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GENETIC DISTANCES IN HENS ESTIMATED WITH PROTEIN GENES FREQUENCIES AND PROCEDURES OF DNA ANALYSIS

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ABSTRACT

Egg yolk and white proteins from hens kept in eight conservation flocks in Poland were separated with horizontal polyacrylamide gel electrophoresis. The analyses were done on 100-120-egg samples from each of the following breeds: Green-Legged Partridge, ZKF and ZK11, Yellow-Legged Partridge, Z33, Polbar, PB, Leghorn, H22 and G99, Rhode Island Red, RD2, and Sussex, S66. After estimating phenotype frequency based on 30-35 bands on the electrophoregrams, the following were identified: gene frequencies of fast-migrating prealbumins, Pa-F, and transferrins, TF, in egg yolk, as well as ovalbumins Ov-A, ovoglobulins, G3, G4, G2, and conalbumins, Co, in the white of egg. Similarity and Nei's genetic distances were calcula-ted according to gene frequency (GF) and procedures used in analysing DNA polymorphism, based on frequencies of particular protein bands (BF) and band sharing (BS). Application of GF and BF in the evaluation of genetic distances gave similar results. The shortest distances were noted between two Leghorn lines and among hens originated from the common breed, i.e. Green-Legged Partridge. The breeds evaluated as similar on GF and BF were practically identical according to BS. The results show that some methods used in analysis of DNA polymorphism can be successfully applied to proteinograms analysis without a necessity to describe protein phenotypes and genotypes.

Key words: protein polymorphism, genetic distance, chickens.

INTRODUCTION

Genetic biodiversity of animals can be determined through morphological traits, polymorphic proteins, immunogenetic markers, or molecular markers. Molecular markers include polymorphism of minisatellite or microsatellite DNA sequences [1, 14]. The data read from protein polymorphism considerably differ from those obtained from DNA polymorphism, which may result from different variability of protein-coding DNA in relation to the non-coding fragments, or depend on estimation methods of the parameters [2]. Hillel et al. [7], basing on the polymorphism of 22 microsatellite DNA loci, compared the methods used for biodiversity evaluation of 43 chicken flocks belonging to various breeds farmed in Europe.

A large number of protein bands in polyacrylamide gel electrophoretic separation may hamper their proper usability in genetic-population studies, due to a possibility of misidentification of particular phenotypes or due to a lack of a genetic model of heritability of phenotypes from particular regions [2].

Application of analytic procedures of DNA minisatellite polymorphism in development of proteinograms would allow better utilisation of the results of protein electrophoresis in biodiversity evaluation of various animal species. Moreover, it may lead to explanation of the discrepancies between the results of genetic evaluation of herds using class I and II markers [11]. The reasons why such discrepancies occur in evaluation of population genetic indices, such as average heterozygosity per locus, the coefficient of inbreeding, or the levels of similarities and differences between the stocks, based on class I markers, i.e. coding sequences of DNA, and class II markers, i.e. non-coding sequences of DNA, may lie in different polymorphism of intron and exon DNA. Also different statistical methods applied in studies may underlie the discrepancies. The variability of non-coding DNA sequences is higher than that of coding DNA sequences, which has been explained with the fact that the previous accumulate more mutations that are more indifferent for the population than those in exons.

The aim of this study was to compare the genetic distances estimated basing on the frequencies of egg yolk and white proteins encoding genes using DNA-analysis procedures.

