Genetic Diversity of Old Chicken Breeds Kept in Poland

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Summary

The aim of this study was to compare the genetic variation of five local chicken breeds reared in Poland. Twenty-seven microsatellite markers were investigated in 138 birds belonging to five breeds: Miniature Cochin (MCO), Gold Italian (GI), Green Legged Partridge (GLP), Silver Italian (SI) and White Leghorn (WL). One hundred eighty five alleles were detected in the overall population, with a mean number of 6.85 ± 3.32 alleles per locus. For the local breeds, the observed and expected heterozygosity ranged from a minimum of 0.287 to a maximum of 0.458 and from 0.397 to 0.499 for the GI and SI breeds, respectively. The overall population heterozygote deficiency was 0.430, the average Wright's inbreeding coefficient (F_{IS}) was 0.061 and the heterozygote deficiency due to breed subdivision was 0.393. Wright's fixation index was slightly positive for all breeds excluding MCO (F_{IS} = -0.476) and the estimated molecular inbreeding (f_{ij}) within breed ranged from 0.296 (GLP and SI) to 0.361 (WL) evidencing limited coancestry. Mean allelic richness, obtained with rarefaction method based on sixteen observations, was 2.12 being the WL the less variable (1.79). Tomiuk and Loeschcke's D_{TL} genetic distance values were used to draw a neighbor-net network which separated the cluster made of MCO and GLP from the cluster of GI, WL and SI. The results arising from our microsatellites analysis represent a starting point for the valorization of these local Polish chicken breeds for monitoring and preserving their genetic variability.

Key words

polish chicken breeds, genetic variability, genetic differentiation, microsatellite

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Aim

The conservation of biodiversity is fundamental in the management of local and less diffused breeds but before taking any protective measure, data covering molecular genetic diversity of breeds is an absolute requirement. Microsatellite markers have been proven to be efficient in evaluating genetic diversity and relationships of farm animals and in particular local chicken breeds (Hillel et al. 2003, Dávila et al. 2009, Tadano et al. 2007, Zanetti et al. 2011). The objective of this study was to estimate the intra- and inter genetic variability of five old local chicken populations reared in Poland.

Material and methods

A total of 138 individual were randomly selected from conservation flocks: Miniature Cochin (MCO) n = 22; Gold Italian (GI) n = 17; Green Legged Partridge (GLP) n = 22; Silver Italian (SI) n= 39; White Leghorn (WL) n = 38.

Miniature Cochin is a variety derived from the original Cochin Chinese breed; the breed has been kept in Europe since nineteenth century. Over the last decades, Gold and Silver Italian breeds became the most popular breeds around Europe; both breeds originated from Italy but, nowadays, they are kept as separated small flocks in several European countries (including Poland). Green-legged Partridge hens were recognized as a breed in the late nineteenth century and were previously known as Galician breed. It should be stressed that in 1930s almost 70% of Poland's area was designated for GLP breeding whereas, forty years later, the population was considerably decreased (1-2%) and, as a consequence of the dwindling of this native breed, a conservation programme was set up for its protection. The first Leghorn hens were brought to Poland before World War I. Over many decades the breed was object of strong genetic improvement however the WL chickens analyzed in the present study can be perceived as "old type" Leghorn without intensive selection pressure. Brief characteristics of these breeds studied are given in Table 1.

Blood samples were collected from the wing vein and genomic DNA was isolated using a modified DNA isolation kit (Gentra System Puregene DNA, QIAGEN, Hilden, Germany).

A set of 27 microsatellite markers, included in the list of recommended microsatellites for chicken analysis by the ISAG/FAO Standing Committee, were amplified using three multiplex fluorescent PCR reactions. Amplification was performed in a GeneAmp 9700 thermal cycler (Life Technologies, USA) starting from 50 ng of purified DNA. The 27 microsatellites were amplified with the following conditions: initial denaturation step of 5 min at 95°C, 35 cycles of 30 s at 95 °C, 1 min 30 s at 61 °C and 30 s at 72 °C and a final extension of 30 min at 60°C using the Type-IT Microsatellite PCR Kit (Qiagen, Hilden, Germany). Allele size was determined with a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA).

