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DNA fingerprinting of chicken lines divergently selected for high or low incidence of skeletal malformations*

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Genetic analysis was conducted in chicken belonging to lines divergently selected for high or low frequency of skeletal defects (line H and L, respectively). The analysis was based on the DNA fingerprinting technique, using enzyme *Hinf*I and Jeffrey's 33.6 probe. Principal aims were: 1) to determine the effects of selection on the genetic variation within and between lines, and 2) to search for the presence of line-specific minisatellite alleles (as bands in the DNA fingerprinting pattern) which could be used as markers for the predisposition to skeletal defects. DNA fingerprinting pattern (DFP) displayed differences in position of bands, some of them appearing characteristic only for one out of two chicken lines studied.

KEY WORDS: chicken / divergent selection / DNA fingerprinting / minisatellites / skeletal defects

In modern animal breeding and production, with welfare and optimum nutrition, the major health problems arise mainly from genetic background. Skeletal defects are one of the most important issues in poultry breeding, particularly in meat type birds. The etiology as well as pathogenesis of skeletal defects are still poorly understood. However, it is known that reasons for skeletal defects in chicken are multiple and

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hereditary [Mercer and Hill 1984].

The most important inherited skeletal defects are 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 and spondylolisthesis [Somes 1990]. Poultry losses caused by skeletal defects result from increased mortality and number of birds culled (caused also by septicaemia-toxaemia) and downgrading of breast and legs quality [Sullivan 1994].

From the studies of Riggins *et al*. [1977] and McCarrey *et al*. [1981] on the inherited form of scoliosis it appears that variation in the expression of scoliosis is attributed to an incomplete penetration of major genes, additive effects of minor modifying genes and possible correlation with sexual maturity. Pryszcz [1996] estimated the realized heritability of embryo defects of the thoracic region of the vertebral column in chickens. Results of scoliosis-based divergent selection of embryos confirmed a feasibility of reduction 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 genetic source of these defects. By applying the divergent selection of RIR chickens for the high or low frequency of scoliosis (of dorsosacral vertebrate in embryos dead on day 17-21 of incubation), the frequency of scoliosis in high-frequency line appeared 10-fold higher than in low-frequency line (40 *vs* 4.1%) – Pryszcz [1996].

Obviously the selection does not affect the genome, but rather changes gene frequencies in the population and modifies the range of genetic variation of selected and correlated traits. These effects can be detected at the DNA level with the use of appropriate tools. Due to relatively small size of the chicken genome total size of repeating sequences represented by microsatellites is reduced. It was assessed [Primmer *et al*. 1997, Roots and Baker 2002] that with the total 30-70 thousands of microsatellites (>20 bp), their frequency is 1 per the 20-39 kbp in comparison to human frequency amounting to 1 per the 6 kbp. As the avian genome is characterized by a low frequency of microsatellite repeats, minisatellite markers are exceptionally valuable sequences in the search for major genes of poultry production traits [Haley 1991]. Their *loci*, therefore, have extremely high polymorphism arising from differences in the number of the core sequence of the repeating units [Jeffreys *et al*. 1991]. Simultaneous screening of many polymorphic *loci* in the genome provides an effective tool for assessing genetic variation and diversity [Brockmann *et al.* 1993] as well as identifying markers associated with *loci* determining selected traits [Dunnington *et al*. 1990, Plotsky *et al*. 1993].

In the present study we aimed to find out to what extent the two phenotypically distinct chicken lines (*i.e*. selected divergently for high or low frequency of skeletal defects) were differentiated with respect to their genotypic profiles. For each chicken line, estimation was made of the degree of polymorphism. We expect that the genetic data obtained in this study may provide valuable information that could be used in the search for genes affecting skeletal malformations in chickens.

Material and methods

Animals

Rhode Island Red (RIR) chickens were used, divergently selected over six generations for high (H) or low (L) incidence of skeletal defects in embryos. A detailed description of the material was previously provided by Pryszcz *et al.* [1999]. The selection was performed over six generations on the basis of the analysis of frequency of scoliosis of dorsosacral part of vertebral column found in embyos dead on day 17-21 of incubation in full families of commercial RIR line. The birds of the examined lines evaluated according to the frequency of defects in the progeny (at least five 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 H line chickens was 10-fold higher than in L line (40% *vs* 4.1%) – Pryszcz *et al.* [1999].

