

Testing the breeding strategy of Hungarian Bronze turkey strains for maintaining genetic diversity with microsatellites

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Abstract

The aim of the study was to provide information on the genetic variability of the Hungarian Bronze turkey gene reserve population and its difference from the Broad-breasted turkey, and offer guidance and proposals for its future conservation strategies. Altogether, 239 Hungarian Bronze turkeys from 10 strains and 13 Broad-breasted turkeys as a control population were genotyped for 15 microsatellites. All loci were polymorphic with the average number of alleles per locus 3.20 ± 1.146 in the Hungarian Bronze turkey. The mean expected (H_{exp}) and observed heterozygosity (H_{obs}) were not different (0.392 and 0.376, respectively) in the overall population, and similar values were obtained for hens and bucks and among hen strains. Inbreeding coefficient (F_{is}) and Shannon index (I) indicated that there was low inbreeding within hens and bucks. Our results confirm that the genetic diversity in the Hungarian Bronze turkey population has been preserved by the rotational mating system. Differences between the Hungarian Bronze turkey and the Broad-breasted turkey populations were determined. Nei's unbiased values clearly indicated that the two populations are highly genetically differentiated.

Keywords: Hungarian Bronze turkey, Broad-breasted turkey, genetic diversity, microsatellite, conservation of gene reserve population

Zusammenfassung

Prüfung der Zuchtstrategie mittels Mikrosatelliten zur Erhaltung der genetischen Vielfalt von ungarischen Bronzeputen

Das Ziel der Studie war es, Informationen über die genetische Vielfalt der ungarischen Bronzeputen als Genreserve im Vergleich zur breitbrüstigen Pute für zukünftige Zuchtstrategien zu erhalten. Insgesamt wurden 239 ungarische Bronzeputen aus 10 Linien und 13 breitbrüstige Puten als Kontrollgruppe für 15 Mikrosatelliten genotypisiert. Alle Loci waren polymorph mit einer durchschnittlichen Anzahl von $3,20 \pm 1,146$ Allelen pro Locus bei der ungarischen Bronzepute. Die mittlere erwartete (H_{exp}) und beobachtete (H_{obs}) Heterozygotität unterschieden sich nicht (0,392 bzw. 0,376). Ähnliche Werte wurden für Puten und Puter sowie Putenlinien gefunden. Inzuchtkoeffizient (F_{IS}) und Shannon Index (I) für

Puten und Puter waren niedrig. Es bestätigte sich, dass durch das Rotationspaarungssystem die genetische Vielfalt erhalten wurde. Zwischen den ungarischen Bronzeputen und den breitbrüstigen Puten bestehen deutliche genetische Unterschiede.

Schlüsselwörter: Hungarian Bronze turkey, Broad-breasted turkey, genetic diversity, microsatellite, conservation of gene reserve population

Introduction

The turkey (*Meleagris gallopavo*) is the second most important poultry species agriculturally after the chicken. Most literature about poultry concerns genetic background and structure of the chicken genome; however, as the importance of the turkey grows, so biological information about this species must increase. According to the American Poultry Association, turkey variants are considered a single breed (Kamara *et al.* 2007). Knowledge of the genetic diversity of turkey populations, strains and breeds is important for the diversity of turkey varieties. This essential genetic resource could help breeders improve their birds' health and vigour or respond to changing environmental conditions, production systems and consumer needs (Christman & Hawes 1999).

The *Meleagris* genus originates in America; however, the turkey (*Meleagris gallopavo*) has been bred in Hungary since the end of 16th century (Mihók 2000). One native Hungarian turkey breed is the Hungarian Bronze turkey. The Black turkey had previously been common, but is now only kept on small farms. Its population has decreased because of crossing with Bronze and other breeds at the beginning of the previous century. The Bronze turkey has adapted to local conditions to become a native breed. There is another native turkey type – the Hungarian Copper turkey – that is kept in the south of the country (Szalay *et al.* 2009). Performance and meat production are similar for all three types; therefore, it remains a question whether they are different breeds or only colour varieties. Some meat quality parameters of the Hungarian Bronze turkey, such as water-holding capacity, consistency and tenderness, are better and production level is usually less than that of the intensive breeds (Mihók *et al.* 1999). The adult weight of a male is 10-12 kg and 5-7 kg for females. The main advantage of this breed is its excellent feed-seeking ability and vitality. In the 1960s, the number of stocks decreased as intensive production systems became widespread (Mihók 2000). The Broad-breasted Bronze turkey is an improved type of Bronze turkey bred in the USA. Adult males weigh 12-13 kg and females 7-9 kg with a broad breast and good carcass performance (Sütő 2006).