The MSA software (Dieringer and Schlötterer, 2003) was employed in calculations of the total number of alleles per locus (TNA), allelic frequencies, observed (H_o) and expected (H_e) heterozygosity, allelic richness (AR, mean number of alleles per locus corrected by sample size), gene diversity (GD) (Nei, 1987), Wright's F-statistics and Wright's fixation index (F_{IS}, Weir

and Cockerham, 1984). Exact tests for deviation from Hardy-Weinberg equilibrium (HWE) were applied using a Markov Chain Monte Carlo simulation (100 batches, 5,000 iterations per batch, and a dememorization number of 10,000) as implemented in GENEPOP v4.0 (Raymond and Rousset, 1995). Allelic richness and private alleles per population were calculated using rarefaction method to adjust for different population sizes using ADZE (Szpiech et al. 2008). Molecular inbreeding coefficients (f_{ij}) and Tomiuk and Loeschcke's (1995) genetic distances (D_{TL}) were measured using MOLKIN 3.0 (Gutierrez et al., 2005). D_{TL} distances among populations were represented by a neighbor-net tree using SplitsTree4 (Huson and Bryant, 2006).

Results and discussion

The total number of alleles detected across the 27 microsatellites markers was 185. All the microsatellite markers were polymorphic with an average of 6.85 ± 3.32 alleles per locus (Table 2). The most polymorphic locus was LEI0192 with 18 alleles and the least number of alleles was detected for MCW0103 (3). Allelic richness values ranged from 2.21 (MCW0103) to 9.32 (LEI0192) with an average of 4.68 ± 1.76 across all markers. The GD over all the loci showed a mean value of 0.631 ± 0.141, ranging from 0.295 to 0.854 in MCW0103 and LEI0192, respectively. The Wright's inbreeding coefficient (F_{IS}) showed a slight excess of homozygotes among all microsatellite markers (0.061, $P < 0.01$). Considering all populations Wright's F-estimates were $F_{IS} = 0.061$, $F_{IT} = 0.430$ and $F_{ST} = 0.393$ indicating that 39% of the observed variability was attributable to among breed variation. The results underline a high degree of breed differentiation that is in accordance with the values reported by Tadano et al. (2008) and Zanetti et al. (2011) for Japanese and Italian local chicken breeds.

The genetic variability in each population was studied in terms of mean number of alleles (Na), allelic richness obtained with rarefaction method (N_{AR}), private allelic richness (P_{AR}), Wright's fixation index (F_{IS}), molecular inbreeding (f_{ij}), observed (H_o) and expected (H_e) heterozygosity and number of loci deviating from Hardy–Weinberg equilibrium (HWE, Table 3). The White Leghorn breed showed the least number of alleles (2.56) while SI and GLP had the largest Na values (3.44 and 3.41 respectively); due to unequal sample sizes of breeds, the rarefaction method was used (Szpiech et al. 2008) to adjust allelic richness and private alleles values per population. Results confirmed the lowest variability of WL ($N_{AR} = 1.79$) and a larger variability present in

Table 1. Mean values of some performance traits of analysed chicken breeds. BW: body weight; LA laying ability; EW: egg weight. Breed names: MCO, Cochin; GI, Gold Italian; GLP, Green Legged Partridge; SI, Silver Italian; WL, White Leghorn

Breed	BW (kg) roosters	BW (kg) hens	LA (eggs/year)	EW (g)
MCO	0.85	0.75	80	30
GI	2.5 - 3.0	1.75 - 2.00	180 - 200	55
GLP	1.7 - 2.2	1.50 - 1.80	180 - 190	55 - 58
SI	2.5 - 3.0	1.75 - 2.00	180 - 200	55
WL	2.0 - 2.7	1.70 - 2.20	250	55

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Table 2. Analyzed loci, dyes and pool, chromosome (chr), fragment size (bp), total number of detected alleles (TNA), allelic richness (AR), gene diversity (GD) and Wright's inbreeding coefficient (F_{IS})

