Journal of Central European Agriculture, 2013, 14(1), p.11-22 DOI: 10.5513/JCEA01/14.1.1147

Preliminary Studies on Genetic Diversity of Selected Polish Local Chicken Varieties

Wstępne badania zróżnicowania genetycznego wybranych polskich lokalnych odmian kur

Piotr ANTOS[1], Krzysztof ANDRES[2*] and Ewa KAPKOWSKA[2]

[1]University of Agriculture in Krakow, Department of Animal Physiology and Endocrinology, al. Mickiewicza 24/28, 30 - 059 Kraków, Poland

[2]University of Agriculture in Krakow, Department of Poultry and Fur Animal Breeding and Animal Hygiene, al. Mickiewicza 24/28, 30 - 059 Kraków, Poland *correspondence: k.andres@ur.krakow.pl

Abstract

The study was aimed at evaluation of the applicability of selected microsatellite markers in examining genetic diversity in five Polish local chicken varieties (two groups of native crested chickens, crestless native chickens from Subcarpathian region and two groups of native miniature chickens) and reveal their genetic relationships to five purebreds (Leghorn, Rhode Island Red, Sussex, Greenleg Partridge, Minorca). About 22.8% (overall F_{ST} = 0.228) of the genetic variation was explained by differences between breeds and varieties. The D_A genetic distances between five Polish native varieties and five purebreds varied between 0.154 and 0.440. The results obtained in this study indicate the great value of the collection and suggest the need for formal protection of these local Polish chicken varieties.

Keywords: genetic diversity, local chickens, microsatellite polymorphism

Streszczenie

Celem pracy była ocena przydatności wybranych markerów mikrosatelitarnych do określenia zróżnicowania genetycznego pięciu polskich lokalnych odmian kur (dwóch grup krajowych kur czubatych, lokalnych kur z Podkarpacia, dwóch grup krajowych kur karłowatych) oraz przedstawienie zależności genetycznych tych odmian w stosunku do pięciu ras (leghorn, rhode island red, sussex, zielononóżka kuropatwiana, minorka). Około 22,8% całkowitej zmienności genetycznej (F_{ST} = 0,228) wynikało z różnic pomiędzy rasami i odmianami. Dystanse genetyczne D_A pomiędzy pięcioma lokalnymi odmianami a pięcioma rasami przybierały wartości od 0,154 do 0,440. Uzyskane wyniki wskazują na dużą wartość kolekcji polskich lokalnych odmian kur oraz sugerują potrzebę objęcia ich formalną ochroną.

Słowa kluczowe: kury lokalne, polimorfizm mikrosatelitarny, zróżnicowanie genetyczne

Detailed abstract

Celem pracy była ocena przydatności wybranych markerów mikrosatelitarnych do określenia zróżnicowania genetycznego pięciu polskich lokalnych odmian kur (dwóch grup krajowych kur czubatych, lokalnych kur z Podkarpacia, dwóch grup krajowych kur karłowatych) oraz przedstawienie ich zależności genetycznych w stosunku do pięciu ras (leghorn, rhode island red, sussex, zielononóżka kuropatwiana, minorka). W tym celu zastosowano 10 wysoce polimorficznych markerów mikrosatelitarnych. Łącznie wykryto 62 allele, z których 15 było unikalnych. Około 22,8% całkowitej zmienności genetycznej (F_{ST} = 0,228) wynikało z różnic pomiędzy rasami i odmianami. Wartości średniej liczby alleli, heterozygotyczności obserwowanej i oczekiwanej, indeksu stopnia polimorfizmu (PIC) oraz współczynnika inbredu (F_{IS}) oszacowane łącznie dla pięciu polskich odmian lokalnych były wyższe niż dla pięciu ras czystych. Dystanse genetyczne D_A pomiędzy lokalnymi odmianami, wynoszące od 0,110 do 0,381, wskazują na wyraźną odrębność tych odmian za wyjątkiem dwóch grup kur czubatych. Dystanse genetyczne D_A pomiędzy pięcioma lokalnymi odmianami a pięcioma rasami przybierały wartości od 0,154 do 0,440. Uzyskane wyniki wskazują na dużą wartość kolekcji polskich lokalnych odmian kur oraz sugerują potrzebę objęcia ich formalną ochroną.

