corresponded to a penetrance of 80%. One dam family appeared to exhibit a very low penetrance (Tab. I), although environmental conditions were identical for all dams and all incubated eggs. An average penetrance close to 80% was described previously with larger numbers of chickens: 1 612 polydactyly chicks and 2 454 normal chicks were obtained after backcrossing heterozygous males $*PO$ to homozygous normal females [28]. In the family studied here, two female chicks, that did not exhibit polydactyly, were mated later on to a heterozygous polydactyly male, and produced a high proportion of polydactyly progeny (10 over 12 offspring for each dam). This result suggested that these two females were carrying the mutant allele $*PO$, although they did not exhibit the polydactyly phenotype, consequently their genotype was considered to be PO^*PO/PO^*N (Tab. I).

3.2. Informativeness of markers

Out of the total number of 156 markers typed on the pools, 23 did not amplify correctly, 51 were homozygous, 16 were heterozygous for the same alleles in the sire and the dams, which left 66 markers showing either 1 (for 39 markers) or 2 (for 27 markers) sire specific alleles. These 66 markers covered a total distance of approximately 1 700 cM but were not evenly spaced. The

Table I. Distribution of phenotypes and corresponding genotypes in the progeny of a male, double heterozygous for the naked neck $^{*}NA$ and polydactyly $^{*}PO$ mutations, mated to homozygous normal females.

Dam number	Number of chicks for each phenotype				Total	Number of carriers	
	normal	naked neck	with extra-toe(s)	with both conditions		$^{*}NA$	$^{*}PO$
1	3	5	0	1	9	6	1
2	3	5	5	2	15	7	7
3	3	3	5	3	14	6	8
4	2	0	0	1	3	1	1
5	4	6	3	1	14	7	$4 + 1^{*}$
6	2	0	1	1	4	1	$2 + 1^{*}$
7	3	3	1	4	11	7	5
total	20	22	15	13	70	35	$28 + 2^{*}$

*: two female chicks of normal phenotype were considered to carry the $*PO$ allele because, at adult age, they produced a high number of polydactyly chicks (10 over 12) after being mated to a heterozygous polydactyly male.

heterozygous sire was obtained by crossing a polydactyly non-naked neck male with a naked neck non-polydactyly female, coming from two different lines, so that the sire was expected to be heterozygous for markers flanking the mutations that were not shared between his parents.

3.3 Mapping the *PO* locus

Out of the markers with 2 sire specific alleles, MCW0082 sire alleles showed the most unbalanced distribution (Fig. 1), with a much higher peak for one of the sire alleles in the pool of affected PO offspring as compared to offspring unaffected for PO (pools NP and NA). The MCW0082 marker was located on the distal region of the short arm of chromosome 2. Two other markers, MCW0247 and ADL0228, located at about 30 cM on each side from MCW0082, also showed an unbalanced distribution of sire alleles, by a factor of two for ADL0228, whereas, in the case of MCW0247, one of the sire alleles shared with the dams was absent, surprisingly, in the PO sample, and was found in the NP and NA samples. These observations suggested the selection of more markers in the vicinity of MCW0082 for individual typings, so the following markers were chosen: ADL0336, MCW0071 typed with the SSCP method, MCW0184 and MCW0247. The two-point distance between PO and MCW0082 was estimated to be 19.8 cM with a lod score of 5.75 over the entire family of 70 chicks. When the linkage analysis was also applied to all markers and all progeny with a penetrance coefficient of 80%, the lod score curve showed a maximum of 9 for a position between MCW0082 and MCW0071, at 5 cM from MCW0071 (Fig. 2). Another peak could be seen with a lod score of 8.4 just on the other side of MCW0071, between MCW0071 and MCW0184.

