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GENETIC DIVERSITY OF SLOVAK ORIGIN GEESE POPULATIONS

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ABSTRACT

The genetic diversity was assessed in 102 individual genotypes in two national goose breeds Suchovska and Slovak and one extinct production crossbreed Tesedik Goose. A total of 40 alleles were found across 6 detected microsatellite loci with a mean number of 6.67 alleles per locus. The mean observed heterozygosity in total population was 0.40. The degree of inbreeding of Suchovska, Slovak and Tesedik calculated as a mean F_{IS} was 0.15, 0.11 and 0.07 respectively. The populations were low differentiated, with a mean F_{ST} value 0.075 in total population. The highest genetic distance was estimated between Slovak and Tesedik (0.087). The results of genetic diversity showed that Suchovska and Slovak Goose satisfies criteria for endangered breeds.

Keywords: genetic diversity, genetic distance, microsatellite, goose/geese

INTRODUCTION

Genetic diversity study is an important requirement for understanding genetic variation within and among breeds, strains and lines and future animal breeding strategies **(Lenstra *et al.*, 2012)**, also can be used to identify genetically vulnerable breeds **(Wilkinson *et al.*, 2012)**, for understanding phenotypic variation **(FAO, 2007)** and for reconstructing the history of livestock **(Groeneveld *et al.*, 2010)**.
In Slovakia exist two recognize national goose breeds Suchovska and Slovak, as a combination of old indigenous breeds and foreign introduced breeds and extinct crossbreed Tesedik Goose. The Suchovska Goose is a result of crossbreeding of local yellow fathering geese with French (Toulouse, Landes) and German (Pomorany, Steinbach) geese, originated at the end of the 1980`s in the village of Suchá nad Parnou and recognized as a breed in 1995 **(Kadlečík *et al.*, 2004)**. It required breeding geese of bigger body frame, firm constitution and of compact and solid body. The geese are by suitable for pasture and for small farming because of the preservation of the clucking instinct of the geese **(Weis and Hrnčár, 2007)**.
The Slovak Goose was created from regional breeds from South-Western Slovakia. Regional German and Hungarian types of goose were used during the breeding process. A year of recognition and initial numbers of birds are unknown **(Kadlečík *et al.*, 2004)**. The aim of breeding was to create a medium weight triple purpose (meat, liver, feather) geese suitable for corn areas, strong resistant geese with a good pasturing ability and with preserved clucking instinct achieved **(Weis and Hrnčár, 2007)**.
Tesedik Goose was created as a commercial crossbreed on the basis of three crossbreed lines: Ivagees (SVK), 2891 (CZE), Babati (HU), recognized in 2002. The aim of breeding programme were preservation and revitalization of origin Ivagees line as a crossbreed of Slovak, Landes, Italian and Rhine Goose. From 1994 in pedigree breeding reproduction and selections were in closed herd turnover **(Mindek, 2001)**.
The main tool in the characterization of the genetic diversity of farm animals is DNA polymorphism analysis of microsatellite loci **(Simianer, 2006)**. Currently there are known same microsatellite markers isolated and evaluated in the wild form of geese as greylag goose *Anser anser* **(Wieß *et al.*, 2008)**, Canada goose *Branta canadensis* L. **(Cathey *et al.*, 1998)**, swan goose *Anser cygnoides* L. **(Tu *et al.*, 2006**, **Li *et al.*, 2007)**, white-fronted goose *Anser albifrons* **(Fields *et al.*, 1997)**, pink-footed goose *Anser brachyrhynchus* **(Noreikiene, 2012)**, *Anser fabalis* **(Kleven *et al.*, 2016)**. *Anatidae* specific microsatellite markers for study of genetic diversity were used in Chinese **(Liu *et al.*, 2006**; **Tu *et al.*, 2006**; **Li *et al.*, 2007**; **Cao *et al.*, 2014)**, Hungarian, Embden **(Aliczky, 2007)** and Zatorska **(Andres and Kapkowska, 2011)** domestic goose breeds.
The objective if this study was preliminary characterization of the levels of the genetic diversity of two critically endangered and one extinct national breeds of goose, using the microsatellite loci analysis.