Introduction

A huge loss of diversity of farm animals has taken place during the last couple of decades mainly due to consolidation of the breeding industry and intensive selection. Chicken genetic resources have been particularly damaged (Woelders, et al., 2006). According to FAO (2007), 33 percent of chicken breeds are classified as being at risk. In Poland in the second half of the XX century the exponential reduction in the number of traditional chickens kept by smallholders was also observed. Despite this fact, some native chickens were said to still exist in the countryside. From 2004 to 2007 we found and recorded chickens, that strongly resemble historical Polish varieties. These chickens were discovered in household flocks in the villages of southeastern Poland, where there was little likelihood of the influence of commercial hybrids. The hatching eggs were obtained and breeding flocks were set up. The collection was composed of native crested and crestless chickens of standard and bantamized size.

In Poland there is a long tradition of protecting chicken genetic resources. Currently we maintained 19 preserved flocks that account for 9 chicken breeds. The objective of this study was to evaluate the applicability of selected microsatellite markers in determining the genetic diversity in recently found and currently restored Polish local chicken varieties and reveal their genetic relationships to preserved strains of utility purebreds maintained in Poland (Leghorn, Rhode Island Red, Sussex, Greenleg Partridge) and to flock of exhibition Spanish Minorca.

Materials and methods

The current study was performed on five populations of Polish local chicken varieties and five chicken purebreds. The local varieties studied were: 2 flocks of native crested chickens originated from birds found in two neighbouring villages (Ryczki and Hucisko) in southern-east Poland, 1 flock of crestless native chickens from Subcarpathian region and 2 flocks of native miniature chickens, commonly called Lilliputians, obtained from two localizations in south-eastern Poland (Podolszynka Ordynacka and Pilchów villages), that are about 30 kilometers apart. The five purebreds were: Leghorn (LG), Rhode Island Red (RI), Sussex (SX), Greenleg Partridge (GP), originated from the conservation flocks (G-99, R-11, S-66 and Z-11, respectively) of The Experimental Station of the National Research Institute of Animal Production in Chorzelów, Poland and Minorca chickens (MN), obtained from fancy breeders.

Crested chickens from Ryczki (CR; Fig.1a) are characterized by black plumage and grayish-yellow shanks whereas birds from Hucisko (CH; Fig.1b) are silver wheaten and yellow or willow legged. Birds of both flocks are single-combed with medium-size globular feather crest and the body weight at 21 week is around 2400 g for males and 1650 g for females. Native chickens from Subcarpathian region (NS; Fig.1c) are wheaten or mahogany red feathered, frequently white mottled and have greenish or bluish shanks and single or rose comb. Body weight at 21 week is around 2050 g for males and 1400 g for females. The miniatures from Podolszynka Ordynacka (LO; Fig.1d) are black-red mottled or brassy-backed mottled and show abundant, soft plumage. They are characterized by single or rose comb and yellow shanks. Lilliputians from Pilchów (LP; Fig.1e) are black-red or golden birchen, frequently white mottled. Their plumage is hard and quite short. They are characterized by single comb, shank colours are yellow, willow, green, blue or white. The body weight of males and female of miniature chickens of both flocks at 21 week is 650 g and 550 g, respectively. Comprehensive characteristics and pictures of LG, RI, SX, GP chickens are available on the “Breed data sheet” for Poland of Domestic Animal Diversity Information System (http://dad.fao.org). MN chickens comply with the description presented in British Poultry Standards (Roberts, 1997). All the birds were kept in the Experimental Unit of the Department of Poultry and Fur Animal Breeding and Animal Hygiene of the University of Agriculture in Kraków, Poland. A total of 80 chickens from ten populations were used in the study (8 birds randomly selected by population).