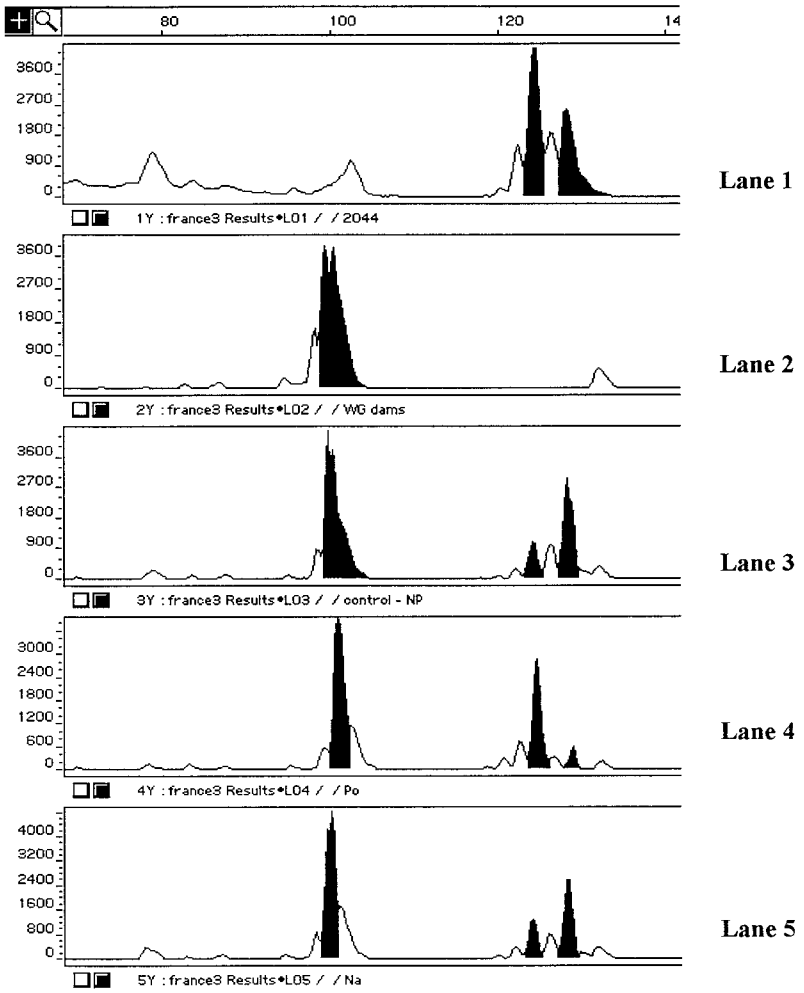

Figure 1. Amplification profiles obtained on the ABI automatic sequencer 373 for the MCW0082 microsatellite marker: the peaks filled in black correspond to the position of microsatellite alleles validated with GeneScan 2.

– lane 1: individual sire sample heterozygous for $^{*}PO$ and $^{*}NA$ mutations,
– lane 2: pooled sample of dams not carrying any of the mutations,
– lane 3: pooled sample NP of progeny not carrying any of the mutations,
– lane 4: pooled sample PO of progeny carrying the $^{*}PO$ mutation,
– lane 5: pooled sample NA of progeny carrying only the $^{*}NA$ mutation.

The correction factor for differential amplification of the sire alleles was calculated to be 1.6, that is the peak read for the sire allele of the smallest size (124 bp) was 1.6 times higher than the peak read for the longer allele (128 bp). The ratio of peak heights for these two sire alleles (124 bp/128 bp) was then calculated to be 0.39 in pool NP, 3.96 for pool PO and 0.45 for pool NA; after dividing by 1.6 to correct for preferential amplification of the 124 bp allele, the ratios became 0.24, 2.48 and 0.28 respectively. Thus, before or after adjustment for differential amplification, the ratios of peak heights of sire alleles varied in a range of 1 to 10 between pool PO and either pool NP or pool NA.