The analysis of minisatellite polymorphism was performed for two families within each line. Each family included 10 full-sib individuals.

The protocol of the experiments on live chickens was approved by the Third Local Ethics Commissionon on Animal Experimentation.

DNA fingerrprinting method

High-molecular-weight genomic DNAs were prepared from blood using standard methods of proteinase K digestion and by phenol/chloroform/isoamyl alcohol extraction. Proteinase K solution was added to the lysis buffer (50 mM KCl; 10 mM Tris-HCl, pH 9; 0.1% Triton X-100) until a final concentration of 0.12 mg/ml, and the mixture was incubated overnight at 56°C. DNA was extracted in 500 µl of phenol:chloroform: isoamyl-alcohol (25:24:1), precipitated with 2 vol. of 100% ethanol, transferred to a sterile microcentrifuge tube, washed with 70% ethanol, centrifuged at 10,000 × g and dried. The resulting pellet was dissolved in 50 µl of distilled water. The DNA concentration and quality was estimated spectrophotometrically (at 270, 320, 360 nm) and with electrophoresis. Eight µg of DNA were digested with restriction enzyme *Hinf* I using three units enzyme per 1 µg of DNA. The digests were separated electrophoretically in 0.8% agarose gel during 48 h. DNA was transferred onto standard Hybond-Npf nylon filters in 20 × SSC using the standard capillary method, and remained so overnight. The filters were pre-hybridized for 40 min at 50°C and next hybridized to probes 33.6 and 33.15 [NICE CELLMARK DIAGNOSTIC] for 30 min in the same temperature. The chemiluminescent signal was detected using Lumi-Phos® 530 solution [CELLMARK DIAGNOSTIC].

The technical problems and high costs associated with the use of individual DNA fingerprinting patterns (DFPs) for inter-population comparisons were partially reduced by using pooled DNA samples [Dunnington *et al*. 1990]. Pooled DNAs from both lines were studied to quantify genomic variation between and within lines. The DFP analysis included only bands representing fragments larger than 2 kb. The gels were

scored by two independent reviewers. Banding patterns were compared between lanes to classify shared and non-shared bands. Bands were regarded as non-shared if they differed in their position more than the half of the band width and if the ratio of the intensities is less than 1:2.

DNA fingerprinting pattern (DFP) analyses

The measures chosen to estimate genetic variation were based on band sharing and band frequency, the two fundamental parametres. To arrive at relative frequencies, optical densities of bands were considered to have the same distance from the origin within the material studied. The band with the highest optical density was assigned the value of 1, and the densities of the remaining bands were expressed relative to the optical density of that band, thus reaching values between 1 and 0.

Based on the results of patterns generated by DNA fingerprinting mathematical calculations were done to compare analysed individuals and to assess genetic distance between lines. Main statistical parametre of band patterns, *i.e*. band sharing (BS), based on the number of common bands between two individual samples, was used to describe the similarity between profiles of DFP. On the basis of BS parametres the probability of identity – P [Wetton *et al*. 1987], the total number of distinct and recombinationally separable hypervariable *loci* – L [Lynch 1990], average genetic variation between individuals – AVB [Kuhnlein *et al*. 1989], heterozygosity – H [Stephens *et al.* 1992], and genetic distance between lines – DL [Lynch 1990] were determined to compare analysed individuals within and between lines. All means were compared using the General Linear Models procedure [SAS Institute 1990]. A detailed description of the mathematical calculations is quoted by Sacharczuk *et al*. [2005].

Results and discussion

The breeding strategy, based on selection conducted independently for each chicken line in several families, renders it possible to eliminate a random occurrence of typical markers, which in a different situation could be, for instance, the effect of a genetic drift. As result of the analysis performed, a similar genetic variation was observed within each line. The principal variation indicator, *i.e*. variation coefficient (V) as well as H and L in the DFP pattern were not found significantly different between lines (Tab. 1). However, a dramatically low inter-family variation was observed within line L when compared with that recorded for line H (Tab. 2 and 3).