MATERIAL AND METHODS

Samples for analysis were taken from 102 geese, included Suchovska (n = 32), Slovak (n = 20) and Tesedik (n = 50) Goose. Samples from Suchovska and Slovak Goose were collected on Nationwide Exhibition of Animals in 2003. Birds came from different breeders, predominantly from west part of Slovakia. Samples from Tesedik Goose were collected from pedigree breeding in 2005. Blood samples were used to isolated genomic DNA followed the protocol of Wizard Genomic DNA Purification Kit (Promega).
For diversity studies were used six *Anatidae* species-specific microsatellite markers (Aalμ1, Bcaμ1, CKW21, TTUCG5, Ans2 and Ans25). Genotyping was conducted using standard laboratory procedure for PCR. Primers for PCR amplification for loci Ans2 and Ans25 **(Weiß *et al.*, 2008)** were designed for greylag goose (*Anser anser*), TTUCG5 **(Cathey *et al.*, 2006)** and Bcaμ1 **(Buchholz, 1998)** were designed for Canada goose (*Branta canadensis* L.), Aalμ1 **(Fields, 1997)** and CKW21 **(Liu *et al.*, 2006)**, were designed for swan (*Anser cygnoides* L.) and white fronted (*Anser albifrons)* goose respectively *(Table 1)*.

Table 1 Characteristic of microsatellite loci

Locus	Source species	Ref.	Repeat motif	Primer sequence 5´ - 3´	Allele size range (bp)
TTUCG5	*Branta canadensis*	a	TCTAT	GGGTGTTTTCCAACTCAG CACTTTCCTTACCTCATCTTG	176 - 288
CKW21	*Anser cygnoides*	b	$(TTA)_{10}$	CAAGGTAGTCATAAACCCAGAACA ACAAAACTAATGGCAGGAAAC	351 - 379
Aalμ1	*Anser albifrons*	c	TG	CATGCGTGTTTAAGGGGTAT TAAGACTTGCGTGAGGAATA	85 - 89
Ans25	*Anser anser*	d	$(GT)_{18}$	CACTTATTAATGGCACTTGAAA GTTCTCTTGTCACAACTGGA	261 - 267
Ans2	*Anser anser*	d	$(AG)_{17}$	TTCTGTGCAGGGGCGAGTT AGGGAACCGATCACGACATG	207 - 228
Bcaμ1	*Branta canadensis*	e	$(TA)_{15}(CA)$	TGCTTTTTACCCCCAGTGTTCT AGAATCTGCTATATTATTTCCAGCTC	114 - 124

Ref.: [a]Cathey *et al.* (2006), [b]Liu (2006), [c]Fields (1997), [d]Weiß *et al.* (2008), [e]Buchholz (1998)

Six pairs of primers were amplified in one multiplex PCR reaction (1U Ampli*Taq*Gold). PCR amplification was performed on a thermal cycler MJ Research (anneling 59^0C/60s., 35 cycles). Amplified PCR products were electrophoresed on sequencer ABI 310 (Applied Biosystems). The size of the analyzed DNA fragments were determined in base pairs using computer package GeneScan v.3.7 (Applied Biosystems), by comparing to an internal size standard (LIZ 500, Applied Biosystems).

The total number of alleles, the average number of alleles per locus, expected and observed heterozygosity and polymorphic information content (PIC) of microsatellite loci **(Botstein *et al.*, 1980**; **Weir, 1996)** were estimated using PoweMarker 3.25 **(Liu and Muse, 2005)**. Number of effective alleles and privat (breed–specific) alleles were calculated by GenAIEx software **(Peakall and Smouse, 2006)**. The extent of genotyping linkage disequilibrium (LD) between pairs of loci in each group of breeds by performing probability test and deviations from Hardy-Weinberg equilibrium (HWE) across all loci for each population were performed by estimation of exact P-value by the Markov chain method using GENEPOP 4.2 software **(Rousset, 2008)**.

