Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wroclaw.

ELECTRONIC JOURNAL OF POLISH AGRICULTURAL UNIVERSITIES

2001
Volume 4
Issue 2
Series ANIMAL HUSBANDRY

BRODACKI A., ZIĘBA G., CYWA-BENKO K. 2001. GENETIC DISTANCE BETWEEN SELECTED BREEDS AND LINES OF LAYING HENS **Electronic Journal of Polish Agricultural Universities**, Animal Husbandry, Volume 4, Issue 2.
Available Online http://www.ejpau.media.pl

GENETIC DISTANCE BETWEEN SELECTED BREEDS AND LINES OF LAYING HENS

Antoni Brodacki[1], Grzegorz Zięba[1], Katarzyna Cywa–Benko[2]
[1]*Department of Biological Bases of Animal Production, Agricultural University of Lublin, Poland*
[2]*National Research Institute of Animal Production, Department of Poultry Breeding and Production, Balice, Poland*

ABSTRACT

The investigations were aimed at the determination of genetic distance among eight lines of laying hens kept in Poland and belonging to the following breeds: Green–Leg ZKF and Z11, Leghorn H22 and G99, Rhode Island Red RD2, Yellow–Leg Ż33, Polbar (Pb) and Sussex (Sx). The phenotype frequencies of fast–migrating prealbumin (Pa–F), egg–yolk transferrin and ovalbumin (Ov–A), ovoglobulins: G_3, G_4 and G_2 and conalbumin were obtained from the electrophoregrams of horizontal polyacrylamide gel electrophoresis.

For each of the gene pool stocks managed in Felin and Zyczyn, Poland, three Pa–F protein phenotypes, B, BD and D, were observed in Pb and ZKF and Z11, six phenotypes: A, AB, AD, B, BD and D were observed in H22 Leghorns, and five phenotypes were recorded in the G99 line. Additional phenotypes, AC, BC, CD and C, were observed in Sussex hens.

A range of alleles determined the fast–migrating prealbumin phenotypes. The alleles were composed of four genes, A, B, C and D, in Sx hens, of three genes in H22, G99 and Ż33 hens, and of two genes in Pb, Z11 and ZKF. The frequencies of ovoglobulin G_3 were identical as the frequencies of ovalbumin in all the lines. It has been presumed that the genes that encode ovalbumin and ovoglobulin G_3 polymorphism are strictly coupled. The frequencies of three ovoglobulin G_2 and G_4 genotypes were similar in both lines of Leghorn, but different in ZKF and Z11 Green–Legs. In all the studied birds, except for one Green–Leg individual and two Yellow–Legs, only conalbumin B was observed. The proteins of transferrin region were encoded by two alleles with substantial advantage of allele B.

Average heterozygosity per single loci, estimated basing on seven protein sets of egg content, ranged from 0.087 in RD2 to 0.287 in G99. The highest genetic similarity was observed for two Leghorn lines, G99 and H22, and for the Green–Legs, ZKF and Z11. The most distant breeds were Z11 and RD2.

Key words: hens, protein polymorphism, genetic distance

INTRODUCTION

Among its other applications, protein polymorphism can be used in phylogenetic studies or to create genetic characteristics of herds and breeds of animals. According to Okabayashi *et al.* [12], genetic differences among animal groups may be determined basing on genetic distances. The genetic distance allows determination of similarities between the stocks of animals with a single estimated number. Genetic distances in hens, based on blood serum and egg content protein or enzyme polymorphism, were presented by, among others, Hashiguchi *et al.* [5], Cewa–Benko *et al.* [2] and Moiseeva *et al.* [10].

The most recent achievements in molecular biology allow determination of DNA polymorphism–based distances with the random amplification of polymorphic DNA (RAPD), with DNA fingerprinting [11, 14, 16, 18] or basing on microsatellite DNA polymorphism [1, 6].

The aim of the study was to determine egg–content protein genetic polymorphism and to estimate the genetic distance among the breeds and lines of hens belonging to gene pool stocks that are managed in Poland. The studies may contribute to more efficient preservation of biodiversity in this domestic species of birds.