Considering that few data are available about native turkey types and their genetic backgrounds, the purpose of this work was to determine the genetic diversity of a Hungarian Bronze turkey gene reserve strains, evaluate the effectiveness of the present mating system to preserve genetic diversity and finally compare this native population to the Broad-breasted turkey breed using modern molecular genetics tools.

Material and methods

Animals

The examined Hungarian Bronze turkey has been owned by the University of Debrecen, Hungary for more than 15 years in order to maintain a gene reserve population. In 1999, 12 unrelated strains were generated containing 14-16 hens and 3-4 tons per strain. To keep the genetic diversity the following breeding program has been used since 2000. Hens in a given strain provide the next generation in the same strain, and the bucks will be the next generation of bucks of the neighbouring strain. In the reproduction cycle, animals mate at random within each strain. Hens lay 19 eggs on average and are kept for two years. Unfortunately, the number of animals in two strains decreased so much that these strains were divided out among the other strains over the past six years.

In this study, 239 Hungarian Bronze turkeys from 10 strains and 13 Broad-breasted turkeys as a control population were examined (Table 5). Blood samples were collected from both strains in 2007.

DNA extraction and microsatellite analyses

Genomic DNA from total blood was extracted using the Zsolnai & Orbán (1999) procedure. Microsatellites were chosen based on their chromosomal location (Table 1).

Polymerase chain reactions (PCRs) were prepared in a total volume of 10 µl containing 30 mM MgCl₂, 2 mM of each dNTP, 2.5 µM of each primer, 5 U Taq polymerase and 100-200 ng genomic DNA. Amplifications were performed using a Perkin Elmer Gene Amp PCR System 9700 Thermocycler (Applied Biosystems, Foster City, CA, USA) and DNA Engine Peltier Thermal Cycle (Bio-Rad, USA). Running conditions consisted of 10 min at 95 °C followed by 35 cycles of 15 s at 95 °C, 30 s at 51-62 °C (depending on the microsatellites, Table 1), 30 s at 73 °C and a final extension step of 20 min at 73 °C. PCR products were run in two multiplexes and analysed using an ABI Prism 310 Genetic Analyser (Applied Biosystems, Foster City, CA, USA). The size of each fragment was determined relative to the LIZ 500 size standards (Applied Biosystems). GeneMapper 3.7 software (ABI; Perkin Elmer, Foster City, CA, USA) was used for genotype scoring.

Statistical analysis

Popgene version 1.31 software package was used for statistical analysis (Yeh & Yong 1999). Population structure was analysed using Wright's fixation indexes (Weir & Cockerham 1984). Expected (H_{exp}) and observed heterozygosity (H_{obs}) were computed using Levene (1949). The effective number of alleles (N_e) was calculated by the Kimura & Crow (1964) formula. Shannon index (I) was calculated to determine genetic diversity (Lewontin 1972). Based on microsatellite data, Nei's (1987) unbiased genetic distance was used for dendrogram construction according to the neighbour-joining algorithm.