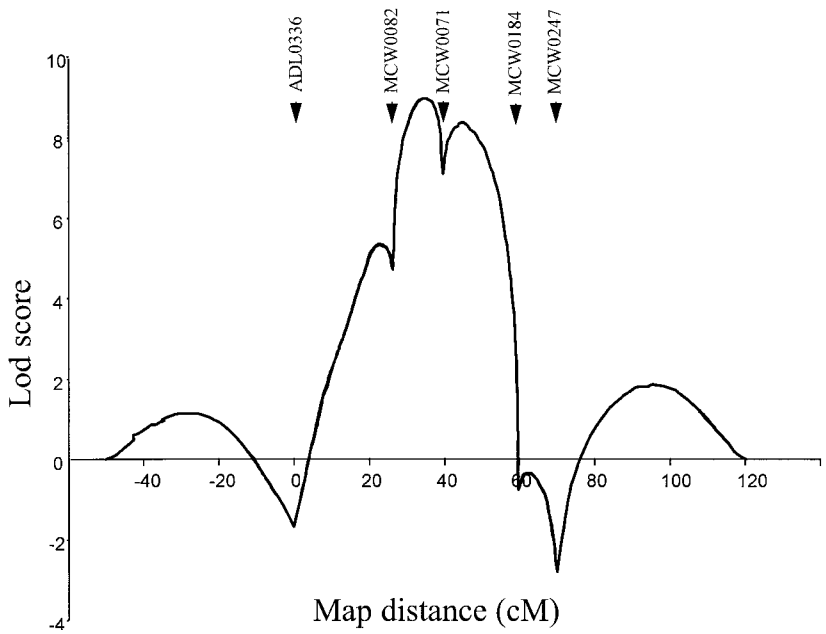

Figure 2. LOD score curve showing the mapping of *PO* locus in the interval between ADL0336 and MCW0247 on chicken chromosome 2p; position in cM is given relative to ADL0336 which is located in the telomeric region of chromosome 2p (average map position of 1.3 cM). Data were obtained from an informative family of 70 progeny produced by mating a sire heterozygous for the mutant allele to homozygous normal females; the linkage analysis accounted for a penetrance coefficient of 80% of the polydactyly phenotype in heterozygous carriers.

3.4. Mapping the *NA* locus

Marker ADL0237, located in region qter of chromosome 3, showed one sire specific allele which was not observed in pools NP and PO but was found in pool NA (Fig. 3). However, the analysis was not clear-cut because the other sire allele was not seen at all in pools NP and PO. This could be due to the fact that this other sire allele differed by only one base pair from the allele most frequently found in dams, and was not easy to detect in the progeny pools (Fig. 3). Yet, chromosome 3 was a good candidate because it also carries the *CPPP* gene linked to NA, and more markers from this region were used for individual typings. The NA mutation mapped between MCW0040 and ADL0237 (Tab. II). The distance between NA and ADL0237 was estimated to be 5.7 cM with a lod score of 13. According to the East Lansing map, the *CPPP* gene is located at 40 map units from ADL0237. Percentage recombination between NA and *CPPP* was estimated to lie between 26 and 32 depending on the data set [5, 6]. Taking into account sample size and accuracy of mapping, these values would appear consistent with the map distance of 34 cM between NA and *CPPP* that can be extrapolated from the present data and the current map.