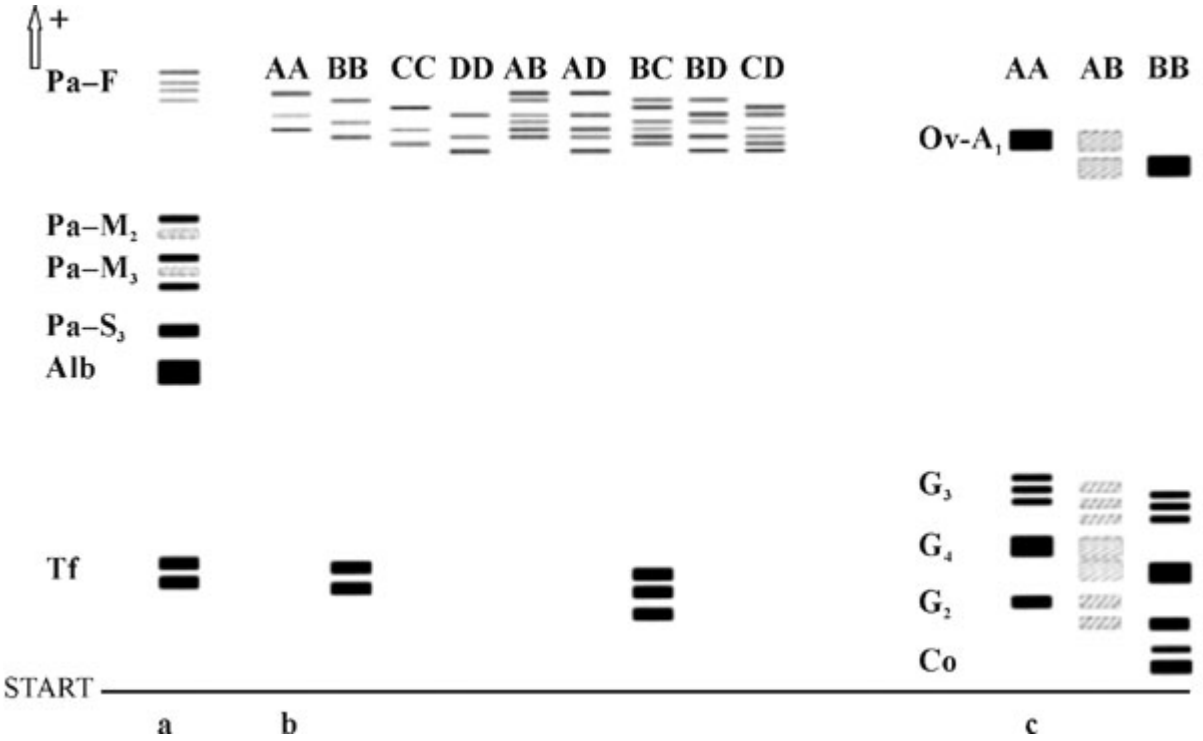
MATERIALS AND METHODS

Egg–content proteins were separated with horizontal polyacrylamide gel electrophoresis, according to the method by Gahne *et al*. [3], with own modifications by Głuchowski *et al*. [4]. The number of 90–120 eggs had been collected form the laying hens of the following breeds: Green–Leg ZKF and Polbar (Pb) – from the experimental station in Felin, Poland – and Green–Leg Z11, Yellow–Leg Z33, Leghorn (H22 and G99), Rhode Island Red RD2 and Sussex S66 – from gene pool stocks in Zyczyn, Poland.

Prior to electrophoresis, the egg white was tenfold diluted, and the yolk was fourfold diluted, both with distilled water. After the electrophoresis, the gel plates were dehydrated in ethyl alcohol, and next laminated at 125°C in order to increase their durability, which facilitated collecting and storing of the research documentation.

Egg–yolk proteins were separated into approx. 30 bands (Fig. 1), which were classified as belonging to the following regions, according to migration speed: prealbumin (Pa), albumin (Alb) and transferrin (Tf). In the white of egg, the following proteins were identified: ovalbumins (Ov–A), ovoglobulins: G_2, G_3, and G_4 and conalbumins (Co).