Table 1
Main characteristics of used primers

Name	Chrom.	Primers (5'-3')	Temp., °C	Length, bp	Accession no.
ADL0149	M12	Forward: ATA GCA TAC ACC CAG CCA CC Reverse: GAA TAA GAA TGT TNC CCT GC	57	224-238	G01574
ADL0266	M4	Forward: GTG GCA TTC AGG CAG AGC AG Reverse: AAT GCA TTG CAG GAT GTA TG	50	94-109	G01686
ADL0292	M5	Forward: CCA AAT CAG GCA AAA CTT CT Reverse: AAA TGG CCT AAG GAT GAG GA	59	128	G01710
ADL0293	M12	Forward: GTA ATC TAG AAA CCC CAT CT Reverse: ACA TAC CGC AGT CTT TGT TC	50	99	G01711
MCW0080	M13	Forward: CCG TGC ATT CTT AAT TGA CAG Reverse: GAA ATG GTA CAG TGC AGT TGG	50	276-288	L40045
MNT106	M18	Forward: TGC AGT GTG AAT ATT GGC TTG Reverse: AAA TAA TGA AGA CAC CGA CAT TTT C	58	158-168	AF540409
MNT162	M6	Forward: AAA AAC CTG AAA ATG TAA ATC CA Reverse: TTC AGA TCT TTT ATT TTT CGA AGC	59	94-100	AY235131
MNT174	M1	Forward: AAA ATT CAG TCC CCC AGA GG Reverse: CTC AGG ATG CAA GCC TTC TC	62	204-216	AY235142
MNT192	M26	Forward: ATT GGT CAG GGT GCC AAT AG Reverse: AGC ACA TTG CAG TTG TTT GC	62	203-219	AY235160
MNT197	M8	Forward: GCT TAC GGA GAT AAG AGC TTT GG Reverse: CCA CAT TGC AGA GGG TCA C	58	105-117	AY235165
MNT199	M3	Forward: AGC TTC CTA TTC AAG AGT TTT GG Reverse: AGT CCA AGA CCA GCC ACC AG	58	136-150	AY235166
MNT214	M4	Forward: GCC ATG AAT GTC AAA AGG AAC Reverse: GGG TGA GCC TGG GTA GAA TG	60	190-206	AY235179
MNT327	M24	Forward: TTG TGT TAT GCA AGT AAA AGC ATC Reverse: GGC TAA CCA GAG CTT CAT GC	60	205-213	AY552897
MNT332	M15	Forward: TTG GTC AAC ATT TGG AAG ACC Reverse: ATT AGC TAA CAG CTG CAA AAT GG	58	149-161	AY552902
MNT387	M14	Forward: AAG CGT TCC ATC TGT TTT GG Reverse: TTC CTA GCC TCT CAT CTG TGC	56	130-135	AY552955

Results

Genetic structure of the Hungarian Bronze turkey strains

Altogether, 48 alleles were detected on the 15 microsatellite loci. All loci were polymorphic with the number of alleles per locus (N_a) varying from two (ADL0149, ADL0266, ADL0293, MCW0080) to six (MNT162). The average number of alleles per locus was 3.20 ± 1.146 . The effective number of alleles per locus ranged from 1.062 (ADL0293) to 3.615 (MNT162) (Table 2). Rare alleles (with a frequency of less than 0.05) were found for 10 loci (Table 3). The rest of the allele frequencies are available from the authors on request.

Expected heterozygosity ranged from 0.059 (ADL0293) to 0.725 (MNT162) and the mean of H_{exp} was 0.392 ± 0.217 among loci (Table 2).

The mean of H_{obs} was 0.376 ± 0.224 among loci, ranging between 0.000 (ADL0293) and 0.722 (MNT162). Higher H_{exp} than H_{obs} were obtained in case of nine microsatellites. A significant deviation from the Hardy-Weinberg equilibrium was observed for only three microsatellites at $P < 0.01$ (Table 2). The inbreeding coefficient (F_{is}) among loci varied from 1.000 (ADL0292) to -0.186 (MNT199) (Table 2).

Table 2

Number of alleles, heterozygosity per locus and inbreeding coefficient in the Hungarian Bronze turkey strains

Locus	N _a	N _e	H _{exp}	H _{obs}	F _{IS}
ADL0149	2	1.385	0.279	0.312	-0.124
ADL0266	2	1.741	0.428	0.448	-0.052
ADL0292	3	1.147	0.128	0.130	-0.013
ADL0293*	2	1.062	0.059	0.000	1.000
MCW0080	2	1.097	0.089	0.093	-0.049
MNT106*	3	2.356	0.577	0.552	0.042
MNT162	6	3.615	0.725	0.722	0.002
MNT174	3	2.794	0.645	0.632	0.016
MNT192	5	2.778	0.642	0.565	0.117
MNT197	4	1.691	0.410	0.392	0.041
MNT199	3	2.395	0.584	0.691	-0.186
MNT214	4	1.733	0.424	0.369	0.127
MNT327	3	1.313	0.239	0.212	0.108
MNT332	3	1.252	0.202	0.211	-0.049
MNT387*	3	1.808	0.448	0.316	0.293
Mean	3.200±1.146	1.878±0.756	0.392±0.217	0.376±0.224	0.037