MATERIALS AND METHODS

Proteins of yolk and white of eggs from hens kept in 8 conservation flocks in Poland were separated in horizontal polyacrylamide (paa) gel electrophoresis, according to the method by Gahne et al. [5]. The analysis covered 100-120 eggs from hens of each of the following breeds: Green-Legged Partridge, lines ZKF and Z11, Yellow-Legged Partridge, Z33, Polbar, PB, Leghorn, H22 and G99, Rhode Island Red, RD2, and Sussex, S66. From obtained 60-70 bands on both yolk and white electrophoregrams, 25 were selected from the regions for which heritability had previously been established. Figure 1 presents the names of the regions and, within them, the bands ordered according to the speed of their migration to the anode, as well as the phenotypes found in the studied populations of chickens. After estimating phenotype frequencies, gene frequencies (GF) were estimated for the genes encoding the proteins of the following subregions: fast-migrating prealbumins, Pa-F, and transferrins, TF, in yolk, and ovalbumins, Ov-A, ovoglobulins, G3, G4, and G2, and conalbumins, Co, in egg-white (Table 1). Band frequencies (BF) as well as the presence or lack of bands in hens of a given breed are presented in Tables 2 and 3.

Fig. 1. Diagram of hen egg content proteins electrophoregram with numbered bands

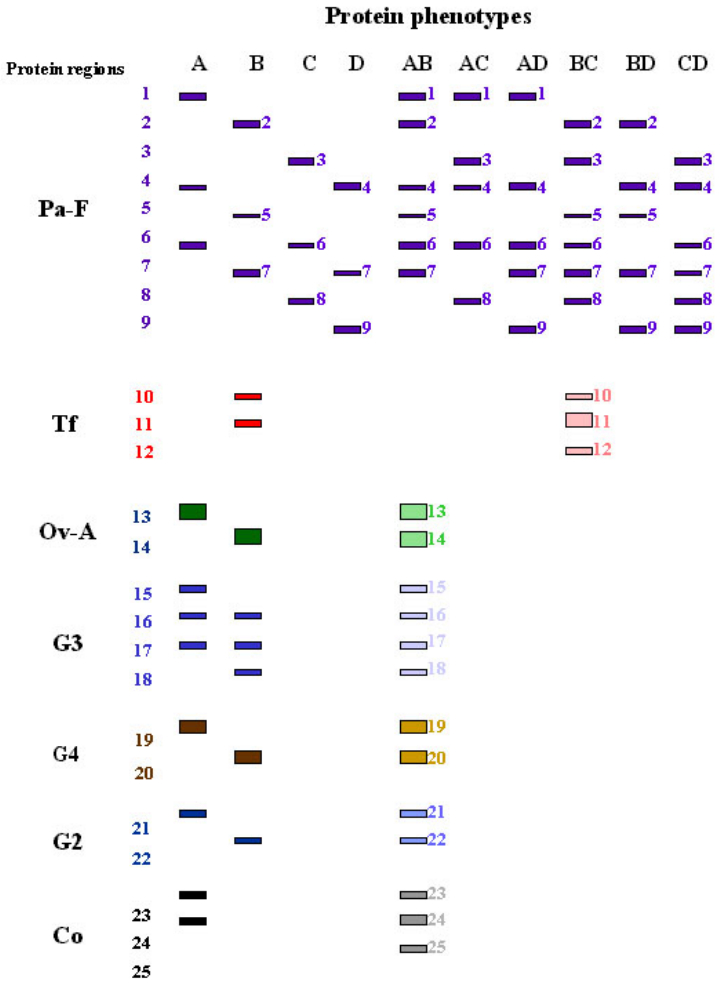


Table 1. Frequency of genes of egg yolk and white proteins in conservation flocks of chickens

Region	Band	Z11	ZKF	Z33	PB	H22	G99	RD2	S66
Pa-F	A	0.000	0.000	0.000	0.000	0.217	0.235	0.000	0.326
	B	0.455	0.602	0.440	0.529	0.194	0.175	0.000	0.152
	C	0.000	0.000	0.010	0.000	0.000	0.000	0.011	0.182
	D	0.545	0.398	0.550	0.472	0.589	0.590	0.989	0.338
Tf	B	0.961	0.975	0.950	0.991	0.978	0.990	0.983	1.000
	C	0.039	0.025	0.050	0.009	0.022	0.010	0.017	0.000
Ov-A	A	0.110	0.061	0.000	0.010	0.083	0.165	0.000	0.000
	B	0.890	0.939	1.000	0.990	0.917	0.835	1.000	1.000
G4	A	0.600	0.740	0.818	0.744	0.556	0.550	0.916	0.612
	B	0.400	0.260	0.182	0.256	0.444	0.450	0.084	0.388
G3	A	0.110	0.061	0.000	0.010	0.083	0.165	0.000	0.000
	B	0.890	0.939	1.000	0.990	0.917	0.835	1.000	1.000
G2	B	0.261	0.146	0.225	0.525	0.250	0.240	0.273	0.200
	C	0.739	0.854	0.775	0.475	0.750	0.760	0.727	0.800
Co	B	0.995	1.000	0.990	1.000	1.000	1.000	1.000	1.000
	C	0.005	0.000	0.010	0.000	0.000	0.000	0.000	0.000