The effective population size was estimated by **Simon and Buchenauer (1993)**:

*Ne = 4*M*F/(M+F)*

where: M is the number of males, F is the number of females.

The ratio of the effective population size to census population size (Ne/N) is an indicator of the extent of genetic variation expected in a population. Male: female ratio (Nm/Nf) is defined as the number of breeding males upon the number of breeding females in a population. Population genetic differentiation was examined using pairwise population fixation index (F_{ST}) values and F-statistics estimated over all populations for each locus, computed by GenAIEx software **(Peakall and Smouse, 2006)**, within-population inbreeding coefficient (F_{IS}) were computed using PowerMarker 3.25 **(Liu and Muse, 2005)**. Nei's standard genetic distance with sample size correction from a small number of individuals (1978) and construction of a neighbor-joining (NJ) tree were performed by POPTREE 2 **(Takezaki *et al.*, 2010)**. To represent geometric relationship among the populations and individuals, a factorial component analysis (FCA) was applied using gene frequencies of all variable loci with Genetix4 **(Belkhir *et al.*, 1996–98)**.

RESULTS AND DISCUSSION

Genotyping in total population of 102 individuals for 6 polymorphic microsatellite loci a total of 40 different alleles were detected. The mean number of alleles was 6.67, with the range extending from 4.17 (Suchovska) to 4.67 (Tesedik). Number of effective alleles across all population range near 2, with a mean number of effective alleles 2.19. A total of 11 private (breed-specific) alleles were detected. Private alleles with a frequency < 0.1% occurred only in Slovak breed (*Table 2*).

Table 2 Genetic diversity

Population	Sample size	Mean No. of allele	Mean No. of effect. allele	No of private allele[1]	Mean expected heterozyg.	Mean observed heterozyg.	LD[2]	HW[3]
Suchovska	32	4.17	2.03	4/0	0.38	0.33	5	1
Slovak	20	4.33	2.33	4/2	0.45	0.42	2	2
Tesedik	50	4.67	2.21	3/0	0.45	0.43	3	1
Total	102	6.67	2.19	11/2	0.46	0.40	6	3

[1] total number of private alleles / number of alleles with a frequency < 0.1%

[2] number of significant test of linkage disequilibrium (LD) out of 15 possible pairs of loci

[3] number of significant disequilibrium of Hardy-Weinberg test

The average expected heterozygosity over all loci ranged from 0.38 in Suchovska to 0.45 in Slovak and Tesedik, while observed heterozygosity varied from 0.33 in Suchovska to 0.43 in Slovak and Tesedik. Mean expected and observed heterozygosity over all loci and group were 0.46 and 0.40 respectively. In population significant deviations from HWE were revealed in TTUCG5, Ans2 and Bcaμ1 locus. In a total population LD was found in 6 out of 15 pairs of loci, with the highest number of locus-pairs with significant LD in Suchovska (5) breed (*Table 2*).

Table 3 Genetic diversity per loci in the total population

Locus	No. of obs.	Genotype No	Major Allele Frequency	Allele No	Expected Heterozyg.	Observed Heterozyg.	PIC	Fis	Fst
TTUCG5	102	32	0.23	13	0.84	0.73	0.82	0.081	0.081
CKW21	100	12	0.76	7	0.41	0.39	0.39	0.011	0.070
Aalμ1	102	6	0.84	3	0.27	0.23	0.26	0.069	0.136
Ans25	102	7	0.63	4	0.54	0.51	0.48	0.005	0.083
Ans2	102	10	0.84	7	0.28	0.23	0.27	0.154	0.042
Bcaμ1	102	11	0.75	6	0.41	0.27	0.39	0.324	0.040
Mean	101.7	13.0	0.68	6.7	0.46	0.40	0.43	0.100	0.075