Locus	Dye	Pool	Chr	Fragment Size	TNA	AR	GD	F_{IS}[1]
ADL0268	D3	A	1	94 - 118	9	5,36	0,762	-0,069
MCW0216	D3	A	13	141 - 149	5	3,82	0,475	0,194
MCW0248	D3	A	1	217 - 225	5	4,34	0,714	0,350
ADL0278	D4	A	8	112 - 124	7	4,93	0,706	0,078
MCW0081	D2	A	5	113 - 137	7	4,77	0,756	0,047
MCW0069	D4	A	26	150 - 168	6	3,82	0,561	0,132
MCW0222	D4	A	3	219 - 227	5	2,42	0,582	-0,028
MCW0034	D2	A	2	222 - 246	11	8,36	0,803	-0,037
LEI0166	D2	A	3	251 - 263	4	3,98	0,597	-0,009
LEI0094	D4	A	4	249 - 277	8	5,69	0,737	0,075
MCW0111	D3	B	1	94 - 106	5	4,53	0,606	0,100
MCW0103	D3	B	3	268 - 274	3	2,21	0,295	-0,059
MCW0295	D4	B	4	88 - 104	7	6,02	0,779	0,149
MCW0037	D2	B	3	147 - 157	4	3,16	0,580	0,131
MCW0016	D4	B	3	142 - 150	4	3,21	0,650	0,055
MCW0014	D4	B	6	168 - 184	4	3,01	0,260	0,400
MCW0183	D4	B	7	295 - 345	8	5,11	0,580	-0,152
LEI0234	D2	B	2	265 - 373	14	8,44	0,812	0,224
MCW0123	D3	C	14	144 - 154	5	3,95	0,497	-0,068
MCW0020	D3	C	1	181 - 191	6	4,25	0,706	-0,066
MCW0165	D3	C	23	110 - 120	5	4,05	0,608	0,293
MCW0078	D4	C	5	136 - 160	7	4,35	0,566	-0,011
MCW0067	D4	C	10	174 - 182	5	3,35	0,606	-0,008
MCW0080	D2	C	15	267 - 319	8	4,39	0,646	0,055
MCW0206	D3	C	2	226 - 246	5	3,42	0,603	-0,110
LEI0192	D2	C	6	231 - 355	18	9,32	0,854	0,094
MCW0104	D4	C	13	187 - 231	10	6,16	0,710	-0,016
Average (±SD)					6,85 ± 3,32	4,68 ± 1,76	0,631 ± 0,141	0.061

[1]F_{IS} values inside 95% confidence interval

Table 3. Sample sizes (N), mean number of alleles (Na), allelic richness obtained with rarefaction method (N_{AR}), private allelic richness (P_{AR}); Wright's fixation index (F_{IS}, Weir & Cockerham (1984)); molecular inbreeding (f_{ij}) expected heterozygosity (He), observed heterozygosity (H_o) and number of loci significantly deviating from Hardy-Weinberg equilibrium (HWE). Breed names: MCO, Miniature Cochin; GI, Gold Italian; GLP, Green Legged Partridge; SI, Silver Italian; WL, White Leghorn

Breed	N	Na	$N_{AR}(16)$[1]	$P_{AR}(16)$[1]	F_{IS}[2]	f_{ij}(SD)	H_o(SD)	H_e(SD)	HWE[3]
MCO	22	3.22	2.15	0.63	-0.476	0.314(0.022)	0.421(0.279)	0.429(0.211)	1
GI	17	3.15	2.08	0.40	0.120	0.329(0.048)	0.287(0.227)	0.397(0.216)	2
GLP	22	3.41	2.35	0.40	0.100	0.296(0.024)	0.451(0.222)	0.499(0.193)	1
SI	39	3.44	2.27	0.50	0.107	0.296(0.012)	0.458(0.279)	0.472(0.230)	-
WL	38	2.56	1.79	0.31	0.142	0.361(0.014)	0.316(0.221)	0.323(0.220)	-

[1]Number of observations in each breed; [2]F_{IS} values inside 95% confidence interval; [3]$P < 0.05$ after Bonferroni correction