Figure 1. Electrophoregram of hen egg yolk and white proteins
A - serum, B - Yolk, C - White



The frequencies of genotypes and genes that determine egg protein phenotypes were derived for the hens in individual stocks, and genetic equilibrium of the stocks was verified. Basing on the established gene frequencies, average heterozygosity per single locus, genetic similarities and genetic distances among the stocks were estimated, and a dendrogram of genetic distances for the studied stocks was plotted.

RESULTS

Figure 1 presents a diagram of electrophoregrams for egg yolk and egg white proteins with the subregions highlighted that are characterized by genetic polymorphism. An exemplary electrophoregram of hen serum proteins is presented as well.

In the subregion of fast–migrating yolk prealbumin, Pa–F, ten different phenotypes were observed, which reflect the genotypes whose frequencies are presented in Table 1. In the Z11, ZFK and Pb hens, three phenotypes appeared in each, i.e. B, BD and D, whereas in Ż33, H22 and G99 hens, five or six different phenotypes of the protein were observed. The most polymorphic were S66 hens, for which ten phenotypes were recorded, while the lowest level of polymorphism was observed among RD2 hens, with two phenotypes. In all the studied lines, the phenotype B appeared in the transferrin region, while only a few individuals had the triple–band BC phenotype.

Table 1. Genotype frequencies of egg yolk proteins Pa–F and Tf in gene pool hen stocks

Stocks	n	Pa–F										Tf	
		A	AB	B	AD	BD	D	AC	BC	CD	C	B	BC
Green–Leg Z–11 (Szczytno)	90	0.00	0.00	24.40	0.00	42.20	33.3	0.00	0.00	0.00	0.00	92.2	7.8
Green–Leg (Felin)	98	0.00	0.00	34.70	0.00	51.00	14.3	0.00	0.00	0.00	0.00	94.9	5.1
Yellow–Leg Ż–33	100	0.00	0.00	22.00	0.00	43.00	33.0	0.00	1.00	1.00	0.00	90.0	10.0
Polbar (Felin)	120	0.00	0.00	28.30	0.00	49.10	22.5	0.00	0.00	0.00	0.00	98.3	1.7
Leghorn H–22	90	7.80	3.30	4.40	24.40	26.70	33.3	0.00	0.00	0.00	0.00	95.6	4.4
Leghorn G–99	100	4.00	13.00	0.00	26.00	22.00	35.0	0.00	0.00	0.00	0.00	98.0	2.0
Rhode Island Red RD–2	90	0.00	0.00	0.00	0.00	0.00	97.8	0.00	0.00	2.20	0.00	96.7	3.3
Sussex S–66	120	9.57	9.57	6.96	26.10	6.96	10.4	11.30	0.90	12.20	5.22	100.0	0.0

The frequencies of the genes that encode the proteins Pa–F and Tf are presented in Table 2. In the stocks of Z11, ZKF, Pb and Ż33, alleles Pa–F^B and Pa–F^D occurred, whose frequencies were similar. In Leghorn hens, an additional allele Pa–F^A occurred, and in S66, RD2 and Ż33 hens – an allele Pa–F^C.

Phenotype frequencies of ovalbumins (Ov–A), ovoglobulins G_3, G_4 and G_2, as well as of conalbumins (Co) are presented in Table 3. The Ov–A proteins of Ż33, RD2 and S66 hens were monomorphic, Z11 and G99 hens were observed to have three phenotypes each, whereas the remaining breeds had phenotypes B and AB. Similar polymorphism and phenotype frequencies were observed for ovoglobulins G_3. Ovoglobulins G_4 were represented by all three phenotypes, i.e. A, AB and B, in all the groups, except for RD2 hens, similarly to ovoglobulins G_2, where also generally three phenotypes, i.e. B, BC and C, were observed, except for the lines ZKF and S66. Conalbumins were polymorphic only in two stocks, Z11 and Ż33, while in the others they were monomorphic.