Figure 1. Males (left) and females (right) of five Polish local chicken varieties: (a) crested chickens from Ryczki; (b) crested chickens from Hucisko; (c) chickens from Subcarpathian region; (d) miniature chickens from Podolszynka Ordynacka; (e) miniature chickens from Pilchów

For each individual DNA was extracted by the following method. Fresh blood was taken from the ulnar vein into tubes with EDTA-K_2 as anticoagulant, and stored at -20^{o}C until use. About 20 µl of blood was dissolved in 400 µl of lysis buffer (100 mM NaCl, 10 mM TRIS, 10 mM EDTA, 2 % SDS) with proteinase K (75 ng/µl). After overnight incubation at 56^{o}C, DNA was purified with phenol and chloroform-isoamyl alcohol (24:1) extractions. Genomic DNA was precipitated by isopropyl alcohol. The DNA was quantified spectrophotometrically and the concentration was adjusted to 35 ng/µl. Ten microsatellite loci which covered 7 chromosomes, were chosen for the study (Table 1). Each of the markers is recommended by a joint ISAG-FAO Advisory Group on Animal Genetic Diversity (FAO, 2004). Amplifications of microsatellite loci were conducted on the Mastercycler® Gradient Thermal Cycler (Eppendorf). Each PCR amplification was performed in a 25 µL reaction mixture, which included 2× reaction buffer, 3 mM $MgCl_2$, 0.3 mM of each dNTP, 0.8 U Taq DNA polymerase, 5 pmol of each primer and approximately 60 ng of genomic DNA as a template. The reaction procedure was as follows: first denaturation step at 95°C for 2 min, 35 cycles of denaturation at 94°C for 30 s, annealing at primer-specific temperature (60°C) for 45 s, extension at 72°C for 1 min, followed by final extension at 72°C for 8 min. The amplified products were separated by electrophoresis on 6 % denaturating polyacrylamide gel with a 10 bp DNA Ladder (Invitrogen) using Sequi-Gen® GT

electrophoresis apparatus (Bio-Rad). DNA fragments were visualized by silver staining and the length of microsatellite alleles in each locus was estimated by comparison with DNA size marker.

Total number of alleles (TNA), mean number of alleles per locus (MNA), observed heterozygosity (H_O), unbiased expected heterozygosity (H_E; Nei, 1987) and polymorphic information content (PIC; Botstein, et al., 1980) were calculated using the CERVUS version 3.0.3 software package (Kalinowski, et al., 2007). Departures from the Hardy–Weinberg equilibrium (HWE) for each group at each locus were tested based on the Markov chain method (Markov chain length, 100,000; dememorization steps, 10,000), using ARLEQUIN version 3.11 (Excoffier, et al., 2005). Inbreeding coefficients for each group (F_{IS}; Weir and Cockerham, 1984), F_{ST} values per each microsatellite locus across all groups and pairwise F_{ST} (Weir and Cockerham, 1984) were estimated with FSTAT version 2.9.3.2 (Goudet, 2001). Nei's D_A genetic distances (Nei, et al., 1983) between each pair of breeds and varieties were evaluated and the tree was constructed based on D_A by using the neighbor-joining method (Saitou and Nei, 1987). The robustness of tree topologies was evaluated with a bootstrap test of 1,000 resamplings across loci. These processes were conducted using POPULATIONS version 1.2.30 (Langella, 2002). The tree was edited using TreeView version 1.6.6 (Page, 1996).

Results

The characteristics of 10 microsatellite markers are summarized in Table 1. A total of 62 distinct alleles were detected, of which 15 alleles (24.2%) were unique to only one breed. The unique alleles were observed in 7 loci. The highest number of unique alleles was detected in LEI0094, which had 4 unique alleles. The average number of alleles per locus was 6.2, with the range from 3 in MCW0037 to 14 in LEI0234. The H_E and PIC per locus ranged from 0.485 (MCW0014) to 0.858 (LEI0234) and from 0.418 (MCW0014) to 0.837 (LEI0234), respectively. The F_{ST} ranged from 0.106 (MCW0067) to 0.342 (MCW0295) with the mean value of 0.228.