Table 2. Frequency of egg-content protein bands in studied flocks of chickens

Region	Band no.	Z11	ZKF	Ż33	Pb	H-22	G99	RD2	S-66
Pa-F	1	0.000	0.000	0.000	0.000	0.355	0.430	0.000	0.566
	2	0.666	0.857	0.660	0.740	0.344	0.350	0.000	0.326
	3	0.000	0.000	0.020	0.000	0.000	0.000	0.022	0.377
	4	0.756	0.653	0.760	0.717	0.955	1.000	1.000	0.862
	5	0.666	0.857	0.660	0.774	0.344	0.350	0.000	0.326
	6	0.000	0.000	0.020	0.000	0.355	0.430	0.022	0.830
	7	1.000	1.000	1.000	1.000	0.921	0.960	1.000	0.813
	8	0.000	0.000	0.020	0.000	0.000	0.000	0.022	0.377
	9	0.756	0.653	0.880	0.717	0.884	0.830	1.000	0.557
Tf	10	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	11	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	12	0.078	0.051	0.100	0.017	0.044	0.020	0.033	0.000
Ov-A	13	0.200	0.122	0.000	0.017	0.167	0.310	0.000	0.000
	14	0.989	1.000	1.000	1.000	1.000	0.980	1.000	1.000
G3	15	0.200	0.122	0.000	0.017	0.167	0.310	0.000	0.000
	16	1.000	1.000	1.000	1.000	1.000	0.980	1.000	1.000
	17	1.000	1.000	1.000	1.000	1.000	0.980	1.000	1.000
	18	0.989	1.000	1.000	1.000	1.000	0.980	1.000	1.000
G4	19	0.200	0.122	0.970	0.923	0.866	0.800	1.000	0.867
	20	0.989	1.000	0.340	0.434	0.755	0.700	0.167	0.133
G2	21	0.500	0.949	0.440	0.833	0.402	0.410	0.489	0.400
	22	0.978	0.469	0.990	0.783	0.912	0.930	0.811	1.000
Co	23	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	24	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	25	0.011	0.000	0.020	0.000	0.000	0.000	0.000	0.000

Table 3. Presence (1) or absence (0) of bands of egg-content proteins in studied flocks of chickens

Region	Band no.	Z11	ZKF	Ż33	PB	H22	G99	RD2	S66
Pa-F	1	0	0	0	0	1	1	0	1
	2	1	1	1	1	1	1	0	1
	3	0	0	1	0	0	0	1	1
	4	1	1	1	1	1	1	1	1
	5	1	1	1	1	1	1	0	1
	6	0	0	1	0	1	1	1	1
	7	1	1	1	1	1	1	1	1
	8	0	0	1	0	0	0	1	1
	9	1	1	1	1	1	1	1	1
Tf	10	1	1	1	1	1	1	1	1
	11	1	1	1	1	1	1	1	1
	12	1	1	1	1	1	1	1	0
Ov-A	13	1	1	0	1	1	1	0	0
	14	1	1	1	1	1	1	1	1
G3	15	1	1	0	1	1	1	0	0
	16	1	1	1	1	1	1	1	1
	17	1	1	1	1	1	1	1	1
	18	1	1	1	1	1	1	1	1
G4	19	1	1	1	1	1	1	1	1
	20	1	1	1	1	1	1	1	1
G2	21	1	1	1	1	1	1	1	1
	22	1	1	1	1	1	1	1	1
Co	23	1	1	1	1	1	1	1	1
	24	1	1	1	1	1	1	1	1
	25	1	0	1	0	0	0	0	0

