

Divergent selection for skeletal malformations in chickens alters polymorphism at microsatellite loci

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Abstract. The objective of this study was to determine microsatellite polymorphism in Rhode Island Red (RIR) and Sussex (SX) chickens, divergently selected over six generations for high (H) or low (L) incidence of skeletal defects in embryos (30.7% for H lines, 3.7% for L lines). The polymorphism analysis covered 15 microsatellite markers within four lines (a total of 60 individuals). Eight alleles were identified as specific to H lines and six alleles as specific to L lines. The selection for skeletal malformation appears to have affected the frequency of microsatellite alleles. The experimental material examined constitutes a valuable source for identification of real genes causing skeletal defects.

Key words: chicken, microsatellites, polymorphism, skeletal defects.

Introduction

Skeletal defects are an important problem in poultry flocks, particularly in meat-type birds. Reasons for such disorders are multiple and the aetiology as well as pathogenesis is as yet poorly defined. However, it is known that some pathological changes in the skeleton are hereditary (MERCER, HILL 1984).

In the United States the estimated annual losses caused by skeletal problems amount to 80-120 million dollars in broiler chickens and 40 million dollars in turkeys (MORRIS 1993). Poultry losses caused by skeletal problems are the result of an increased mortality and number of birds culled (caused also by septicaemia-toxaemia) and by the downgrading of breasts and legs (SULLIVAN 1994).

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Skeletal problems are recognised as one of the four major factors limiting the performance of meat-type birds, because fast-growing broilers, reaching market weights at earlier ages, are considered to have more leg problems and suffer from greater losses than slower-growing birds (DAY 1990, JULIAN 1998).

The most important inherited skeletal defects include abnormalities of the axial skeleton, including mutations affecting the beak, head, neck and body: cerebral hernia, hereditary exencephaly, crossed beak, short mandible, short upper beak, supernumerary ribs, kyphoscoliosis, spondylolisthesis. The genetic basis of most of these skeletal disorders is still not fully understood (SOMES 1990).

The genetics of an inherited form of scoliosis in chickens was studied by RIGGINS et al. (1977) and MCCARREY et al. (1981). Variation in the expression of scoliosis is attributed to an incomplete penetration of major genes, additive effects of minor modifying genes, and primarily to environmental effects, and seems to be correlated with sexual maturity (MCCARREY et al. 1981). It was shown that plasma-free hydroxyproline concentration in scoliotic birds is about twice that observed in normal chickens and may reflect an abnormality in collagen metabolism (LIN et al. 1980). It is probable that both the curvature of the spine (scoliosis) and an increase in the vertical arch of synsacrum (kyphosis) are affected by some genes in common (TAYLOR 1971).

PRYSZCZ et al. (1996a) estimated the realised heritability of embryo defects of the thoracic region of the vertebral column in chickens. Results of scoliosis-based divergent selection performed in embryos confirm the possibility of limitation of these defects through such early selection in families. That study showed that the other axial skeletal defects were similarly affected by the selection for scoliosis, indicating a common source of these defects. PRYSZCZ (1996b) described the relation between scoliosis and abnormalities in the joints of adult chickens.

Microsatellites are simple sequence repeats (STRs) of mono-, di-, tri-, tetra- or penta-nucleotide units, widely dispersed throughout animal genomes (TAUZ 1989, BECKMANN, WEBER 1992). STRs are primary genetic markers; they are highly polymorphic loci that have been used to map quantitative trait loci (QTL), to estimate genetic variation, to determine parentage, and to determine the phylogeny of organisms (CHENG 1997, ORTI et al. 1997, ZHOU, LAMONT 1999, ZHANG et al. 2002).

Compared to other DNA polymorphism analyses, the detection of microsatellite polymorphism results in the greatest expected heterozygosity (POWELL et al. 1996). Microsatellite polymorphism enables a clearer differentiation, even between closely related breeds, and increases the accuracy of the predicted divergence (ZHANG et al. 2002).

Highly polymorphic microsatellite markers have been used in a new efficient method known as 'DNA pooling' for the identification of complex disease loci (CARMI et al. 1995). DNA pooling relies on differences observed in the allelic distribution between pools from affected and unaffected individuals. One of the dif-

ferences consists in a reduced number of alleles in the affected pool; this indicates the sharing of a chromosomal region (SHEFFIELD et al. 1995). Application of that strategy leads to identification of several linked disease loci in the human genome (WINICK et al. 1999, TURNPENNY et al. 1999, BETARD et al. 2000).