Table 2. Allele frequencies of egg yolk proteins Pa–F and Tf in gene pool hen stocks

Stocks	Pa–F				Tf	
	A	B	C	D	B	C
Green–Leg Z–11 (Szczytno)	0.00	0.455	0.00	0.545	0.961	0.039
Green–Leg (Felin)	0.00	0.602	0.00	0.398	0.975	0.025
Yellow–Leg Ż–33	0.00	0.440	0.01	0.550	0.950	0.050
Polbar (Felin)	0.00	0.529	0.00	0.472	0.991	0.009
Leghorn H–22	0.217	0.194	0.00	0.589	0.978	0.022
Leghorn G–99	0.235	0.175	0.00	0.590	0.990	0.010
Rhode Island Red RD–2	0.00	0.00	0.011	0.989	0.983	0.017
Sussex S–66	0.328	0.152	0.182	0.338	1.000	0.000

Table 3. Frequencies of ovalbumin, ovoglobulins G_4, G_3 and G_2 and conalbumin genotypes in the gene pool hen stocks

Stocks	Ov–A			G_4			G_3			G_2			Co	
	A	AB	B	A	AB	B	A	AB	B	B	BC	C	B	BC
Green–Leg Z–11 (Szczytno)	1.1	18.9	80.0	33.3	53.3	13.3	1.1	18.9	80.0	2.2	47.8	50.0	98.9	1.1
Green–Leg (Felin)	0.0	12.2	87.8	53.1	41.8	5.0	0.0	12.2	87.8	0	28.6	71.4	100.0	0.0
Yellow–Leg Ż–33	0.0	0	100.0	66.0	31.0	3.0	0.0	0.0	100.0	1.0	43.0	56.0	98.0	2.0
Polbar (Felin)	0.0	1.7	98.3	56.8	35.6	7.8	0.0	1.7	98.3	21.7	61.6	16.7	100.0	0.0
Leghorn H–22	0.0	16.7	83.3	24.4	62.2	13.3	0.0	16.7	83.3	8.9	31.3	59.8	100.0	0.0
Leghorn G–99	2.0	29.0	69.0	30.0	50.0	20.0	2.0	29.0	89.0	7.0	34.0	59.0	100.0	0.0
Rhode Island Red RD–2	0.0	0.0	100.0	83.6	16.7	0.0	0.0	0.0	100.0	5.6	43.3	37.8	100.0	0.0
Sussex S–66	0.0	0.0	100.0	35.8	50.8	13.3	0.0	0.0	100.0	0.0	40.0	60.0	100.0	0.0

The frequencies of the genes that encode egg–white ovalbumins, ovoglobulins and conalbumins are presented in Table 4. It can be noticed that the frequency of one of the alleles that encodes ovalbumins, ovoglobulins G_3 and conalbumins was very high, and reached from 0.834 $Ov–A^B$ in the G99 hens to 1.00 in Ż33, RD2 and S66. The frequency of the G_3^B gene was similar. The alleles encoding the proteins G_4 and G_2 occurred with more balanced frequency among the particular groups than those encoding $Ov–A^B$, G_3 and Co. One may observe, analyzing the frequencies of egg protein encoding genes, that there are no alleles that would be specific for a particular breed. All the studied hen stocks were in the state of genetic equilibrium in respect to the frequencies of the genes that encode the egg proteins of yolk or white.

Table 4. Frequencies of ovalbumin, ovoglobulins G_4, G_3 and G_2 and conalbumin alleles in gene pool hen stocks

STOCKS	Ov–A		G_4		G_3		G_2		Co	
	A	B	A	B	A	B	B	C	B	C
Green–Leg Z–11 (Szczytno)	0.110	0.890	0.600	0.400	0.110	0.890	0.261	0.739	0.995	0.005
Green–Leg (Felin)	0.061	0.939	0.740	0.260	0.061	0.939	0.146	0.854	1.000	0.000
Yellow–Leg Ż–33	0.000	1.000	0.818	0.182	0.000	1.000	0.225	0.775	0.990	0.010
Polbar (Felin)	0.010	0.990	0.744	0.256	0.010	0.990	0.525	0.475	1.000	0.000
Leghorn H–22	0.083	0.917	0.556	0.444	0.083	0.917	0.250	0.750	1.000	0.000
Leghorn G–99	0.165	0.835	0.550	0.450	0.165	0.835	0.240	0.760	1.000	0.000
Rhode Island Red RD–2	0.000	1.000	0.916	0.084	0.000	1.000	0.273	0.727	1.000	0.000
Sussex S–66	0.000	1.000	0.612	0.388	0.000	1.000	0.200	0.800	1.000	0.000