*significant deviation from Hardy-Weinberg equilibrium ($P < 0.001$), N_a: observed number of alleles, N_e: effective number of alleles, H_{ob}: observed heterozygosity, H_{exp}: expected heterozygosity, F_{IS}: inbreeding coefficient

Genetic differentiation between Hungarian Bronze turkey hens and bucks

The genetic structure of hens (n=194) and bucks (n=45) were analysed separately. From the examined 15 loci, 47 alleles were detected in hens and 43 in bucks. The same number of alleles was found in 11 loci in hens and bucks. Two more alleles were detected on locus MNT162 and MNT214: one (MNT327) when comparing hens to bucks and one (MNT387) when comparing bucks to hens. Each of those alleles, which were found only in hens or in bucks, had less than a 0.05 frequency in the overall population (Table 3).

Table 3

Frequency of rare alleles in the Hungarian Bronze turkey strains

Locus	Alleles	F in the whole strains	F in hens	F in bucks
ADL0292	117	0.039	0.041	0.033
	131	0.029	0.030	0.022
ADL0293	89	0.030	0.011	
MCW0080	279	0.046	0.047	0.046
MNT162	86	0.003	0.003	
	88	0.003	0.003	
MNT192	207	0.013	0.007	0.033
	217	0.008	0.007	0.011
MNT197	97	0.039	0.044	0.022
MNT214	200	0.008	0.011	
	192	0.005	0.007	
MNT327	201	0.005	0.007	
MNT332	150	0.026	0.024	0.033
MNT387	145	0.003		0.011

F: allele frequency

Mean of N_a was higher in hens than in bucks, however, the mean of N_e was slightly higher in bucks than in hens (Table 4).

Table 4

Number of alleles, heterozygosity, inbreeding coefficient and Shannon index per hen and buck

Population	N_a	N_e	H_{exp}	H_{obs}	F_{IS}	I
Hens	3.133±1.187	1.868±0.756	0.389±0.218	0.383±0.223	0.011	0.667±0.366
Bucks	2.867±0.916	1.896±0.724	0.408±0.209	0.354±0.231	0.119	0.684±0.346

N_a : observed number of alleles, N_e : effective number of alleles, H_{obs} : observed heterozygosity, H_{exp} : expected heterozygosity, F_{IS} : inbreeding coefficient, I: Shannon index

This might be the result of the lower number of bucks than hens. Significant deviation from the Hardy-Weinberg equilibrium was observed for three loci in bucks (MNT327 [$P<0.05$], ADL0293 and MNT332 [$P<0.01$]), and for six loci in hens (ADL0149, MNT197 and MNT199 [$P<0.05$] and ADL0293, MNT106 and MNT387 [$P<0.001$]).

Mean H_{exp} was higher than mean H_{obs} in both hens and bucks. F_{IS} and Shannon index indicated that there was a low inbreeding within hens and bucks (Table 4). Multilocus fixation index (F_{ST}) indicated that around 0.4% of the total genetic variation could be explained by a sex difference; the remaining corresponded to differences among individuals. Nei's unbiased genetic distance value was low between hens and bucks (0.003).

Genetic differentiation among the Hungarian Bronze turkey hen strains

The genetic structure of the 10 strains was investigated separately and differentiation was determined among them. Three loci (ADL0292, ADL0293 and MNT332) were found to be not polymorphic in at least one strain. All hens were homozygous for one of these loci in strains 1, 3 and 5-8, two loci in strains 2, 9 and 10 and three loci in strain 4. The number of alleles per locus varied between one and five in the strains. The mean number of alleles per locus per strain ranged from 2.333±0.724 (strain 9) to 2.600±0.828 (strain 3). The examined hen strains had similar levels of allelic variations. N_e ranged from 1.598±0.655 (strain 1) to 1.934±0.753 (strain 8) (Table 5).