Table 1. Description of microsatellite markers used in the study

Locus	Primer sequence (5' → 3')	Chromosome	Ta (°C)	Na	Nua	Allele size range (bp)	H_E	PIC	F_{ST}
ADL0112	F: GGCTTAAGCTGACCCATTAT R: ATCTCAAATGTAATGCGTGC	10	60	6	0	124 - 134	0.553	0.490	0.124
LEI0094	F: GATCTCACCAGTATGAGCTGC R: TCTCACACTGTAACACAGTGC	4	60	9	4	259 - 285	0.770	0.729	0.244
LEI0234	F: ATGCATCAGATTGGTATTCAA R: CGTGGCTGTGAACAAATATG	2	60	14	3	213 - 309	0.858	0.837	0.230
MCW0014	F: AAAATATTGGCTCTAGGAACTGTC R: ACCGGAAATGAAGGTAAGACTAGC	6	60	5	2	172 - 188	0.485	0.418	0.178
MCW0037	F: ACCGGTGCCATCAATTACCTATTA R: GAAAGCTCACATGACACTGCGAAA	3	60	3	0	153 - 157	0.605	0.532	0.325
MCW0067	F: GCACTACTGTGTGCTGCAGTTT R: GAGATGTAGTTGCCACATTCCGAC	10	60	4	1	176 - 184	0.619	0.540	0.106
MCW0111	F: GCTCCATGTGAAGTGGTTTA R: ATGTCCACTTGTCAATGATG	1	60	5	1	98 - 108	0.697	0.644	0.153
MCW0222	F: GCAGTTACATTGAAATGATTCC R: TTCTCAAAACACCTAGAAGAC	3	60	4	0	218 - 224	0.593	0.547	0.199
MCW0295	F: ATCACTACAGAACACCCTCTC R: TATGTATGCACGCAGATATCC	4	60	6	2	91 - 109	0.721	0.669	0.342
MCW0330	F: TGGACCTCATCAGTCTGACAG R: AATGTTCTCATAGAGTTCCTGC	17	60	6	2	257 - 299	0.713	0.653	0.323
Overall	-	-	-	62	15	-	-	-	0.228

Ta, annealing temperature; Na, number of alleles; Nua, number of unique alleles; H_E, expected heterozygosity; PIC, polymorphic information content; F_{ST}, fixation index

The values of genetic diversity parameters within each group are summarized in Table 2. The highest TNA (38) and MNA (3.8) was observed in CH and the smallest TNA (22) and MNA (2.2) was observed in the LO. The highest H_O was observed in NS with the value of 0.563 and the lowest value of 0.334 was observed in RI. The highest H_E and PIC were observed in CH with the values of 0.655 and 0.545, respectively, and the lowest H_E and PIC values of 0.399 and 0.310, respectively, were in LO. One group (SX), having a negative fixation coefficient, showed a heterozygosity excess (F_{IS} = -0.017) and 9 groups had positive F_{IS} values which ranged from 0.029 (LO) to 0.370 (RI). Mean values of the following diversity measures: MNA, H_O, H_E, PIC and F_{IS} among five purebreds (GP, RI, SX, LG, MN) were 2.76; 0.422; 0.489; 0.397 and 0.143, respectively, and among five indigenous chicken varieties (NS, CR, CH, LO, LP) were 3.20; 0.490; 0.548; 0.452 and 0.104, respectively. Of all 100 possible HWE tests, six locus-group combinations were not tested because of monomorphic loci in the group. For the remaining 94 cases, 10 significant departures from the Hardy-Weinberg equilibrium at the 5 % level were observed. In these departures, one single case had a heterozygosity excess (CH; MCW0295) and 9 cases had a heterozygosity deficit. Number of deviated loci in groups amounted to one in 5 groups (LG, SX, LP, CR, NS), two in RI or three in CH. At least one private allele was observed in each group and the highest number of unique alleles, namely three, was detected in the MN and LP group.