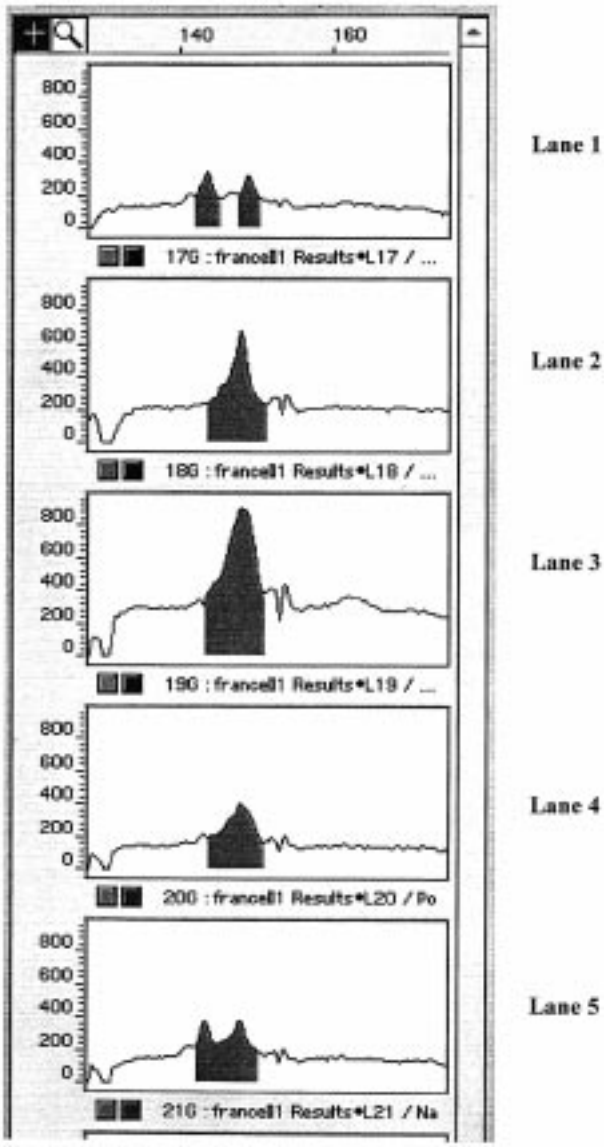

Figure 3. Amplification profiles obtained on the ABI automatic sequencer 373 for the ADL0237 microsatellite marker: the peaks filled in black correspond to the position of microsatellite alleles validated with GeneScan 2.

– lane 1: individual sire sample heterozygous for $^{*}PO$ and $^{*}NA$ mutations,
– lane 2: pooled sample of dams not carrying any of the mutations,
– lane 3: pooled sample NP of progeny not carrying any of the mutations,
– lane 4: pooled sample PO of progeny carrying the $^{*}PO$ mutation,
– lane 5: pooled sample NA of progeny carrying only the $^{*}NA$ mutation.

The sizes of the sire alleles were 144 and 149 bp and differential amplification of the smaller allele was negligeable (peak height ratio of 1.07). The pool of dams showed one sharp peak at 148 bp. Both pools NP and PO showed also one peak, not so sharp, estimated to correspond also to 148 bp, no sire allele was scored in these lanes, probably because the 149 bp and the 148 bp fragments could not be separated. The lane with pool NA was the only one to exhibit the sire specific allele of 144 bp, with the same peak height as the 148 bp allele of the dams, that could be confounded partially with the 149 bp allele of the sire. Thus, this marker exhibited a markedly unbalanced distribution of sire alleles in the progeny pools, but this was not quantified because of the difficulty in separating one of the sire alleles from the dams allele.

Table II. Linkage mapping of the naked neck mutation (*NA*) with four microsatellite markers on chicken chromosome 3q in an informative family of 70 progeny. The loci are ordered from centromere to telomere.

Locus	% recombination between adjacent loci	Cumulated distance in cM
MCW0207		0
	14	
MCW0048		16
	2	
ADL0237*		18
	6	
NA		24
	7	
MCW0040**		31

* According to the chicken genome database, the estimated distance between *CPPP* and ADL0237 is 40 cM.
** Average map position on chromosome 3 is 283 cM for this marker.