The genetic linkage maps of the chicken contain over 1900 loci, of which nearly 800 are highly polymorphic microsatellite markers (GROENEN et al. 2000). Recently, the development of resolution genetic maps and the necessary powerful statistical methods have initiated QTL mapping experiments for a variety of complex diseases and economically important traits, including those affecting growth (GROENEN et al. 1997, VAN KAAM et al. 1998, 1999a, TATSUDA, FUJINAKA 2001), feed efficiency (VAN KAAM et al. 1999a), carcass traits (VAN KAAM et al. 1999b), and Marek's disease (VALLEJO et al. 1998, XU, GOODRIDGE 1998, YONASH et al. 2001).

The aim of this study was to determine the genetic diversity at microsatellite loci in Rhode Island Red and Sussex chickens, divergently selected over six generations for the incidence of skeletal defects in embryos. For each chicken line, estimation was made of the allelic composition and frequencies as well as the degree of polymorphism (number of alleles, heterozygosity, polymorphic information content). We expected that the genetic data obtained in this study would provide valuable information that could be used in the search for genes affecting skeletal malformations in chickens.

Material and methods

Experimental material

The experimental material consisted of chickens of two breeds (Rhode Island Red, RIR, and Sussex, SX) divergently selected over six generations for high (H) or low (L) incidence of skeletal defects in embryos. A description of the material was previously provided by PRYSZCZ et al. (1999). The divergent selection was performed over six generations on the basis of the analysis frequency of scoliosis of dorsosacral vertebrae found in dead embryos on day 17-21 of incubation in full families of commercial RIR and SX lines. The birds of the lines examined, evaluated according to the frequency of defects in the progeny (at least 5 embryos), were crossed within the groups significantly differing in the level of scoliotic defects in relation to the mean for the embryo population (high and low). The frequency of scoliosis in dead embryos in RIR chickens was 40% in H lines and 4.1% in L lines, while in SX chickens, it was 19.5% in H lines and 3.3% in L lines.

The analysis of polymorphism of 15 microsatellite DNA markers was performed for 60 individuals – 30 per breed (15 per line with high or low incidence of skeletal defects).

DNA Samples

Blood samples were collected into vacuum tubes containing EDTA, and stored at -20°C . The DNA was extracted by standard methods, its concentration was determined spectrophotometrically, after which the DNA was diluted to a final concentration of 0.1 g/ L .

Microsatellite markers

The microsatellite loci were chosen from among MCW (CROOIJMANS et al. 1996, GROENEN et al. 1997) and ADL markers (CHENG et al. 1995, BURT et al. 1996). In total, 15 polymorphic markers on chromosomes 1-5 and 8 were genotyped. The microsatellite markers were obtained from the Roslin Institute database (<http://www.ri.bbsrc.ac.uk>). The microsatellite repeat MCW41 is situated within the *Y* gene of the chicken ovalbumin family, while MCW51 in the chicken vitamin D-induced calbinding *D 28 K* gene.

DNA genotyping

The PCR was carried out in a volume of 7.5 L comprising 100 ng of template DNA, 2.5 pmol of each primer, 100 M of each dNTP, 0.5 unit of DNA Taq polymerase, 10 mM tris- HCl (pH 8.8), 1.5 mM MgCl_2 , 50 mM KCl, and 0.1% Triton X-100. One primer for each locus was labelled with fluorescein. The following amplification conditions were applied: 5 min. of denaturation at 94°C , followed by 25-35 cycles of denaturation at 94°C for 45 s, annealing at $50\text{-}65^{\circ}\text{C}$, and extension at 72°C for 60 s. The PCR was performed by using a PTC-200 Programmable Thermal Controller (MJ-Research).

The fluorescent PCR products were separated on 6% denaturing polyacrylamide gels, by an Automated Laser Fluorescent (ALFexpress) DNA Sequencer. The PCR products were analysed after 5 min. of denaturation in a 50% formamide solution containing blue dextran. In each lane, 1-2 PCR products, differing in size range, were loaded together with a standard size marker. Results were visualised and the genotyping performed with the Allele Links 1.01 software. After automated allele calling within Allele Links 1.01, individual genotypes were checked by manual inspection before exporting the genotypes to Excel.

Heterozygosity and PIC

Two genetic parameters were estimated from marker allelic frequencies: the probability of heterozygosity for a marker locus in the chicken lines (OTT 1992, WEIR 1990) and polymorphic information contents (PIC) (BOSTEIN et al. 1980).

Results

At the 15 microsatellite loci examined, the total number of alleles amounted to 44 and 49 for RIR and SX chickens, respectively. The number of alleles at a single locus ranged from 1 to 6, the average number being 2.9 and 3.6 for RIR and SX chicken, respectively. Breed-specific alleles were observed for 14 of the 15 microsatellite markers.