Genetic similarities and genetic distances among the studied breeds of hens are presented in Table 5 and in Figure 2. The averages of heterozygosity, calculated for individual groups of hens, are presented as well. The lines H22 and G22, ZKF and Ż33, as well as ZKF and Z11 were the most similar to each other. In contrast, the most distant lines from each other were the RD2 and Z11. The average heterozygosity per single locus was estimated basing on seven loci of egg content protein sets, and ranged between 0.087, for the RD2, and 0.287, for the G99 hens.

Figure 2. Dendrogram of Nei's genetic distances between the studied stocks of laying hens

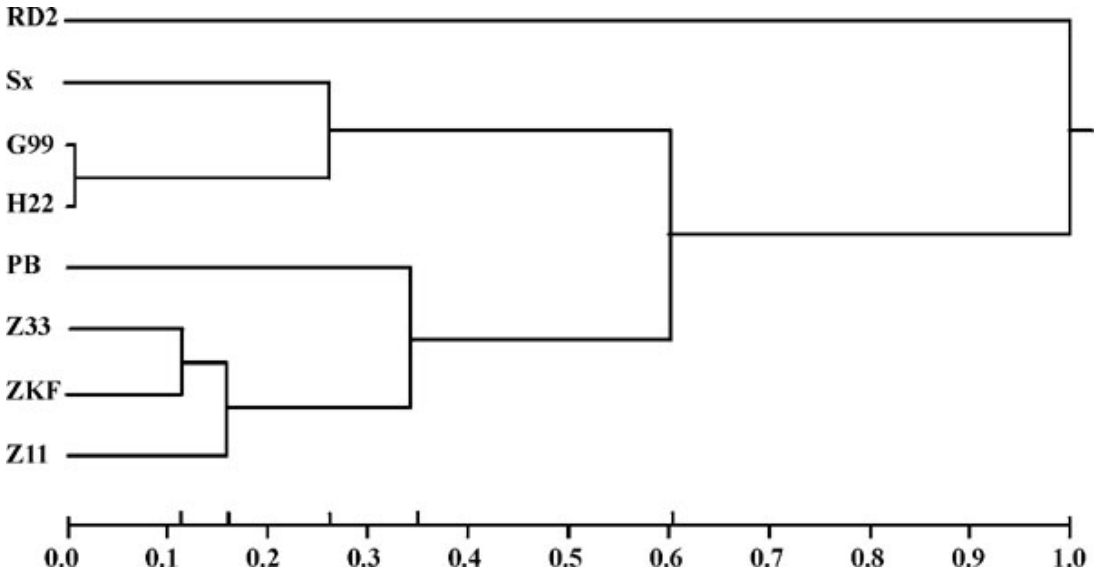


Table 5. Genetic similarities (above diagonal) and Nei's genetic distances among gene pool hen stocks in Poland

	ZK11	ZKF	Z33	PB	H22	G99	RD2	S66	H
ZK11	0.0000	0.9914	0.9894	0.9804	0.9899	0.9868	0.9500	0.9705	0.264
ZKF	0.0086	0.0000	0.9932	0.9734	0.9706	0.9647	0.9344	0.9650	0.200
Z33	0.0106	0.0067	0.0000	0.9827	0.9772	0.9680	0.9682	0.9695	0.182
PB	0.0197	0.0268	0.0174	0.0000	0.9633	0.9528	0.9417	0.9516	0.206
H22	0.0101	0.0297	0.0229	0.0373	0.0000	0.9994	0.9602	0.9878	0.256
G99	0.0132	0.0359	0.0324	0.0482	0.0005	0.0000	0.9535	0.9810	0.287
RD2	0.0512	0.0678	0.0323	0.0600	0.0405	0.0475	0.0000	0.9379	0.087
S66	0.0299	0.0355	0.0309	0.0496	0.0122	0.0191	0.0641	0.0000	0.217

The yolk proteins, denoted as Pa–F, correspond to the fraction marked Pr–2 in Tanabe and Ogawa [17]. The number of bands observed for homozygotes was the same as in the mentioned study. However, the five or six bands observed in this subregion of heterozygotes do not correspond to those presented by Tanabe and Ogawa [17].

