GENETIC DIVERSITY AND STRUCTURE OF SLOVAK DOMESTIC GOOSE BREEDS

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Abstract. Knowledge of the genetic diversity and structure is particulary important in the case of endagered breeds. The preliminary characterization of the genetic diversity and structure were assessed in two critically endangered national goose breeds Suchovska and Slovak, based on 6 microsatellite loci analysis in a total population of 50 birds. A total of 31 alleles were found across 6 microsatellite loci with a mean number of 5.17 alleles per locus. The mean observed heterozygosity was 0.33 in Suchovska and 0.39 in Slovak. The degree of inbreeding of Suchovska and Slovak calculated as a mean F_{IS} was 0.13 and 0.15 respectively. The F_{ST} value between Suchovska and Slovak breeds was 0.058. Nei´s genetic distance between Suchovska and Slovak goose was 0.093. The low level of diversity indicated as heterozygosity deficiency coupled with low population size confirm, that Slovak national breeds of geese are critically endangered and required attention for the preservation.
Keywords: genetic diversity, genetic structure, microsatellite, goose/geese.

Introduction

Knowledge of the genetic diversity and structure of breeds can inform sustainable management initiatives and decision-making processes for conservation of breed biodiversity (Wilkinson et al., 2012). There have been several collaborative European-wide initiatives on the characterization of farm animal genetic resources (FanGR), for example in cattle, pigs, goats, sheep (Wilkinson et al., 2012). However, in the case of domestic poultry composed of various avian species, the study of genetic diversity focuses mainly on chickens (AVIANDIV).

Geese are one of the oldest species of domestic poultry. There are 181 recognized breeds of domestic geese in the world with 158 local populations distributed mainly in Europe and Asia. In 2006, two thirds of distinguished breeds (121) faced the risk of being lost or their risk status was unknown (FAO, 2007). Gandini & Villa (2003) recorded that local breeds are an evidence of great achievements of many generations of breeding. For centuries, farmers have been adapting chickens (poultry) to local conditions, cultural needs and preferences. Unfortunately, over the last decades, because of the industrialization of agriculture the old local breeds of Europe mostly suffered a graduate decrease in members. On the other hand, native goose breeds have better adaptability to extensive management, higher disease resistance, higher reproduction rate and better meat quality, which is due to the natural gene pool and good original material of crossbreed predominance and high performance. Such excellent native breeds may contain the genes and alleles permitting adaptation to unforeseen breeding requirements in the future and a source of research materials (Romanov et al., 1996).

There exist two recognized national goose breeds Suchovska and Slovak in Slovakia, as a combination of old indigenous breeds and still genetically undefined foreign introduced breeds. The Suchovska Goose is a result of crossbreeding of local yellow fathering geese with French (Toulouse, Landes) and German (Pomorany, Steinbach) geese. This breed of geese originated at the end of the 1980`s in the village of Suchá nad Parnou and was recognized as a breed in 1995, with a number of 45 birds: 21 males and 24 females (Kadlečík et al., 2004). It was produced seeking to breed geese of bigger body frame, firm constitution and of compact and solid body. The geese are suitable for pasture. They also are suitable for small farming because of the preservation of the clucking instinct (Weis & Hrnčár, 2007). In case of the Suchovska goose its lowest population occurred in 2003 (67 birds - 26 males and 41 females) and the highest in 2005 (143 birds - 43 males and 100 females). Minimal detected effective population size of the Suchovska goose was 57.778 (year 2001), the maximum being 125.333 (year 2006) with an average of the 96.717 (Weis et al., 2010).

The Slovak Goose was created from regional breeds from South-Western Slovakia in 1940s. Regional German and Hungarian types of goose were used during the breeding process. The aim of breeding was to create a medium weight triple purpose (meat, liver, feather) geese suitable for corn areas, a strong resistant geese with a good pasturing ability and with preserved clucking instinct (Weis & Hrnčár, 2007). Literary sources do not indicate
the year of recognition and initial number of birds at that time (Kadlečík et al., 2004). The lowest population of Slovak Goose was in 2001 (34 birds - 10 males and 24 females), the highest in 2005 (83 birds - 24 males and 59 females). The effective population size of Slovak Goose

varied widely from 28.235 (year 2001) to 76.861 (year 2008) with an average of 56.261. The Suchovska and Slovak geese were categorized as endangered breeds (Weis et al., 2010).