Across all population the number of alleles per locus ranged from 3.0 (Aalμ1) to 13 (TTUCG5). Major allele frequencies differed notably in their distribution in the different loci, ranging from 0.23 (TTUCG5) to 0.84 (Ans2, Aalμ1), with an average value 0.68 per locus. The number of observed genotype varied widely from 6 (Aalμ1) to 32 (TTUCG5), with an average value 13.0 per locus. In all estimated loci the observed heterozygosity is similar or lower to their expectation. The lowest and the greatest heterozygosity per locus was 0.23 (Aalμ1, Ans2) and 0.73 (TTUCG5) respectively, with an average value 0.40. The estimated PIC ranged from 0.26 (Aalμ1) to 0.82 (TTUCG5), with a mean PIC value 0.43 *(Table 3).*

The Pairwise Population F_{ST} Values between Suchovska and Slovak and between Slovak and Tesedik was 0.050 and 0.051 respectively. The F_{ST} value among Suchovska and Tesedik was 0.038. Mean genetic differentiation (F_{ST}) estimated over all populations for each locus was 0.075. All populations had positive within-population (F_{IS}) estimates, where mean F_{IS} values were 0.07 for Tesedik, 0.13 for Slovak and 0.15 for Suchovska. Mean F_{IS} value in total population was 0.14 (Figure 1). Significant deficiency of heterozygotes occurred in locus TTUGC5 (Slovak), Ans2 (Slovak) and Bcaμ1 (Suchovska, Tesedik), *(Table 3).*

All estimated microsatellite loci were polymorphic as was published in previously studies **(Aliczki 2007**; **Wieß *et al*. 2008**; **Andres and Kapkowska, 2011)**, with a varying number of alleles. The number of alleles detected in locus TTUCG5, Aalμ1, Bcaμ1 were similar as in other breeds **(Bucholz *et. al*; 1998**, **Aliczki 2007**; **Andres and Kapkowska, 2011)**. Considerable lower number of detected alleles was in CKW21, Ans2 and Ans25 locus, compare to other published papers **(Liu *et al.*, 2006**; **Aliczki 2007**; **Wieß *et al*. 2008**; **Andres and Kapkowska, 2011)**. **Barker (1994)** suggested that microsatellite loci used in genetic distance studies should have more than four alleles in order to reduce the distance estimate standard error. The mean numbers of allele in some loci in this study does not meet this condition. At the same time, several private alleles across the breeds were found. On the other hand, private alleles are not applicable as breed markers due to low frequency. The existence of private allele can be explain by multi-origin of the breeds, little subsequent genetic exchange between them, or by genetic drift (Agha *et al.*, 2008).

The mean expected heterozygosity across all population were similar as in other European geese **(Aliczki *et al.*, 2007**; **Andres and Kapkowska, 2011**; **Noreikiene *et al.*, 2012)**, but considerably lower than in Chinese breeds **(Tu *et al.*, 2006**; **Li *et al.*, 2007)**. The results of the expected heterozygosity were consistent with that of PIC. Over all population the mean observed heterozygosity within populations were lower than mean expected. The deficiency of heterozygotes was reflected in the considerably higher estimates of within-population inbreeding coefficient (F_{IS}), than was reported in Hungarian breeds **(Aliczki *et al.*, 2007)**. Estimated number of alleles and level of heterozygosity as an available parameter to assess within breeds genetic diversity refer about low genetic diversity of Suchovska and Slovak breeds. These results point out to inbreeding caused by small number of individuals. In decade 2001-2010 the census population size of Suchovska and Slovak Goose varied widely, with highest numbers of individuals in 2005 (Suchovska 143, Slovak 83). But last five years the population size gradually decreases, which is related to the decline of the effective population size with it historically the lowest value (Suchovska 13.750 and Slovak 26.182) (*Table 4*).