Table 5

Number of alleles, heterozygosity and inbreeding coefficient and Shannon index per Hungarian Bronze turkey hen strain

Strains	N	N_a	N_e	H_{exp}	H_{obs}	F_{IS}	I
1	23	2.400±0.828	1.598±0.655	0.306±0.234	0.342±0.272	-0.075	0.504±0.369
2	18	2.467±0.834	1.664±0.575	0.349±0.216	0.364±0.233	-0.087	0.569±0.347
3	31	2.600±0.828	1.807±0.667	0.381±0.235	0.385±0.254	-0.014	0.627±0.375
4	24	2.467±1.187	1.779±0.828	0.350±0.253	0.358±0.267	-0.055	0.579±0.431
5	17	2.467±0.990	1.643±0.552	0.344±0.210	0.311±0.210	0.035	0.557±0.340
6	21	2.267±0.704	1.828±0.655	0.394±0.232	0.396±0.281	-0.017	0.606±0.358
7	20	2.467±0.834	1.764±0.607	0.379±0.226	0.429±0.262	-0.035	0.607±0.362
8	10	2.533±0.834	1.934±0.753	0.434±0.238	0.458±0.252	-0.093	0.678±0.385
9	13	2.333±0.724	1.720±0.573	0.371±0.230	0.396±0.309	-0.116	0.585±0.357
10	17	2.533±0.916	1.907±0.736	0.419±0.254	0.431±0.291	-0.052	0.657±0.398
Mean		3.133±1.187	1.868±0.756	0.389±0.218	0.383±0.223	-0.082	0.667±0.366

N: number of examined individuals, N_a : observed number of alleles, N_e : effective number of alleles, H_{obs} : observed heterozygosity, H_{exp} : expected heterozygosity, F_{IS} : inbreeding coefficient, I: Shannon index

Different alleles were found with a low frequency ($F < 0.05$) in the different strains. Rare alleles were not detected in strain 8 (Table 6).

Table 6
Frequency of rare alleles in the 10 Hungarian Bronze turkey hen strains

Locus	Alleles	Alleles frequency in strains								
		1	2	3	4	5	6	7	9	10
ADL0292	117			0.042						
	131	0.039	0.036				0.029			
ADL0149	224								0.046	
MCW0080	279			0.050				0.046		
MNT162	81							0.031		
	86					0.033				
	88				0.026					
	91		0.036							
MNT197	97				0.026					
	119					0.033				
MNT199	152		0.039							0.050
MNT214	200			0.022						0.050
	192									0.050
MNT327	209	0.039						0.031		0.050
	201		0.036	0.021						
MNT106	152					0.033				
	158					0.033				
MNT332	150								0.042	0.050
MNT387	145	0.039				0.033				

H_{exp} and H_{obs} per strains ranged from 0.306 ± 0.234 (strain 1) to 0.434 ± 0.238 (strain 8) and from 0.311 ± 0.210 (strain 5) to 0.458 ± 0.252 (strain 8), respectively. Genetic diversity was highest in strain 8 (Table 5). F_{IS} values were almost negative in all strains and ranged from 0.035 (strain 5) to -0.116 (strain 9).

Nei's unbiased genetic distance values were calculated (Table 7).

Table 7
Nei's unbiased genetic distances (below diagonal) between the Hungarian Bronze turkey hen strains

	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5	Strain 6	Strain 7	Strain 8	Strain 9	Strain 10
Strain 1	0.000									
Strain 2	0.029	0.000								
Strain 3	0.047	0.020	0.000							
Strain 4	0.018	0.012	0.017	0.000						
Strain 5	0.040	0.045	0.074	0.050	0.000					
Strain 6	0.056	0.049	0.049	0.032	0.020	0.000				
Strain 7	0.081	0.063	0.067	0.074	0.059	0.068	0.000			
Strain 8	0.026	0.032	0.038	0.022	0.015	0.011	0.028	0.000		
Strain 9	0.089	0.056	0.037	0.052	0.097	0.056	0.033	0.036	0.000	
Strain 10	0.062	0.044	0.027	0.033	0.059	0.027	0.017	0.012	0.016	0.000

The greatest distance values were found between the following pairs: strain 5/strain 3, strain 6/strain 1, strain 9/strain 1, strain 9/strain 2, strain 9/strain 4, strain 9/strain 5, strain 9/strain 6, strain 10/strain 1 and strain 7/all other nine strains. Figure 1 shows the neighbour-joining dendrogram obtained using Nei's unbiased genetic distance values. The genetic relationship among strains is shown clearly.