The phenotype frequencies recorded in this study were similar to those of the same breeds of hens studied in the years 1970–1980. The allele Tf^{B} frequency ranged between 0.80 and 1.00, whereas the allele Tf^{C} was very rare, and the allele Tf^{A}, which had been quite often present during the 1970s in the hens bred in Poland [8], in this study was not observed at all.

The frequencies of ovalbumin encoding alleles can be compared to those studied by Stratil [15], Kurył [9] and Moiseeva *et al.* [10]. The frequencies calculated here correspond with those presented by these authors. It should be added, however, that the ovalbumin alleles A and B, as well as the alleles of the remaining proteins, were determined in decreasing order of migration speed to anode, which was not in line with the terminology applied by Stratil [15] and Kurył [9].

The shortest genetic distance was noted for two lines of the Leghorn breed, i.e. H22 and G99, which certainly results from the fact of their belonging to the same breed. The numerical values of the genetic distances, presented in Table 5, ranged from 0.00058 within the Leghorn lines to 0.00861 within the stocks of Green–Legs, and are comparable to those obtained by Inafuku *et al.* [7] for 12 Indonesian breeds of hens, which were estimated basing on five loci of the egg proteins: Ov–A, G_1, G_2, G_3 and Co (0.001–0.037). These values were similar to those estimated by Hashiguchi *et al.* [5], i.e. 0.0201–0.041 for both Asian and European hens bred in Japan. Moiseeva *et al.* [10] obtained considerably higher genetic distance indices. The distances were 0.212 between Sx and RiR and 0.296 between Sx and Lg. The genetic distances obtained here are much shorter than those derived from DNA polymorphism [13, 19, 20]. The presented dendrograms, which depict genetic distances, do not correspond with those presented by Wezyk *et al.* [19], which were estimated with RAPD–PCR for the same gene pool stocks bred in Poland. The extensive differences in the values of genetic similarity indices may have also resulted from different ways of their computation.

The lowest average heterozygosity, 0.087, was obtained for the RD2 line of hens. The remaining groups were characterized by substantially higher heterozygosity, reaching from 0.183 for Z33 hens to 0.287 for the G99 line. However, the heterozygosity was much lower than that by Hillel *et al.* [6], which were estimated basing on microsatellite DNA polymorphism. This characteristic ranged between 0.033 and 0.109 [5] or between 0.028 and 0.141 [7] for hens bred in Asia. Higher heterozygosity obtained in this study may have resulted from the fact that the analysis focused on genetic pool stocks, where the influence of selection on genetic polymorphism is reduced.

CONCLUSIONS

1. In gene pool stocks of hens kept in Poland, the fast–migrating egg–yolk protein Pa–F was characterized by ten different phenotypes determined by four alleles, whereas the remaining proteins, i.e. Tf, Ov–A and ovoglobulins G_4, G_3, and G_2, were encoded by two alleles.
2. Nei's genetic distances, estimated basing on seven protein loci of egg content, were the shortest for two stocks of Leghorn and for two Green–Leg stocks, and were the longest between Z11 and RD2.
3. The average heterozygosity of the studied stocks was relatively high and ranged between 0.087 for RD2 and 0.287 for G99 hens.

REFERENCES

1. Bjornstadt G., Gunby E., Roed K.H., 2000. Genetic structure of Norwegian horse breeds. Anim. Breed. Gen. 117: 200 – 201.
2. Cywa–Benko K., Brodacki A., Szwaczkowski T., 1994. Comparative study of blood serum protein polymorphism in three breeds of hens. Rocz. Nauk. Zootech. 21 (1–2): 41–49 [in Polish].
3. Gahne B., Juneja R.K., Grolmus J., 1977. Horizontal polyacrylamide gradient gel electrophoresis for simultaneous phenotyping of transferrin, post–transferrin, albumin and postalbumin in the blood plasma of cattle. Anim. Blood Groups and Biochem. Genet. 8: 127–137.