Table 2. Genetic diversity parameters estimated for 10 microsatellite markers for five purebreds and five Polish local varieties

Group	TNA	MNA	H_O	H_E	PIC	F_{IS}	HWE	Nua
GP	28	2.80	0.379	0.464	0.379	0.194	0 (2)	1
RI	31	3.10	0.334	0.516	0.426	0.370	2 (1)	1
SX	26	2.60	0.488	0.480	0.387	-0.017	1 (0)	1
LG	26	2.60	0.484	0.502	0.401	0.039	1 (1)	1
MN	27	2.70	0.425	0.483	0.391	0.128	0 (2)	3
NS	35	3.50	0.563	0.587	0.488	0.044	1 (0)	1
CR	36	3.60	0.475	0.596	0.498	0.214	1 (0)	1
CH	38	3.80	0.555	0.655	0.545	0.161	3 (0)	1
LO	22	2.20	0.388	0.399	0.310	0.029	0 (0)	2
LP	29	2.90	0.470	0.503	0.418	0.071	1 (0)	3

TNA, total number of alleles; MNA, mean number of alleles per locus; H_O, observed heterozygosity; H_E, unbiased expected heterozygosity; PIC, polymorphic information content; F_{IS}, inbreeding coefficient; HWE, number of loci not in Hardy-Weinberg equilibrium (P <0.05) and the number of monomorphic loci in parentheses; Nua, number of unique alleles

GP, Greenleg Partridge; RI, Rhode Island Red; SX, Sussex; LG, Leghorn; MN, Minorca; NS, chickens from Subcarpathian region; CR, crested chickens from Ryczki; CH, crested chickens from Hucisko; LO, miniature chickens from Podolszynka Ordynacka; LP, miniature chickens from Pilchów

The D_A genetic distance and F_{ST} values between each pair for all ten chicken breeds and varieties are shown in Table 3. The lowest D_A genetic distance was found