Table 1. Number of alleles, heterozygosity, and the polymorphic information contents (PIC) index per locus for 15 microsatellite loci for lines with high (H) or low (L) incidence of skeletal defects

Microsatellite locus	Alleles/locus (n)		Heterozygosity*		PIC index	
	H lines	L lines	H lines	L lines	H lines	L lines
ADL322	5	5	0.70	0.80	0.62	0.74
MCW145	4	4	0.71	0.74	0.63	0.67
MCW114	4	5	0.63	0.62	0.55	0.54
MCW167	2	2	0.51	0.51	0.37	0.37
MCW68	5	4	0.71	0.75	0.63	0.67
MCW18	5	4	0.73	0.59	0.66	0.49
MCW200	5	5	0.67	0.59	0.60	0.53
MCW283	3	3	0.65	0.64	0.56	0.54
MCW32	3	3	0.58	0.49	0.48	0.40
MCW63	4	4	0.71	0.61	0.62	0.52
MCW40	4	6	0.61	0.71	0.52	0.63
MCW41	4	2	0.40	0.18	0.35	0.16
MCW51	2	2	0.51	0.41	0.37	0.33
MCW56	4	5	0.67	0.63	0.58	0.55
MCW170	4	2	0.40	0.51	0.34	0.37
Mean	3.8	3.7	0.22	0.21	0.83	0.82

* heterozygosity corrected for small populations

Table 1 presents characteristics of chicken lines with a high (H) and low (L) level of skeletal defects: number of alleles per locus, heterozygosity, and PIC index. Average heterozygosity and PIC index for 15 analysed markers were nearly the same for H and L lines. The average number of alleles at a single locus was 3.8 for H lines and 3.7 for L lines.

Table 2. Frequency of alleles (%) for RIR and SX chicken lines with high (H) or low (L) incidence of skeletal defects

Locus	Allele (bp)	Frequency of alleles (%)				All lines
		H lines		L lines		
		SX	RIR	SX	RIR	
1	2	3	4	5	6	7
MCW40	130	–	–	3.3	–	0.8
	132	73.3	30	40	20	40.8
	140	–	70	3.3	40	28.3
	142	23.3	–	46.6	36.7	26.6
	148	3.3	–	3.3	3.3	2.5
	150	–	–	3.3	–	0.8
MCW114	248	–	53.3	–	56.6	27.5
	262	63.3	46.6	66.6	43.3	55
	264	–	–	3.3	–	0.8
	270	20	–	10	–	7.5
	274	16.6	–	20	–	9.1
MCW41	160	50.0	100	80	100	83.6
	162	23.0	–	20	–	10.3
	164	23.0	–	–	–	5.1
	166	3.8	–	–	–	0.8
MCW68	172	–	20	–	63.3	20.8
	174	–	3.3	33.3	–	9.1
	178	73.3	–	66.6	–	35
	184	26.6	–	–	–	6.6
	188	–	76	–	–	19.1
	196	–	–	–	36.6	9.1
MCW18	214	70	–	40	32.1	35.5
	220	23.3	–	10	7.1	10.1
	222	–	26.7	–	–	6.7
	224	6.6	–	46.6	60.7	27.9
	228	–	73.3	3.3	–	19.4
MCW56	172	89.2	–	80	30.0	49.1
	174	10.7	–	13.3	10.0	8.4
	196	–	–	6.6	–	1.6
	200	–	73.3	–	53.5	32.2
	204	–	26.7	–	6.7	8.4
MCW32	262	–	16.7	–	–	5.1
	272	100	33.3	81.8	56.7	62.2
	278	–	–	–	10.0	3.0
	290	–	50.0	18.1	33.3	29.5

Table 2 cont.

1	2	3	4	5	6	7
MCW170	260	40	–	83.3	6.7	32.5
	264	3.3	–	–	–	0.8
	270	6.6	–	–	–	1.6
	272	50	100	16.6	93.3	65
ADL322	130	76.6	–	56.6	–	33.3
	134	–	20	3.3	33.3	14.1
	136	–	3.3	–	13.3	4.1
	138	–	76.6	–	50	31.6
	140	23.3	–	40	3.3	16.6
MCW145	178	16.6	–	26.6	–	10.8
	186	83.3	–	43.3	6.6	33.3
	196	–	53.3	–	73.3	31.6
	204	–	46.6	30	20	24.1
MCW167	106	–	100	–	93.3	48.3
	112	100	–	100	6.6	51.6
MCW283	116	13.3	60	66.6	20	40
	124	–	40	–	80	30
	140	86.6	–	33.3	–	30
MCW200	230	21.4	–	43.3	78.5	35.3
	232	50	–	40	–	22.4
	242	10.7	–	–	3.5	3.4
	244	17.8	–	16.6	–	8.6
	252	–	100	–	17.8	30.1
MCW63	142	–	63.3	9.0	13.3	25.5
	144	18.7	–	68.1	–	18.3
	146	–	36.6	18.1	83.3	40.8
	148	81.2	–	4.5	3.3	15.3
MCW51	87	–	100	–	53.3	38.3
	100	100	–	100	46.6	61.6

The frequency of alleles for H and L lines of RIR and SX chickens is presented in Table 2. We observed differences in allelic distribution between the affected (H) and unaffected (L) lines (Table 2).