The effective population size as the indicator of the amount of genetic variation present in the population confirms the low level of genetic variation in Slovak national goose breeds. At the same, the decrease of effective population size leads increases to extent of LD. Higher number of alleles and observed heterozygosity in Tesedik Goose points to higher genetic diversity compare with Slovak national goose breeds, caused by crossbreeding and high number of individuals.

Table 4 Census population size, effective population size and sex ratio per breed and year

	Suchovska Goose			Slovak Goose			Tesedik Goose		
Year	**Ne/N**	**Ne**	**Nm/Nf**	**Ne/N**	**Ne**	**Nm/Nf**	**Ne/N**	**Ne**	**Nm/Nf**
1996							5191	4312.898	0.417
2001	72	57.778	0.417	34	28.235	0.385	2074	1697.213	0.399
2002	82	74.976	0.478	68	59.529	0.547	1850	1509.224	0.402
2003	67	63.642	0.625	52	49.231	0.634	1762	1441.056	0.405
2004	122	98.098	0.415	75	62.187	0.386	1988	1631.378	0.400
2005	143	120.280	0.407	83	68.241	0.430			
2006	141	125.333	0.375	66	52.364	0.500			
2007	140	120.686	0.422	64	53.438	0.458			
2008	136	112.941	0.717	79	76.861	0.417			
2009	114	105.018	0.436	79	66.835	0.562			
2010	82	69.512	0.439	82	69.512	0.439			
2011	47	43.404	0.477	65	56.862	0.567			
2012	35	28.571	0.452	45	38.578	0.400			
2013	31	25.548	0.464	41	35.512	0.409			
2014	26	22.154	0.407	38	31.263	0.444			
2015	19	17.684	0.400	35	28.571	0.583			
2016	16	13.750	0.375	33	26.182	0.455			

Wang *et al.* (1999) demonstrated that fitness declines with Ne of 50 because of detrimental mutations fixation despite natural selection. **Meuwissen** and **Wooliams (1994)** suggested, from theoretical predictions, that Ne between 30 and 250 is needed for natural selection to counteract inbreeding depression. According to **Lynch *et al.* (1995)**, Ne should exceed 500 animals otherwise, this accumulation of slightly deleterious mutations will deem the population to extinction. It is important to monitor Ne, because it can be smaller than expected due to any effect increasing variance of the family size of an animal (e.g. selection, unequal survival rates). A rapid strategy to minimize inbreeding would be therefore to maximize the effective population size in flocks and increase the male: female ratio in some breeds **(Larivière *et al.*, 2011)**.

For reducing the inbreeding rate in population, the effective population size needs to be increased for which many strategies are. These can be equalization of family sizes **(Wang, 1997)**, choice of parents **(Cabarello and Toro, 2000)** and various systems of breeding **(Nomura and Yonezawa, 2000)**.

Genetic distance was calculated based on allelic frequencies in each breed, after 10 000 permutation **(Nei, 1978)**. The highest genetic distance was estimated between Slovak and Tesedik (0.087), compare to the smallest genetic distance between Suchovska and Tesedik (0.054). The Nei´s genetic distance 0.064 point out small genetic distance between Suchovska and Slovak breeds. Based on Nei´s genetic distance matrix Neighbor-Joining tree was constructed (Figure 1).