Specific bands were observed in both lines – six in line L and five in line H (Photo 1). Those bands are markers and the principal determinants of the genetic distance, estimated on the level shown in Tables 4 and 5. The value of the genetic distance was clearly higher when between lines L and H individual families were compared (Tab. 2 and 3). This is a natural phenomenon, as such comparisons do not consider the variation occurring between families, but within the lines selected. One must also emphasize, that lowering the value of the total genetic distance was, in the lines examined, deter-

Table 1. Parameters of genetic variation within lines selected for high and low frequency of skeletal defect

Line	Probe/Enzyme		N[1]	L[2]	P[3]	H[4]	AVB[5]
L	33.6/*Hin*fI	mean	15.00	10.09	1.45×10^{-1}	0.21	0.38
		SD	2.45				
H		mean	13.86	9.45	6.16×10^{-1}	0.18	0.42
		SD	1.95				

[1]Mean number of bands.
[2]Total number of distinct and recombinationally separable hypervariable loci.
[3]Probability of identity.
[4]Heterozygosity.
[5]Genetic variation between individuals.

Table 2. Parameters of genetic variation between families selected for low (L) incidence of skeletal defect

Parameters between families within L line	Line L (Family 1)	Line L (Family 2)
Specific bands	1	2
BS	0.94	
AVB	0.06	
DL	0.06	

Parameters' abbreviations are explained at the bottom of Table 1.

Table 3. Parameters of genetic variation between families selected for high (H) incidence of skeletal defect

Parameters between families within H line	Line H (Family 1)	Line H (Family 2)
Specific bands	3	2
BS	0.86	
AVB	0.14	
DL	0.16	

Parameters' abbreviations are explained at the bottom of Table 1.

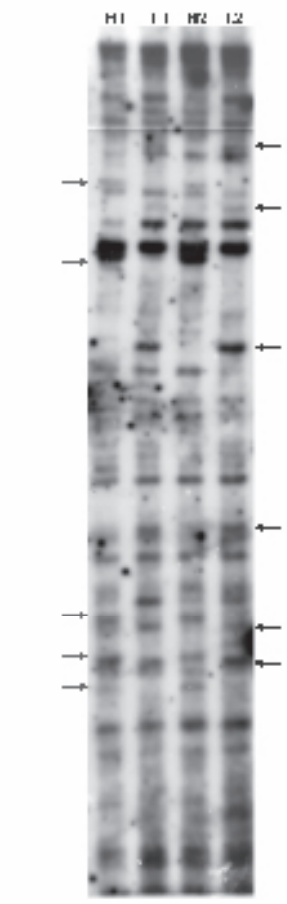

Photo 1. Genetic profiles of chicken families divergently selected for high (Families H1 and H2) and low (Families L1 and L2) incidence of skeletal defects. Arrows indicate specific bands for H and L lines.

Table 4. Parametres of genetic variation between chicken lines selected for low (L) and high (H) frequency of skeletal defects as calculated by lines comparison

Parametres between lines	Line L	Line H
Specific bands	6	5
BS	0.84	
DL	0.17	

Parametres' abbreviations are explained at the bottom of Table 1.

Table 5. Parametres of genetic variation between chicken lines selected for low (Family 1 and 2) and high (Family 1 and 2) frequency of skeletal defects as calculated by families comparison

BS AVB DL	L (Family 1)	L (Family 2)
H (Family 1)	0.71 0.29 0.34	0.69 0.31 0.37
H (Family 2)	0.62 0.38 0.47	0.61 0.39 0.49

mined principally by the high variation between families within line H. Identified marker bands may be used for controlled breeding, as they render it possible to eliminate individuals characterized by the presence of bands specific for line H and promote birds with alleles marked by bands specific for line L.

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 could assume that our experimental material became fixed as regards scoliosis alleles at all major *loci*. The material was obtained from closely related lines (after five generations the coefficient of inbreeding amounted to 20.9% and 14.2% in H and L line, respectively). In H line, a higher mortality of embryos was observed – total losses during the hatching

period in H and L lines were 42.5% and 33.0%, respectively [Pryszcz 1996]. According to Pryszcz *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 those with high incidence of developmental spine disturbances (5.7%). Higher frequency of chromosome abnormalities was also observed in the heavy-weight turkey embryos when compared to the medium-heavy weight ones [Jaszczak *et al*. 2003].