SI and GLP breeds (N_{AR} = 2.27 and 2.35 respectively); presence of private alleles was limited ($P_{AR} < 1$) in all breeds. Even tough the WL animals analyzed in this study belong to an "old type" population, the low genetic diversity values obtained for this particular breed could be attributable to an intensive selection pressure rather than to bottleneck events occurred after their introduction in Poland. F_{IS} values were positive for all breeds with the exception of MCO where a significative excess of heterozygotes was found (F_{IS} = -0.476). Estimation of f_{ij} is another method by which within-breed diversity can be measured, f_{ij} was similar in all the breeds but slightly higher in WL (0.361). Observed heterozygosity was similar to H_e and it was in the range of 0.287 ± 0.227 (GI) to 0.458 ± 0.279 (SI), these values are also in

agreement with those reported by other authors for local chicken breeds (Hillel et al. 2003, Dávila et al. 2009, Tadano et al. 2007, Zanetti et al. 2011). Departure from HWE was limited to 1 microsatellite marker in MCO and GLP breeds and 2 microsatellite markers in GI breed after Bonferroni correction ($P < 0.05$).

The between populations F_{ST} and D_{TL} genetic distances are given in Table 4. The D_{TL} distance was used because it simultaneously accounts for the impact of mutation and genetic drift on gene frequencies (Tomiuk and Loeschcke 1995). In addition, D_{TL} is more robust to deviations in the assumptions concerning mutation and genetic drift (Tapio et al. 2003). The F_{ST} values were rather high when compared to Italian and Japanese chicken breeds (Zanetti et al., 2010 and Tadano et al. 2007) or

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Table 4. Genetic distances for five chicken breeds using F_{ST} ($P < 0.001$, above the diagonal) and D_{TL} (below the diagonal). Breed names: MCO, Miniature Cochin; GI, Gold Italian; GLP, Green Legged Partridge; SI, Silver Italian; WL, White Leghorn

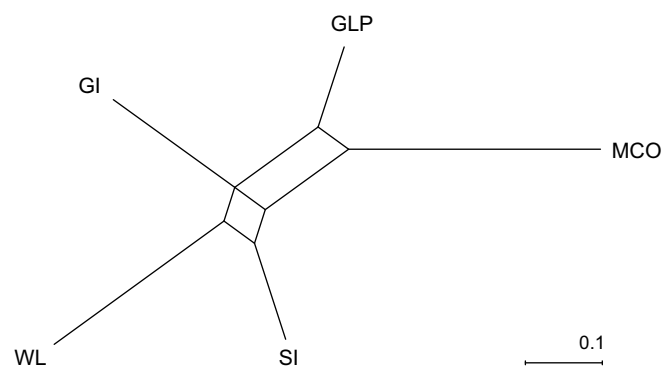|	MCO	GI	GLP	SI	WL
MCO	-	0.418	0.330	0.392	0.523
GI	0.699	-	0.319	0.280	0.473
GLP	0.484	0.440	-	0.305	0.408
SI	0.636	0.394	0.493	-	0.398
WL	0.829	0.536	0.531	0.451	-

Figure 1. Neighbor-Net network of polish chicken breed using D_{TL} genetic distances. Breed names: MCO, Miniature Cochin; GI, Gold Italian; GLP, Green Legged Partridge; SI, Silver Italian; WL, White Leghorn

Indonesian local breeds analyzed with SNP markers (Riztyan et al. 2011). Pairwise F_{ST} values ranged from 0.280 (GI-SI) to 0.523 (WL-MCO) supporting the hypothesis of a closer relationship between the two "Italian" originated breeds opposed to the others. Further evidences of the closer relationship among the "Italian" originated breeds (WL, SI and GI) could be found in the neighbor-net network implemented from D_{TL} distances in Figure 1. The network clearly separates the GLP and MCO from the "Italian" originated cluster (GI, WL and SI), moreover the exotic MCO breed was the most differentiated ones and was placed at the end of the longest branch of the network.

Conclusions

In this study we estimated the intra- and inter- breed genetic variability of five old local chicken populations reared in Poland. According to their recent breeding history the five breeds were clearly separated in terms of genetic identity. Genetic variability is similar to that of other local chicken breeds undergoing in situ conservation but the continuous monitoring of genetic variability parameters is needed to avoid the increase of inbreeding and the loss of biodiversity. These results represent a starting point for the valorization of these local Polish chicken breeds as an important reservoir of genetic diversity.

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