Genetic similarities, marked as I_N, were calculated basing on gene frequencies (GF), according to the formula by Nei [10]:

$$I_N = Gxy/\sqrt{GxGy}$$

where:
I_N – coefficient of similarity,
$G_{xy} = \sum q_x q_y$,
$G_x = \sum q^2_x$,
$G_y = \sum q^2_y$,
q_x – gene frequency in population x,
q_y – gene frequency in population y.

Substituting gene frequency in the above equation with band frequency (BF) allowed calculating the coefficient of similarity, I_B. The procedure applied here was similar to that used by Bumstead et al. [3] in their studies on DNA polymorphism in chickens.

Moreover, according to Deepak et al. [4], the similarity index, BS, was calculated using the data from Table 3 and the following equation:

$$BS = 2Nab / (Na + Nb)$$

where:

Bs – index of band similarity,
Nab – number of bands shared by breeds "a" and "b",
Na and Nb – overall number identified in breed "a" and breed "b".

Next, genetic distances, D_N, D_B, and D_D, were determined between the studied flocks of hens, expressed as the negative natural logarithm of genetic similarities, respectively I_N, I_B, and I_D.

The calculated genetic distances were used for creating dendrograms for each of the 3 measures with the STATGEN software. The mean heterozygosity per locus as well as the error of the mean heterozygosity were also calculated.

$$\overline{H} = \frac{\sum_{k=1}^{r} h_{gk}}{r}$$

where:

$\overline{H}$ – mean heterozygosity per locus,
$h_{gk} = a/N$,
h_{gk} – heterozygosity at given locus,
r – number of loci,
a – number of heterozygotes,
N – size of the studied flock.

RESULTS

The mean values of coefficients of heterozygosity per locus calculated basing on gene frequencies are presented in Table 4, while those calculated on band frequencies are in Table 5. These values, calculated with two methods, were quite similar, ranging from 0.087 in RD2 to 0.287 in the Leghorn line G99. Standard errors of these coefficients estimated using the two criteria of flock characteristics were also very similar. The mean heterozygosity estimated on the basis of gene frequencies and band frequencies appeared to be much lower than that estimated basing on the polymorphism of microsatellite DNA sequences [7].

Table 4. Genetic similarities (I_N) and genetic distances (D_N) as well as mean heterozygosity ($\overline{H}$) estimated basing on gene frequency (GF) of egg-content proteins

D_N \ I_n	Z11	ZKF	Z33	PB	H22	G99	RD2	S66	$\overline{H}$	SE
Z11	1.0000 0.0000	0.9914	0.9894	0.9804	0.9899	0.9868	0.9500	0.9705	0.264	0.073
ZKF	0.0086	1.0000 0.0000	0.9932	0.9734	0.9706	0.9647	0.9344	0.9650	0.200	0.067
Z33	0.0106	0.0067	1.0000 0.0000	0.9827	0.9772	0.9680	0.9682	0.9695	0.182	0.076
PB	0.0197	0.0268	0.0174	1.0000 0.0000	0.9633	0.9528	0.9417	0.9516	0.206	0.091
H22	0.0101	0.0297	0.0229	0.0373	1.0000 0.0000	0.9994	0.9602	0.9878	0.256	0.085
G99	0.0132	0.0359	0.0324	0.0482	0.0005	1.0000 0.0000	0.9535	0.9810	0.287	0.082
RD2	0.0512	0.0678	0.0323	0.0600	0.0405	0.0475	1.0000 0.0000	0.9379	0.087	0.055
S66	0.0299	0.0355	0.0309	0.0496	0.0122	0.0191	0.0641	1.0000 0.0000	0.217	0.101