4. Gluchowski W., Brodacki A., Rupec Z., Wojcik A., 1985. Method of polyacrylamide gel plates fixing. Folia Soc. Sci. Lubl. 27: 141–143 [in Polish].
5. Hashiguchi T., Tsuneyoshi M., Nishida T., Higashiwutoko H., Hiraoka E., 1981. Phylogenetic relationship determined by the blood proteins types of fowls. Jap. J. Zootech. Sci. 52 (10): 713–729.
6. Hillel J., Korol A., Kirzner V., Freidlin P., Weigend S., Barre–Dirie A., Groenen A.M., Crooijmans R.P.M. A. Tixier–Boichard M., Vignal A., Wimmers K., Burke T., Thomson P.A., Maki–Tanila A., Elo K., Zhivotovsky L.A., Feldman M.W., 1999. Biodiversity of chickens based on DNA pools: first results of the EC funded project AVIANDIV. Proc. 10th European Poultry Conf. Jerusalem: 22–29.
7. Inafuku K., Maeda Y., Okamoto S., Ardiningsasi S. M., Hashiguchi T., 1998. Polymorphism of egg white proteins in native chickens in Indonesia. Jap. Poult. Sci. 35 (5): 278–284.
8. Kierek–Jaszczuk D., Gasparska J., 1976. Genetic polymorphism of proteins and enzymes in blood and egg content of hens. III. Polymorphism of transferrins in blood serum and egg content of hens and in semen of roosters. Pr. Mater. Zootech. 10: 29–40 [in Polish].
9. Kuryl J., 1978. Electrophoretic forms and the heredity of prealbumin and albumins in the blood plasma and egg content of hens. Pr. Mater. Zootech. 15: 89–104.
10. Moiseeva I. G., Voloknovich V.A., Tolokonnikova E.V., Altukhov Yu.P., 1984, Differentiation of chicken breeds for biochemical gene markers. Gienietika 4: 671–681.
11. Nei M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 98: 583–590.
12. Okabayashi H., Kamiya S., Tanabe Y., 1998. Phylogenetic relationships among Japanese native chicken breeds based on blood protein polymorphisms. Jap. Poult Sci. 35 (3): 173–181.
13. Plotsky Y., Kaiser M.G., Lamont S.J., 1995. Genetic characterization of highly inbred chickens lines by two DNA methods: DNA fingerprinting and polymerase chain reaction using arbitrary primers. Animal Genetics 26: 163–170.
14. Ponsuksili S., Wimmers K., Horst P., 1998. Evaluation of genetic variation within and between different chicken lines by DNA fingerprinting. J. Hered. 89: 17–23.
15. Stratil A., 1968. Transferrin and albumin loci in chicken, *Gallus gallus* L. Comp. Biochem. and Physiol. 24: 113–121.
16. Takezaki N., Nei M., 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144: 389–399.
17. Tanabe H., Ogawa N, 1980. Comparative studies on physical and chemical property of avian eggs. 5. Horizontal polyacrylamide gradient gel electrophoregrams of chicken (*Gallus domesticus*) egg white proteins. Japan. Polt. Sci. 17: 242–248.
18. Weigend S., 1999. Assessment of biodiversity in poultry with DNA markers. Proc. of Poultry Genetics Symposium, 6–8 October 1999, Mariensee/Germany: 7–14.
19. Wezyk S., Cywa–Benko K., Siwek M., Bednarczyk M., Calik J., 2000. Studies on genetic diversity of gene pool breeds of hens. Rocz. Nauk. Zootech. 27 (1): 115–127 [in Polish].
20. Zhang X., Mc. Daniel G. R., Glambrone J. J., 1995. Random Amplified Polymorphic DNA comparison among broiler lines selected for incidence of tibial dyschondroplasia. Poultry Sci. 74: 1253–1258.

Submited:

Antoni Brodacki, Grzegorz Zięba
Department of Biological Bases of Animal Production
Agricultural University of Lublin
Akademicka 13, 20–950 Lublin, Poland

Katarzyna Cywa-Benko
National Research Institute of Animal Production
Department of Poultry Breeding and Production
32–083 Balice, Poland