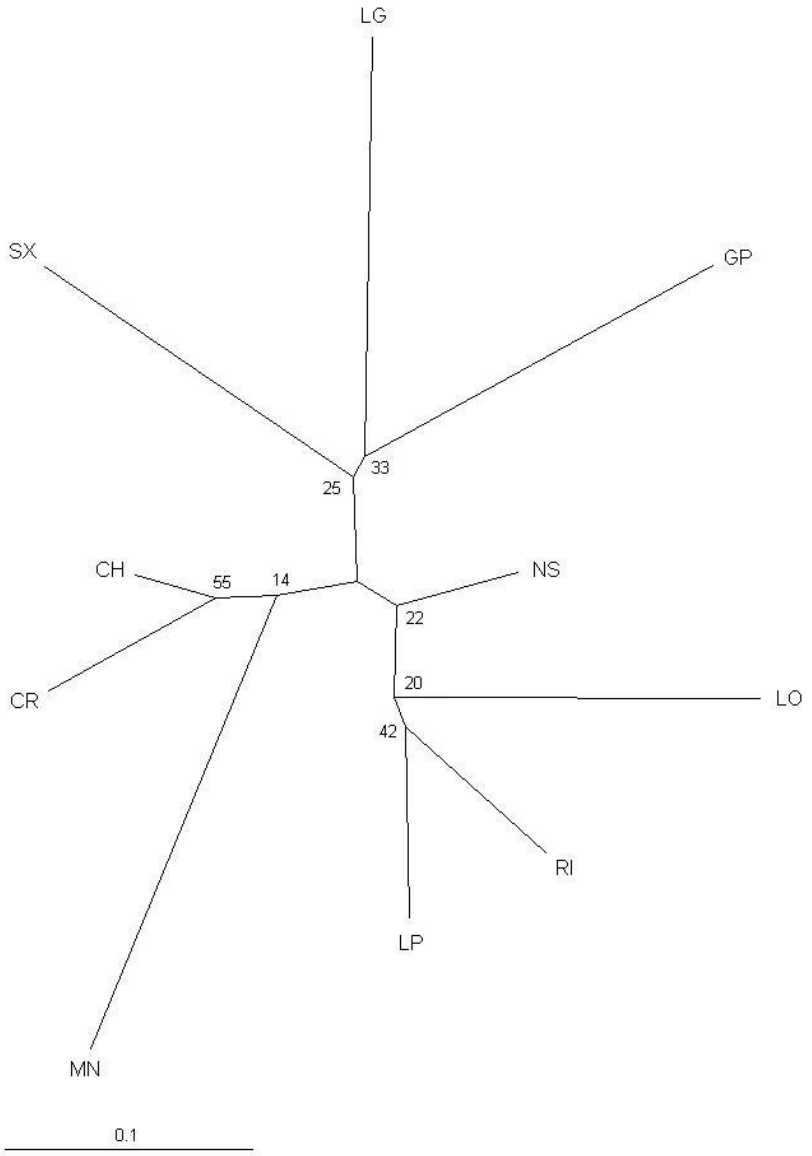
between CR and CH (0.110). The highest D_A value among indigenous chicken varieties was found between CR and LO (0.381). The D_A genetic distance among four preserved chicken breeds and the Minorca ranged from 0.314 (LG and RI) to the value of 0.476 (MN and SX), that was the highest in the study. The D_A genetic distances obtained between five local chicken varieties and five purebreds ranged from 0.154 (LP and RI) to 0.440 (LO and LG). The pairwise F_{ST} value ranged from 0.039 (NS and CH) to 0.390 (GP and LO). A neighbor-joining tree based on the D_A genetic distance is shown in Figure 2.

Table 3. D_A genetic distance (below diagonal) and pairwise F_{ST} (above diagonal) between groups

	GP	RI	SX	LG	MN	NS	CR	CH	LO	LP
GP		0.306	0.243	0.245	0.328	0.244	0.273	0.224	0.390	0.257
RI	0.342		0.302	0.236	0.298	0.109	0.188	0.091	0.183	0.081
SX	0.317	0.388		0.263	0.366	0.180	0.259	0.208	0.386	0.266
LG	0.330	0.314	0.332		0.327	0.255	0.196	0.228	0.372	0.228
MN	0.443	0.378	0.476	0.390		0.247	0.189	0.153	0.268	0.288
NS	0.271	0.195	0.205	0.355	0.340		0.115	0.039	0.146	0.108
CR	0.370	0.290	0.310	0.315	0.277	0.185		0.044	0.288	0.226
CH	0.338	0.221	0.245	0.362	0.278	0.115	0.110		0.185	0.137
LO	0.433	0.240	0.433	0.440	0.366	0.216	0.381	0.297		0.132
LP	0.304	0.154	0.349	0.320	0.395	0.205	0.347	0.269	0.238	

GP, Greenleg Partridge; RI, Rhode Island Red; SX, Sussex; LG, Leghorn; MN, Minorca; NS, chickens from Subcarpathian region; CR, crested chickens from Ryczki; CH, crested chickens from Hucisko; LO, miniature chickens from Podolszynka Ordynacka; LP, miniature chickens from Pilchów

Figure 2. Unrooted neighbor-joining tree of 10 chicken breeds and varieties, based on Nei's D_A genetic distance calculated from 10 microsatellite loci. The number at each node represents percentage of bootstrap value from 1000 replications



Discussion

The use of molecular markers in the characterization of local populations are the important step in genetic resources itemizing (Tixier-Boichard, et al., 2009). According to Kalinowski (2007), the most useful microsatellite markers for diversity studies are these with an H_E value of over 0.5. Of all markers studied, only one did not exceed this value (MCW0014; H_E = 0.485). Two of the markers (MCW0014 and ADL0112), with PIC values slightly below 0.5, were considered as moderately informative and eight markers with PIC values exceeding 0.5 were highly informative in accordance with the criteria adopted by Botstein (1980).