Table 5. Similarities (I_B) and genetic distances (D_B) as well as mean heterozygosity ($\overline{H}$) estimated from band frequencies (BF) of hen egg

D_B \ I_B	Z11	ZKF	Z33	PB	H22	G99	RD2	S66	$\overline{H}$	SE
Z11	1.0000 0.0000	0.9917	0.9909	0.9870	0.9750	0.9686	0.9494	0.9057	0.149	0.033
ZKF	0.0083	1.0000 0.0000	0.9909	0.9829	0.9526	0.9448	0.9157	0.9022	0.119	0.034
Z33	0.0091	0.0091	1.0000 0.0000	0.9884	0.9672	0.9560	0.9568	0.9232	0.114	0.035
PB	0.0131	0.0173	0.0117	1.0000 0.0000	0.9516	0.9413	0.9331	0.9026	0.115	0.034
H22	0.0253	0.0485	0.0333	0.0496	1.0000 0.0000	0.9978	0.9607	0.9461	0.166	0.037
G99	0.0319	0.0568	0.0449	0.0605	0.0021	1.0000 0.0000	0.9505	0.9469	0.187	0.041
RD2	0.0614	0.0860	0.0441	0.0692	0.0400	0.0507	1.0000 0.0000	0.9239	0.051	0.024
S66	0.0990	0.1029	0.0799	0.1024	0.0554	0.0545	0.0791	1.0000 0.0000	0.184	0.042

Genetic similarity between the studied flocks was evaluated using three different procedures: I_N, I_B, and BS, presented respectively in Tables 4 to 6. Irrespectively of the applied procedure, the highest similarities were found between the lines within the breeds. It turned out that similarities between ZKF and PB, as well as between the Leghorn lines H22 and G99 were equal 1 if estimated using the BS index. A high conformity of coefficients of similarity were found for the groups of close origin, while it was much lower for the groups that were more distant from each other.

Tables 4-6 also present the values of genetic distances between the studied flocks of fowl obtained through different procedures, i.e. D_N, D_B, and D_D.

Table 6. Similarities (BS) and genetic distances (D_D) between studied flocks of laying hens estimated with *Band Sharing* (BS)