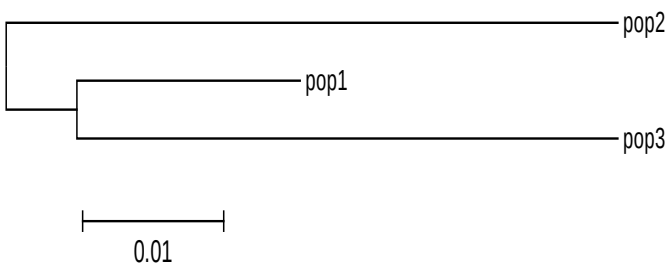

Figure 1 - Neighbor - Joining Tree

Legend: pop1 - Suchovska, pop2 - Slovak, pop3 **–** Tesedik

The mean genetic differentiation (F_{ST}) estimated amongst the populations over all loci was lower with level comparable to Hungarian breeds **(Aliczki *et al.*, 2007)**

and other livestock species **(Laval *et al.*, 2000**; **Lawson-Handley *et al.*, 2007)**. Values of all calculated pairwise population fixation index (F_{ST}), that range near value 0.01 to 0.05, shows a generally low level of genetic differentiation, with quite differences between populations.

Birds are crowded in relatively independent breed´s cluster with a low deviation in both axes. A relatively large number of Suchovska individuals are incorporated in cluster of Tesedik Goose. Although Slovak Goose served as one of the progenitors of Tesedik, lower genetic distance was between Suchovska and Tesedik. Closer genetic relationship confirm with FCA analysis was caused probably by influence of Landes Goose that were introduced during crossbreeding of both Suchovska and Tesedik Goose. Although Suchovska and Slovak Goose are phenotypically different (wildly colored yellow, heavy Suchovska vs. white, medium weight Slovak), between breed diversity are considerable low. Close relationship among the population had obvious association with their historical relations and geographical distribution **(Li *et al.*, 2007)**, when the most goose breeds in Europe were originated from *Anser anser* **(Tu *et al.*, 2006)**. Moreover, Suchovska and Slovak have a common indigenous ancestor. On the other hand, the presence of private (breed-specific) alleles occurred over all estimated populations proved their genetic divergences.

To represent diversity that may correlate with geographical or genetic variability, a factorial component analysis (FCA) was applied using gene frequencies of all variable loci. The axe 1 explained 8.08% and axe 2 explained 7.32% of total variability (Figure 2).

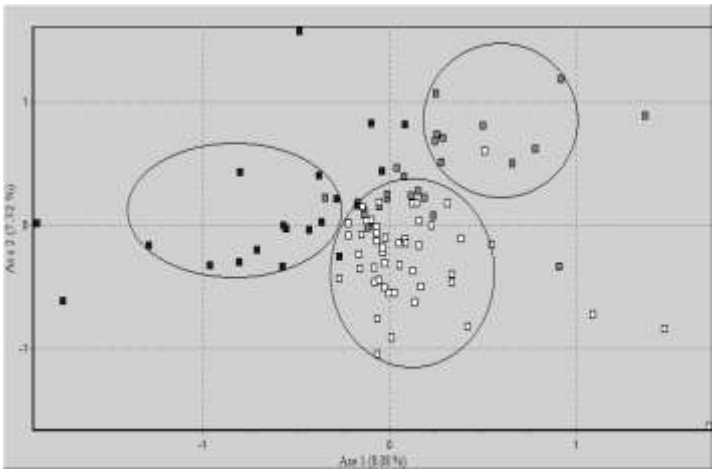

Figure 2 - Factorial correspondence analysis
Legend: Suchovska (grey), Slovak (black), Tesedik (white)

Compare to Chinese goose breeds **(Tu *et al.*, 2006**; **Li *et al.*, 2007)**, genetic distance between evaluated populations are very low. Genetic distances were used to construct a between populations neighbor-joining tree (*Figure 2*), when Tesedik and Suchovska Goose branches are derived from Slovak Goose branch. To represent geometric relationship among the birds and populations, a factorial component analysis (FCA) was applied (*Figure 3*).

CONCLUSIONS

The estimated low genetic diversity and uniqueness coupled with the low effective population size confirm that Suchovska and Slovak Goose are critically endangered and their preservation is required. In addition, these breeds are carriers of the gene pool of the indigenous local geese that no longer exist. For the future application, the increase a number of tested microsatellite markers is necessary.

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