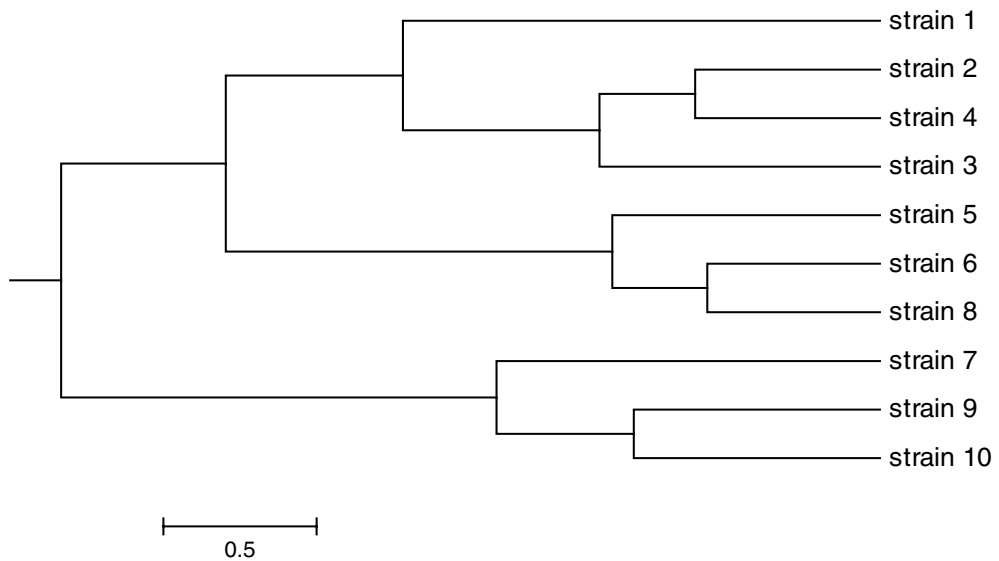


Figure 1
Neighbour-joining dendrogram among the Hungarian Bronze turkey hen strains

Genetic distance between the Hungarian Bronze turkey and Broad-breasted turkey strains

The mean number of observed alleles was lower (2.769 ± 1.013) and the mean number of effective alleles was higher (1.952 ± 0.738) in the Broad-breasted turkey than in the Hungarian Bronze turkey. Mean H_{exp} was higher than mean H_{obs} (0.441 ± 0.219 and 0.419 ± 0.232 , respectively) in the Broad-breasted turkey.

The result of F_{ST} showed that 15.9% of the total genetic variation was explained by differences between the two strains, and the remaining 84.1 % was accounted for differences among individuals. Nei's unbiased genetic distance values were calculated to determine the distance between the Hungarian Bronze and Broad-breasted turkey strains (Table 8).

Table 8
Genetic identity (above diagonal) and genetic distances (below diagonal) between the Hungarian Bronze turkey strains and Broad-breasted turkey

	Hungarian Bronze turkey	Broad-breasted turkey
Hungarian Bronze turkey	0.000	0.745
Broad-breasted turkey	0.293	0.000

Discussion

Microsatellite markers are now used efficiently for determining the genetic diversity of poultry species (Leberg *et al.* 1994, Rhodes *et al.* 1995, Ye *et al.* 1998, Kaiser *et al.* 2000, Romanov & Weigend 2001, Kong *et al.* 2006, Tu *et al.* 2006, Shahbazi *et al.* 2007, Yan *et al.* 2008). In our study, 15 microsatellite markers were used to determine the genetic structure of a Hungarian Bronze turkey gene reserve population and its differences from the Broad-breasted turkey.