The material described here was screened for genomic variation on the basis of microsatellites and growth hormone (GH) polymorphism. As a result of the polymorphism analysis of 15 microsatellite *loci*, eight specific alleles were identified for H line and six for L line [Wardęcka *et al*. 2004]. The analysed microsatellite repeat MCW51 (located in the chicken vitamin D-induced calbinding D 28 gene) was used for searching specific alleles for H and L lines [Wardęcka *et al*. 2004]. This protein plays a fundamental role in the vitamin D-mediated transport of calcium in birds and mammals. However, none of the identified alleles was found to be line-specific. The results of the studies on the GH (our unpublished data) showed high polymorphism in this gene when RFLP was analysed with *Msp*I enzyme, and that 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 *loci*.

The current study was performed with the same chicken lines (Rhode Island Red) selected for high or low incidence of skeletal defects) using DNA fingerprinting method. Pooled DNA was compared from individuals with high and low incidence of skeletal defects (Photo 1). DFP displayed differences in position of bands; some characteristic bands differentiated line H from line L. We anticipate that these bands may be linked to genes controlling the skeletal development. As the hot-spots of recombination events, also the polymorphism in minisatellites longevity may be responsible for chromosome aberrations [Singh 1995].

It is possible that both the curvature of the spine (scoliosis) and an increase in the vertical arch of synsacrum (kyphosis) are determined by common genes [Taylor 1971]. Pryszcz [1996] reported a relation between scoliosis and abnormalities in the joints of adult chickens. Further selection of healthy animals could be possible with this method – an important application in modern chicken-farming.

Because of similarities in the expression of skeletal malformation in chickens and humans, further investigation of the disease *loci* in chickens may provide useful insights in the understanding of idiopathic scoliosis in humans [McCarrey *et al*. 1981, Wardęcka *et al*. 2004].

REFERENCES

1. BROCKMANN G., BUITKAMP J., BUNGER L., EPPLEN J.T., SCHWERIN M., 1993 – DNA fingerprinting of trait-selected mouse lines and linkage analysis in reference families. ***Experientia*** 67, 403-410.
2. DUNNINGTON E.A., GAL O., PLOTSKY Y., 1990 – DNA fingerprints of chickens selected for high and low body weight for 31 generations. ***Animal Genetics*** 21, 247-257.