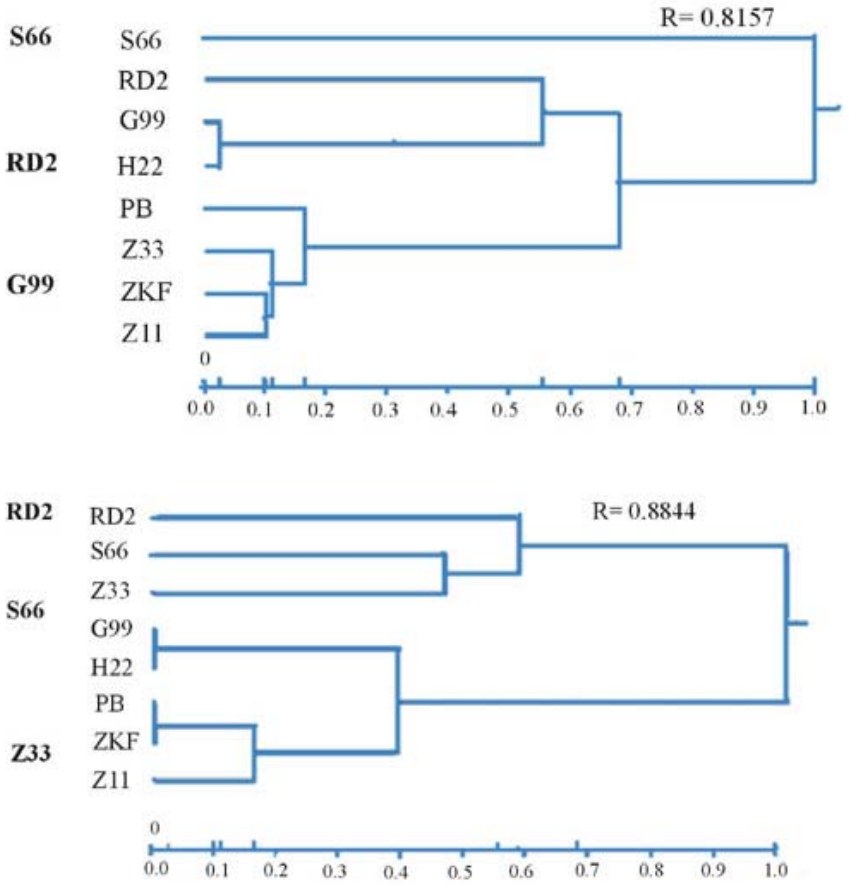
BS / D_D	Z11	ZKF	Z33	PB	H22	G99	RD2	S66
Z11	1.0000 / 0.0000	0.9756	0.8830	0.9756	0.9302	0.9302	0.8000	0.8571
ZKF	0.0247	1.0000 / 0.0000	0.8570	1.0000	0.9523	0.9523	0.9230	0.8290
Z33	0.1244	0.1543	1.0000 / 0.0000	0.8570	0.8636	0.8636	0.9268	0.9302
PB	0.0247	0.0000	0.1543	1.0000 / 0.0000	0.9524	0.9524	0.8205	0.8293
H22	0.0723	0.0488	0.1466	0.0488	1.0000 / 0.0000	1.0000	0.8292	0.8837
G99	0.0723	0.0488	0.1466	0.0488	0.0000	1.0000 / 0.0000	0.8886	0.8837
RD2	0.2231	0.0801	0.0760	0.1978	0.1872	0.1301	1.0000 / 0.0000	0.9000
S66	0.1542	0.1875	0.0723	0.1872	0.1236	0.1236	0.1053	1.0000 / 0.0000

The lowest genetic distances and, what is more, irrespectively of the way of their estimation, were found between the two lines of the Leghorn. These were 0.0, 0.0021, and 0.0005, estimated basing on, respectively, genetic similarity index (BS), band frequency (BF), and gene frequency (GF). Relatively low distances were observed between the Green-Legged Partridge lines, Z11 and ZKF, as well as between the Green-Legged Partridge and Yellow-Legged Partridge lines. A very high similarity to these breeds, estimated with GF and BF, was found for the Polbar breed of chickens.

The observed low genetic distances between the Polbar breed and the group of three flocks, Z11, ZKF, and Z33, result from the common origin of the hens. Both Yellow-Legged Partridge and Polbar have an addition of Green-Legged Partridge genes in their genotypes. The largest distance from the remaining hens was found for the RD2 hens, as estimated on GF, or S66, as estimated on BF. The presented numerical values of the distances, which are 0.0005 between the Leghorn lines or 0.0086 between the Green-Legged flocks, are comparable with those found by Inafuku et al. [8] in Indonesian chickens, the distances being estimated basing on five loci of egg-content proteins, i.e. Ov-A, G1, G2, G3, and CO. The discussed values were also similar to those estimated by Hashiguchi et al. [6] for chickens farmed in Japan, of both Asian and European breeds. However, the results appear to be much lower than those reported by Moiseeva et al. [9] for Sx and RIR, as well as for Sx and Lg, with the values respectively 0.212 and 0.296. The distances found in this study are also much lower then those estimated using DNA polymorphism [13, 15, 16].

The dendrograms of genetic distances (Fig. 2) do not correspond to those presented by Wężyk et al. [15], who applied the RAPD-PCR method to evaluate the same conservation flocks managed in Poland. Considerable differences occurred despite the fact of using similar way of calculation (BS) in both studies. They also may have resulted from using different markers for calculating the indices; we used markers class I in this study, i.e. proteins encoded by coding DNA sequences, while unidentified DNA sequences were used in the studies by Wężyk et al. [15].