High numbers of alleles ($n=48$) were found on the examined 15 loci in the Hungarian Bronze turkey. The use of a mixture of highly variable and less variable markers should reduce the danger of overestimating genetic variability, which might occur if only highly variable loci are used (Wimmers *et al.* 2000). In our study, all loci were polymorphic. The number of observed alleles per locus ranged from two to six, with a mean number of alleles of 3.2 ± 1.1 , which demonstrates the utility of the chosen microsatellites as informative molecular markers in the examined breed. However, the effective number of allele was much lower than the observed alleles in all loci. Larger differences were found in cases of those microsatellites where rare alleles occurred because they are less likely to take part in mating. Altogether, 14 rare alleles were found on 10 loci. Szöke *et al.* (2004) previously studied this Hungarian Bronze turkey five generations earlier using seven microsatellites, from which five (ADL0292, ADL0293, ADL0149, ADL0266 and MCW0080) were the same as we have studied. They found one more allele on every locus except MCW0080, which means that one allele from each of these loci, was lost over the past five years. The frequency of these alleles was lower than 0.01 in their study. They assumed that gender greatly influences the probability of losing a rare allele using the present mating system, which was borne out by their simulation results. They reported that if only one hen had a rare allele from 144 turkeys (allele frequency less than 0.01), it was lost within five generations in 85.5 % of the cases; when the male had the rare allele the value was only 21.5 %. We suppose that those lost alleles were carried by hens in the examined population. In the present study, 6 of the 14 rare alleles were carried only by hens, eight were found in both genders and one was observed only in bucks. The occurrence of the rare alleles among strains was different. Each allele was mainly observed only in one strain (Table 2). We should pay particular attention to the animals carrying the rare alleles because preservation requires more offspring from these animals during selection. However, this is difficult to put it into practice because turkeys have a short generational period and the cost of genotyping is expensive compared to their value.

Concerning heterozygosity, the mean H_{exp} and H_{obs} were not different (0.392 and 0.376, respectively) in the overall population, with similar values obtained for both hens and bucks. Szöke *et al.* (2004) reported a mean H_{exp} of 0.165 and mean H_{obs} of 0.164 for seven loci in this strain. Differences in heterozygosity might be the result of different sample sizes and studied microsatellites. The F_{IS} and Shannon index indicated a low inbreeding within hens and bucks (Table 4). Multilocus F_{ST} indicated that around 0.4 % of the total genetic variation was explained by a sex difference; the remaining amount corresponded to differences among individuals. The H_{exp} and H_{obs} varied marginally among hen strains, and H_{obs} was higher than H_{exp} in all strains except strain 5. The highest heterozygosity was obtained in strain 8, which could be the reason for the absence of rare alleles. Kalinowski (2004) reported that rare alleles affect allelic richness. F_{IS} and I also indicate similar high genetic diversities within all

examined hen strains. All these results confirm that the genetic diversity in the Hungarian Bronze turkey has been preserved by the rotational mating system. Differences between the Hungarian Bronze turkey and Broad-breasted turkey were determined. Nei's unbiased values clearly indicated that the examined two populations were highly genetically differentiated because of their different breeding strategies.

We conclude that the present mating system is suitable for preserving the genetic diversity of the Hungarian Bronze turkey gene reserve population. However, genetic diversity could rapidly decrease by reducing the population size. Bottlenecks cause a rapid loss of rare alleles and also result in the loss of genetic variability because of the effects of genetic drift (Allendorf & Luikart 2007). The Hungarian Bronze turkey population has a similar genetic diversity to other turkeys, suggesting that the studied population has not experienced serious genetic loss from the effects of bottlenecks. Inbreeding or breeding for a long time without fresh blood, especially in small populations, could reduce the level of genetic variability and produce a low heterozygosity value. Besides using the present breeding strategy, bringing fresh blood from other Bronze turkey populations is recommended. The results of this study might be useful as a guide for preserving the Hungarian Bronze turkey.