Specific alleles for H lines were identified at loci: MCW41 (164, 166 bp), MCW68 (184, 188 bp), MCW18 (222 bp), MCW32 (262 bp), and MCW170 (264, 270 bp). Among them, alleles specific to the SX breed included: MCW41 (164 bp, 166 bp), MCW68 (184 bp), MCW170 (264 bp, 270 bp), and for the RIR breed: MCW18 (222 bp), MCW32 (262 bp) and MCW68 (188 bp).

For L lines, 6 specific alleles were found at loci: MCW114 (264 bp), MCW68 (196 bp), MCW56 (196 bp), and MCW32 (278 bp) and MCW40 (130, 150 bp). Among them, alleles specific to the SX breed were: MCW114 (264 bp), MCW56 (196 bp), MCW40 (130, 150 bp), and for the RIR breed: MCW68 (196 bp) and MCW32 (262 bp).

None of the alleles was observed as specific to H or L lines in both breeds simultaneously.

Discussion

According to MCCARREY et al. (1981), the positive response to long-term selection implies that the expression of scoliosis is affected by several genes and that most of the major alleles are recessive. Because of inbreeding and selection, we assumed that our experimental material became fixed as regards scoliosis alleles at all major loci. It was created from closely related lines (after 5 generations the coefficient of inbreeding amounted to 20.9% and 14.2% in RIR chickens and 14.4% and 11.0% in SX chickens for lines H and L, respectively).

In lines selected for high skeletal deformations, a higher mortality of embryos was observed – total losses during the hatching period in H and L lines were 42.5% and 33.0% in the RIR breed and 30.6% and 24.4% in the SX breed, respectively (JASZCZAK et al. 1999). The frequency of chromosome abnormalities occurring at the early embryo stages, was significantly lower in the unaffected lines (2.4%) than in lines with developmental spine disturbances (5.7%) (PRYSZCZ et al. 1999).

The material described was screened for genomic variation on the basis of DNA fingerprinting. An analysis of the representative DNA fingerprinting band patterns led to the identification of specific bands for chicken families with a high or low incidence of skeletal defects. The *Hinf*I enzyme / 33.6 probe combination produced 30 bands with different frequencies in each family. The average band sharing between lines with a high or low incidence of skeletal defects was 0.79, while between families within lines selected for high or low incidence of skeletal defects, it was 0.97 and 0.98, respectively (JASZCZAK et al. 2001).

In this study, 15 microsatellite loci located on macrochromosomes 1, 2, 3, 4, 5 and 8 were chosen from the Roslin Institute database on the basis of their utility and widespread location (potentially linkage to genes affecting skeletal defects). As a result of the analysis of polymorphism of those loci, 8 specific alleles were identified for H lines and 6 specific alleles for L lines. None of the alleles observed was specific to H or L lines in both breeds simultaneously.

The analysed microsatellite repeat MCW51 is located in the chicken vitamin D-induced calbinding D 28 K gene. This protein plays a fundamental role in the vitamin D-mediated transport of calcium in birds and mammals. Deficiency in vitamin D leads to osteomalacia in humans. It has been shown that addition

of 1,25-dihydroxyvitamin D to the diet of young broilers reduces the incidence of tibial dyschondroplasia (MITCHELL et al. 1997). The analysis of microsatellite polymorphism of MCW51 loci shows that none of the identified alleles was specific to H or L lines.

These results confirm that the microsatellite loci analysed are highly polymorphic. The selection for a high or low level of skeletal defects in embryos, respectively, had different effects on the frequencies of alleles of the analysed microsatellite loci. A further analysis is needed to check whether this response reflects a linkage to a neighbouring gene.

Because of similarities in the expression of this disease in chickens and humans, an investigation of the disease loci in chickens may provide useful insights into locating genes affecting adolescent idiopathic scoliosis in humans (MCCARREY et al. 1981). Chicken lines selected for high or low skeletal defects constitute a valuable source enabling the identification of real genes causing the effect of interest.

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