4. DISCUSSION

The bulked segregant approach has been successfully used to identify the chromosomal regions carrying two independent mutations in the chicken. Mapping was done with individual typings, using markers chosen in the regions selected after the typings on pools. From the actual distances estimated with individual typings, it can be concluded that an unbalanced inheritance of a linked marker allele can be detected with typings on pools when the distance between the gene and the marker varies between 5 and 20 cM. The number of typings realised was $((156 \times 5) + (80 \times 9)) = 1\,500$, instead of 10 000 for a typical genome scan in a family of this size. Reading the profile of the amplified products on pools took more time than the analysis of individual typings for the same marker, because saturation of signal intensity often resulted in bifurcated peaks. Thus, the optimal strategy depends on the limiting factor, either marginal costs or manpower: bulked segregant analysis minimises the cost of consumables and the use of the ABI sequencer, but does increase the time spent for analysis and requires a good prior knowledge of microsatellite markers, to allow for multiloading on gels, and to minimise the number of gels for a given number of markers.

It must be noticed that the bulked segregant approach was also successful for mutations showing incomplete penetrance. The accurate mapping of the *PO* locus remained, however, difficult. The *PO* locus mapped close to MCW0071 but finding the accurate position is obviously very much influenced by the phenomenon described as penetrance, the molecular nature of which is not known. The level of penetrance appeared to depend on the dam family, although sampling errors could not be ruled out with small family sizes. The dam line chosen to produce the informative family has never been shown to exhibit the

polydactyly condition, and was considered to be homozygous for the wild-type allele. Another allele, called duplicate polydactyly, is known at the PO locus, but is associated with a much severe condition than the one observed in the present study [27] and was considered to be absent from the family under study. Another locus, $PO-2$, carries a polydactyly mutant allele in the chicken, but the mutant is recessive, is associated with an increased embryonic mortality and with leg deformities in the survivors [27]; there was no observation that could suggest the segregation of this second mutation in the family under study. One could speculate about the possibility that two loci close to MCW0071 could control the inheritance of the polydactyly condition in chickens. Because, these two loci are tightly linked, they would not be identified easily in a single cross, but heterozygosity at one or both loci in the sire could lead to different genotypes in progeny, and explain the differences in expression of the polydactyly phenotype. This hypothesis should be tested in a larger design, including several sires and several steps of successive meiosis in order to be able to distinguish the putative closely linked loci.

The mapping of a mutation can provide a way to propose candidate genes for it. The naked neck mutation has very important effects on heat tolerance and performance of chickens [23], knowing this gene would provide a way to understand how feather coverage is regulated. At the moment, there is no obvious candidate, unfortunately, in the qter region of chromosome 3. The polydactyly mutation is found in some breeds in France or abroad, and could be used as a trademark to contribute to breed identification. Furthermore, polydactyly mutations are also known in mammals, and it would be interesting to check whether the same genes are involved at the molecular level. Some information can already be obtained from comparative mapping. The 2p region of the chicken, carrying PO, has been suggested to show homology with a region on chromosome 5 of the mouse. Indeed, the MCW0071 marker lies within the $EN2$ gene (engrailed) which maps to chicken chromosome 2p [17] and $EN2$ was found to map to mouse chromosome 5 [22]. Interestingly, one mutation associated with extra toes, named Hx, has been mapped 1 cM from $EN2$ on the mouse chromosome 5 [22]. The homologous gene for Hx in the chicken is not known, but might be a candidate for the PO mutation.

A number of classical mutants have now been placed on the molecular map such as pea-comb P and blue-egg shell O [1, 4, 29], inhibitor of dermal melanin ID [12], dominant white I [25], autosomal dwarfism ADW [26]. The mapping of PO on 2p makes it possible to propose markers in the surrounding region to map other mutants from the former linkage group IV. This would involve producing a family informative for the M 'multiple spurrs' mutation, previously located at 33% recombination from PO, and for the D 'duplex comb' mutant, previously located at 27% recombination from M [20]. Regarding other former linkage groups of the classical map, group I carrying the creeper, CP, rose comb, R, uropygial gland, U, lavander plumage color, LAV, and ametapodia, MP, mutations would be the last one not to be connected with the molecular map. The connection between molecular and classical maps would also benefit from mapping of the crest, CR, and frizzle, FR, mutations, which flank the dominant white mutation linked to MCW0188 [25].

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