3. HALEY C.S., 1991 – Use of DNA fingerprints for the detection of major genes for quantitative traits in domestic species. ***Animal Genetics*** 22, 259-277.
4. JASZCZAK K., PARADA R., SACHARCZUK M., 2003 – A note on chromosome abnormalities in early turkey embryos. ***Journal of Animal and Feed Sciences*** 12, 833-837.
5. JEFFREYS A.J., MACLEOD A., TAMAKI K., NEIL D.L., MONCKTON D.G., 1991 – Minisatellite repeat coding as digital approach to DNA typing. ***Nature*** 354, 204-209.
6. JEFFREYS A.J., MORTON D.B., 1987 – DNA fingerprints of dogs and cats. ***Animal Genetics*** 18, 1-15.
7. KUHNLEIN U., DAWE Y., ZADWORNY D., GAVORA J.S., 1989 – DNA fingerprinting: a tool for determining genetic distances between strains of poultry. ***Theoretical and Applied Genetics*** 77, 669-672.
8. LYNCH M., 1990 – The similarity index and DNA fingerprinting. ***Molecular Biology and Evolution*** 7, 478-484.
9. MCCARREY J.R., ABBOTT U.K., BENSON D.R., RIGGINS R.S., 1981 - Genetics of scoliosis in chickens. ***Journal of Heredity*** 72, 6-10.
10. MERCER J.T., HILL W.G., 1984 – Estimation of genetic parameters for skeletal defects in broiler chickens. ***Heredity*** 53, 193-203.
11. PLOTSKY Y., CAHANER A., HABERFELD A., LAVI U., LAMONT S.J., HILLEL J., 1993 – DANN fingerprinting bands applied to linkage analysis with quantitative trait loci in chickens. ***Animal Genetics*** 24, 105–110.
12. PRIMMER C.R., RAUDSEPP T., CHOWDHARY B.P., MOLLER A.P., ELLEGREN H., 1997 – Low frequency of microsatellites in the avian genome. ***Genome Research*** 7, 471-482.
13. PRYSZCZ W., 1996 – Scoliotic changes in adult birds from families selected for low and high frequency of embryonal scoliosis. ***Zivocisna Vyroba*** 11, 527-528.
14. PRYSZCZ W., JASZCZAK K., JASZCZAK J., BRZĘK A., 1999 – Chromosome aberrations in chicken embryos from families with developmental abnormalities of spine. ***Annales Universitatis Mariae Curie-Sklodowska*** 33, 259-263.
15. RIGGINGS R., ABBOTT U., ASHMORE C., RUCKER R., MCCARREY J.R., 1977 – Scoliosis in chicken. ***Journal of Bone and Joint Surgery*** 59, 1020-1026.
16. ROOTS E.H., BAKER R.J., 2002 – Distribution and characterization of microsatellites in the emu (Dromaius novaehollandiae) genome. ***Journal of Heredity*** 93, 100-106.
17. SACHARCZUK M., JEZIERSKI T., DANIEWSKI W., GÓRECKA A., PARADA R., ŚWIERGIEL A.H., JASZCZAK K., 2005 – DNA fingerprinting analysis of rabbits from lines divergently selected for high and low open-field activity. ***Animal Science Papers and Reports*** 23 (2), 107-117.
18. SAS Institute, 1990 – SAS/STAT User's Guide, 4th ed. Cary, N.C.
19. SINGH L., 1995 – Biological significance of minisatellites. ***Electrophoresis*** 16, 1586-1595.
20. SOMES R.G., 1990 – Mutations and major variants of muscles and skeleton in chickens. In: Poultry Breeding and Genetics (R. D. Crawford, ed.), Elsevier.
21. STEPHENS J.C., GILBERT D.A., YUHKI N., O'BRIEN S.J., 1992 – Estimation of heterozygosity for single probe multilocus DNA fingerprints. ***Molecular Biology and Evolution*** 9, 729-743.
22. SULLIVAN T.W., 1994 – Skeletal problems in poultry: estimated annual cost and descriptions. ***Poultry Science*** 73, 869–882.
23. TAYLOR L., 1971 – Kyphoscoliosis in a long-term selection experiment with chickens. ***Avian Diseases*** 15, 367-390.

24. WARDĘCKA B., JASZCZAK K., PIERZCHAŁA M., PARADA R., KORCZAK M., 2004 - Divergent selection for skeletal malformations in chickens alters polymorphism at microsatellite loci. ***Journal of Applied Genetics*** 45, 61-71.
25. WETTON J.H., CARTER R.E., PARKIN D.T., WALTERS D., 1987 – Demographic study of a wild sparrow population by DNA fingerprinting. ***Nature*** 327, 147–152.

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DNA fingerprinting kur selekcjonowanych na częstość występowania wrodzonych wad szkieletu

S t r e s z c z e n i e

Przeprowadzono analizę genetyczną kur z dwóch linii, selekcjonowanych rozbieżnie na wysoką (H) i niską (L) częstość występowania wad szkieletowych. W analizie wykorzystano technikę DNA fingerprinting z użyciem enzymu *Hinf*I i wielopozycyjnej sondy molekularnej Jeffreys'a 33.6. Głównym celem analizy było 1) określenie wpływu selekcji na zmienność genetyczną wewnątrz selekcjonowanych linii i między tymi liniami oraz 2) poszukiwanie specyficznych alleli minisatelitarnych (w postaci prążków we wzorze DNA fingerprinting) dla danej linii, które mogą być markerami predyspozycji do wad szkieletowych. W obrazie DNA fingerprinting stwierdzono prążki specyficzne tylko dla jednej bądź drugiej selekcjonowanej linii kur.