About 22.8% (overall F_{ST} = 0.228) of the genetic variation was explained by differences between breeds and varieties, and the remaining was the result of differences among individuals. Four of the five Polish local chicken varieties (CR, CH,

LP, NS) were characterized by an H_E above 0.5. Surprisingly, the lowest variability (H_E = 0.399; PIC = 0.310) was observed in the LO group of native bantams. The history of local chicken populations is not documented, so we could only indicate isolation of this population as the probable reason for the relatively low variability. Even though, the local chicken varieties showed higher overall genetic diversity measures than the purebreds. The most probable explanation might be the fact that local chickens were raised in the villages in free range flocks without any selection program in contrast to standardized breeds. The genetic variability of Polish local chicken varieties, reflected by the expected heterozygosity index, was comparable with European local chickens. The H_E values of indigenous chickens from France, Hungary and Italy ranged from 0.43 to 0.63 (Berthouly, et al., 2008), from 0.44 to 0.56 (Bodzsár, et al., 2009) and from 0.243 to 0.559 (Zanetti, et al., 2010), respectively. Our results of genetic diversity estimations of Greenleg Partridge were approximately equivalent to the results of the Aviandiv project (H_O = 0.362; H_E = 0.442; F_{IS} = 0.175; Hillel, et al., 2003). In our study the purebreds had a higher mean value of the inbreeding coefficient (F_{IS}), compared to local chicken varieties. It could be explained by nonrandom mating performed to improve desirable traits in utility strains of purebreds in the past. Among local varieties, unexpectedly relatively high inbreeding coefficients were observed in crested chickens (CR, CH).

The decision to classify a given population as a genetic resource should be drawn from its distinctiveness within a species, particularly expressed by genetic distance (Wimmers, et al., 2000). If the separateness between the representatives of bantams and large fowl is clear even on the basis of appearance, the determination of the relatedness between large local varieties of chickens and utility breeds must be revealed by the molecular markers genotyping. The NJ tree based on D_A distances pictures a clear separation of five purebreds (GP, RI, SX, LG, MN). Each of these breeds descend from different countries and have distinct breeding histories. According to the NJ tree topology there is no evidence of close genetic relationships between local varieties and utility strains. Each group of local chickens was also characterized by having at least one unique microsatellite allele, so the introgression of genetic material of utility breeds to local populations appears to be marginal at best. Among local chickens varieties, a quite large distinctiveness can be denoted with the exception of two groups of crested chickens that are most similar. The last two belong to the same variety and what more, they came from adjoining villages. Crestless Subcarpathian chickens were genetically closer to crested chickens than to miniatures, what was in agreement with their exterior appearance. Two groups of miniature chickens, together with Rhode Island breed, form one of the most distant cluster. High genetic distance between miniatures and between miniature chickens and other breeds and varieties can be corroborated by the number of private alleles, which are relatively abundant in miniature chickens.

It is widely agreed that genetic diversity can be depicted at molecular level and by the phenotype and the production performance descriptions (Berthouly, et al., 2008; Hillel, et al., 2003; Tixier-Boichard, et al., 2009; Wimmers, et al., 2000). In our previous study (Andres, et al., 2008), we stated that among 7 breeds and varieties the significantly highest proportion of yolk in the egg was from the Lilliputians from Pilchów. We also reported that egg shell quality of native crested chickens and native Subcarpatian crestless chickens were excellent and surpassed that from the Rhode Island Red and Leghorn.