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References

- Allendorf FW, Luikart G (2007) Conservation and the Genetics of Populations. Blackwell Publishing, Malden USA, 642
- Christman CJ, Hawes RO (1999) Birds of a Feather: Saving Rare Turkeys from Extinction. ALBC, Pittsboro, NC, USA
- Kaiser MG, Yonash N, Cahaner A, Lamont SJ (2000) Microsatellite polymorphism between and within broiler populations. *Poult Sci* 79, 626-628
- Kalinowski ST (2004) Counting alleles with rarefaction: private alleles and hierarchical sampling designs, *Conserv Genet* 5, 1-12
- Kamara D, Gyenai KB, Geng T, Hammade H, Smith EJ (2007) Microsatellite marker-based genetic analysis of relatedness between commercial and heritage turkeys (*Meleagris gallopavo*). *Poult Sci* 86, 46-49
- Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite population. *Genetics* 49, 725-738
- Kong HS, Oh JD, Lee JH, Jo KJ, Sang BD, Choi CH, Kim SD, Lee SJ, Yeon SH, Jeon GJ, Lee HK (2006) Genetic variation and relationships of Korean native chickens and foreign breeds using 15 microsatellite markers. *Asian Austral J Anim Sci* 19, 1546-1550
- Leberg PL, Stangel PW, Hillestad HO, Marchinton RL, Smith MH (1994) Genetic structure of reintroduced wild turkey and white-tailed deer populations. *J Wildl Manage* 58, 698-711
- Levene H (1949) On a matching problem arising in genetics, *Ann Math Stat* 20, 91-94
- Lewontin RC (1972) The apportionment of human diversity. *BMC Evol Biol* 6, 381-398
- Mihók S, Bodó I, Bíró G, Süth M (1999) Meat production of the Bronze turkey reflected in addressing special consumer demands. *Állattenyésztés és Takarmányozás. Hungarian J Anim Prod* 48, 796-800

- Mihók S (2000) Bronze turkey. In: Bodó I (ed.), Living Heritage. Old Historical Hungarian Livestock. Agroinform Publishing and Printing Ltd., Budapest, Hungary, 84-85
- Nei M (1987) Molecular Evolutionary Genetics. Columbia University Press, NY, USA
- Rhodes OE, Buford DJ, Miller MS, Lutz RS (1995) Genetic structure of reintroduced Rio Grande wild turkeys in Kansas. *J Wildl Manage* 59, 771-775
- Romanov MN, Weigend S (2001) Analysis of genetic relationships between various populations of domestic and jungle fowl using microsatellite markers. *Poult Sci* 80, 1057-1063
- Shahbazi S, Mirhosseini SZ, Romanov MN (2007) Genetic diversity in five Iranian native chicken populations estimated by microsatellite markers. *Biochem Genet* 45, 63-75
- Sütő Z (2006) Characterization of turkey breed. In: Mihók S (ed.) Economy animals-Breeds: Chicken, guinea fowl, turkey, duck, muscovy duck, goose. *Mezőgazda Kiadó*, 116-134 [in Hungarian]
- Szalay IT, Kisné DDX, Virág Gy, Szentes K, Bódi L (2009) Prospects for conserving traditional poultry breeds in the Carpathian basin. *Animal Welfare, Ethology and Housing Systems* 5, 119-148 [in Hungarian]
- Szöke Sz, Komlósi I, Korom E, Ispány M, Mihók S (2004) A statistical analysis of population variability in Bronze turkey considering gene conservation. *Arch Tierz* 47, 377-385
- Tu YJ, Chen KW, Zhang SJ, Tang QP, Gao YS, Yang N (2006) Genetic diversity of 14 indigenous grey goose breeds in China based on microsatellite markers. *Asian Austral J Anim Sci* 19, 1-6
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38, 1358-1370
- Wimmers K, Ponsuksili S, Hargreave T, Valle-Zarate A, Mathur PK, Horst P (2000) Genetic distinctness of African, Asian and South American local chickens. *Anim Genet* 31, 159-165
- Yan W, Liu XL, Hau SS, Wei H (2008) Study on genetic diversity of six duck populations with microsatellite DNA. *Asian Austral J Anim Sci* 21, 776-783
- Ye X, Zhu J, Velleman G, Nestor KE (1998) Genetic diversity of commercial turkey primary breeding strain as estimated by DNA fingerprinting. *Poult Sci* 77, 802-807
- Yeh FC, Yong R (1999) POPGENE version 1.31: Microsoft-based Freeware for Population Genetic Analysis. University of Alberta, Edmonton, Canada
- Zsolnai A, Orbán L (1999) Accelerated separation of random complex DNA patterns in gels: comparing the performance of discontinuous and continuous buffers. *Electrophor* 7, 1462-1468

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