Fig. 2. Dendrograms of Nei's genetic distances between studied flocks of chickens estimated with various methods: (A) gene frequency, (B) band frequency, (C) band sharing (BS)



A comparison of the dendrograms (Fig. 2) demonstrates a quite high conformity of the estimations done with GF and BF. In both cases, the studied flocks can be divided into two groups. One group includes Green-Legged Partridge, Polbar, and Yellow-Legged Partridge hens, i.e. those that have nearly never been used as commercial breeds and have not undergone intensive selection. The other group, on the other hand, may comprise the Leghorn lines H22 and G99, as well as the breeds RD2 and S66, which represent commercial flocks selected for laying performance. It may be observed that the BF method provides a more clear division line between the groups than does the GF method. It should also be stressed that some discrepancies were found between both methods of genetic distance estimation. Lower conformity of results of the three methods was found for the breeds between which the genetic distance was high. The GF demonstrates that the RD2 chickens were the most different from the others, whereas such were S66 hens according the BF.

In the case of the breeds separated by a short distance, the conformity of the applied methods was found to be high. These results are similar to those reported by Pepin and Nguyen [12], who basing on polymorphism of proteins and immunogenetic markers demonstrated conformity of estimation methods by Nei, Cavalli-Sforza, Gregorius, and Balakrishan for goats; the coefficients of correlation obtained by the authors between the methods were from 0.93 to 0.98. On the other hand, these correspond less to the results by Hillel et al. [7], who calculated coefficients of correlation between the methods by Nei, Reynolds, and Cavalli-Sforza, as well as $(\delta\mu)^2$, estimating the distances of 43 European breeds of chickens basing on 22 microsatellite DNA markers. The coefficients of correlation ranged from 0.47 between Nei's and $(\delta\mu)^2$ methods to 0.89 between Reynolds' and Cavalli-Sforza's methods.

The dendrogram created basing on BS differed the most from the others. The distance between the lines of Leghorn, ZKF and, derived therefrom, Polbar was equal zero. Using either GF or BF, a strong similarities were found between ZKF and PB as well as between H22 and G99, while the recorded strong similarity between Z33 and S66, estimated on BS, cannot be explained neither with their origin nor with the applied selection methods. This fact, as well as the zero genetic distances between the two Leghorn lines and between ZKF and PB may

demonstrate that such a method allows estimation of distances with a large approximation only. The method seems of little use in estimating distances between groups that are genetically very close. This conclusion does not, however, correspond to the statements by Weigend and Romanov [14], who concluded that DNA polymorphism is very useful in analysing genetic associations, especially between closely related populations.

The results of this study can contribute to better use of electrophoregrams of proteins separated in polyacrylamide gel. The electrophoresis in this type of gel allows obtaining up to 40 protein bands, which causes difficulties in determining phenotypes and gene frequencies, which in turn would enable estimation of genetic characteristics of flocks. Using only the frequencies of particular bands will allow avoiding problems related to classification of particular bands to appropriate subgroups. This will also allow avoiding errors committed in classification of particular phenotypes, by which it will contribute to better utilisation of information hidden in protein electrophoregrams. Moreover, it will also be possible to jointly use the information from both protein electrophoregrams and DNA profiles.

The results indicate that some of the methods of DNA polymorphism analysis can successfully be used in proteinogram analysis, without determination of phenotypes and genotypes of proteins.

CONCLUSIONS

1. Mean heterozygosity estimated with the methods used in processing the data on DNA polymorphism was lower than that obtained basing on the frequencies of genes that encode for the proteins of egg content.
2. The methods displayed strong conformity in the case of very similar populations, whereas the conformity was poor for the breeds separated with a high genetic distance.
3. A high similarity was found between the flocks derived from the Green-Legged Partridge.
4. The RD2 (GF) and S66 (BF) chickens were found to have the largest genetic distance from the other breeds, whereas the smallest distance was found between the lines of the Leghorns.
5. The methods applied in studies on DNA polymorphism, mainly those based on BF, were found to be applicable in proteinogram analysis.

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