In conclusion, our results confirmed high polymorphism of selected microsatellite markers. The Polish local chicken varieties showed higher overall genetic variability measures than the purebreds. No direct evidence of close genetic relationships between local varieties and utility strains was found, which is of great importance, especially because the survival of several distinct local chicken populations in the early 21st century in Europe was unexpected. It was also found through the present study, that the genetic distances between local crested, crestless and bantams chickens were considerable. Together with the detection of frequent unique microsatellite alleles, the obtained results point to the separateness of the local varieties and suggest the need for their formal protection in the future.

Acknowledgements

The work was financed by DS3241/KHDZFiZ/2012 and BM4213/KHDZFiZ/2012

References

Andres, K., Kapkowska, E., Wójtowicz, M., (2008) Charakterystyka lokalnych odmian kur pod względem wybranych cech użytkowych. Roczniki Naukowe Zootechniki, 35, 2, 119-129.

Berthouly, C., Bed'Hom, B., Tixier-Boichard, M., Chen, C.F., Lee, Y.P., Laloe, D., Legros, H., Verrier, E., Rognon, X., (2008) Using molecular markers and multivariate methods to study the genetic diversity of local European and Asian chicken breeds. Animal Genetics, 39, 121-129.

Bodzsár, N., Eding, H., Revay, T., Hidas, A., Weigend, S., (2009) Genetic diversity of Hungarian indigenous chicken breeds based on microsatellite markers. Animal Genetics, 40, 516-523.

Botstein, D., White, R., Skolnick, M., Davis, R., (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics, 32, 314-331.

Excoffier, L., Laval, L., Schneider, S., (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online, 1, 47-50.

FAO Standing Committee, Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans. Measurement of Domestic Animal Diversity (MoDAD): Recommended Microsatellite Markers, 2004

FAO, The State of the World's Animal Genetic Resources for Food and Agriculture, edited by Barbara Rischkowsky & Dafydd Pilling, Rome, 2007.

Goudet, J., (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3).

Hillel, J., Groenen, M.A., Tixier-Boichard, M., Korol, A.B., David, L., Kirzhner, V.M., Burke, T., Barre-Dirie, A., Crooijmans, R.P., Elo, K., Feldman, M.W., Freidlin, P.J., Mäki-Tanila, A., Oortwijn, M., Thomson, P., Vignal, A., Wimmers, K., Weigend, S., (2003) Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. Genetics Selection Evolution, 35, 533-557.

Kalinowski, S.T., Taper, M.L., Marshall, T.C., (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology, 16, 1099-1006.

Langella O., (2002) Populations (Version 1.2.30). CNRS, France, 2002.

Nei, M., Tajima, F., Tateno, Y., (1983) Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. Journal of Molecular Evolution, 19, 153-170.

Nei, M., (1987) Molecular Evolutionary Genetics. Columbia University Press, New York.

Page, R.D.M., (1996) TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences, 12, 357-358.

Roberts V., (1997) British Poultry Standards. 5th ed. Blackwell Science, Oxford.

Saitou, N., Nei, M., (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4, 406-425.

Tixier-Boichard, M., Bordas, A., Rognon, X., (2009) Characterization and monitoring of poultry genetic resources. World's Poultry Science Journal, 65, 272-285.

Weir, B.S., Cockerham, C.C., (1984) Estimating F-statistics for the analysis of population structure. Evolution, 38, 1358-1370.

Wimmers, K., Ponsuksili, S., Hardge, T., Valle-Zarate, A., Mathur, P.K., Horst, P., (2000) Genetic distinctness of African, Asian and South American local chickens. Animal Genetics, 31, 159-165.

Woelders, H., Zuidberg, C.A., Hiemstra, S.J., (2006) Animal Genetic Resources Conservation in The Netherlands and Europe: Poultry Perspective. Poultry Science, 85, 216-222.

Zanetti, E., De Marchi, M., Dalvit, C., Cassandro, M., (2010) Genetic characterization of local Italian breeds of chickens undergoing in situ conservation. Poultry Science, 89, 420-427.