In the 2012, the number of Suchovska Goose was 35, thereof in recognized pedigree breeding 17 (5 males, 12 females). The number of Slovak Goose was 44, thereof in recognized pedigree breeding 22 (5 males, 17 females).

Preservation of the local breed genome is important in order to save its unique genetic traits. The genetic variability of animals, represented by differences among the individual breeds, was evaluated by many types of genetics markers. At a national level, the genetic variability of domestic poultry based on DNA was analysed sparsely. Only the population of Japanese quail was characterized by RAPD method (Židek, 2004). The main tool in the characterization of the genetic diversity of farm animals is DNA polymorphism analysis of microsatellite loci (Simianer, 2006). Currentl,y there are know 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), and pink-footed goose Anser brachyrhynchus (Noreikiene, 2012). 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), Hungarian, Embden (Aliczky, 2007) and Zatorska (Andres & Kapkowska, 2011) domestic goose breeds.

The aim of this study was to preliminary characterise the genetic diversity and structure of two critically endangered national breeds of goose, based on 6 microsatellite loci analysis.

Materials and methods
Samples for analysis were taken from 50 domestic geese, including Suchovska (n = 32) and Slovak (n = 18) breeds. Non-admixed samples from different breeders were collected on Nationwide Exhibition of Animals in 2003. The birds came predominantly from the western part of Slovakia. Blood samples were used to isolate genomic DNA following the protocol of Promega (Wizard Genomic DNA Purification Kit. Six pairs of primers for geese: Aalµ1, Bcaµ1, CKW21, TTUCG5, Ans2, Ans25 (Table 1) 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). Products of the PCR were electrophoresed on sequencer ABI 310 (AB). The size of the analysed DNA was determined in base pairs using computer package GeneScan v.3.7 (AB).

The total number of alleles, the average number of alleles per locus, expected heterozygosity (gene diversity), observered heterozygosity and polymorphic information content (PIC) of microsatellite loci (Botstein et al., 1980; Weir, 1996) were estimated using PoweMarker 3.25 (Liu & Muse, 2005). Number of effective alleles and privat (breed–specific) alleles was calculated by GenAIEx software (Peakall & Smouse, 2006). The extent of genotypic linkage disequilibrium (LD) between pairs of loci in each group of breeds by performing probability test and test for deviations from Hardy-Weinberg equilibrium (HWE) using the Markov chain algorithm was tested by GENEPOP 4.2 software (Rousset, 2008). 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 & Smouse, 2006). Within-population inbreeding, coefficient (F_{IS}) and Nei´s genetic distance (1972) were computed using PoweMarker 3.25 (Liu & Muse, 2005). To represent geometric relationship among the goose breeds, a principal component analysis (PCA) was applied using TANAGRA software (Rakotomalala, 2005).

Table 1 **Characteristic of microsatellite loci**

Locus	Source species	Repeat motif	Primer sequence 5´ - 3´	Allele size range (bp)
TTUCG5[a]	*Branta canadensis*	TCTAT	GGGTGTTTTCCAACTCAG CACTTTCCTTACCTCATCTTG	176–288
CKW21[b]	*Anser cygnoides*	$(TTA)_{10}$	CAAGGTAGTCATAAACCCAGAACA ACAAAACTAATGGCAGGAAAC	351–379
Aalµ1[c]	*Anser albifrons*	TG	CATGCGTGTTTAAGGGGTAT TAAGACTTGCGTGAGGAATA	85–89
Ans25[d]	*Anser anser*	$(GT)_{18}$	CACTTATTAATGGCACTTGAAA GTTCTCTTGTCACAACTGGA	261–267
Ans2[d]	*Anser anser*	$(AG)_{17}$	TTCTGTGCAGGGGCGAGTT AGGGAACCGATCACGACATG	207–228
Bcaµ1[e]	*Branta canadensis*	$(TA)_{15}(CA)$	TGCTTTTTACCCCCAGTGTTCT AGAATCTGCTATATTATTTCCAGCTC	114–124
Ref.: [a]Cathey *et al.* (2006), [b]Liu *et al.* (2006), [c]Fields (1997), [d]Weiß *et al.* (2008), [e]Buchholz (1998				

Results
The investigated loci were chosen from species-specific microsatellite markers isolated and evaluated in the Anatidae species. Six pairs of primers were chosen in accordance with previously published papers, used in diversity studies of ancestor greylag goose and some

European domestic breeds as Emdem, Hungarian and Zatorska. Primers Ans2 and Ans25 were isolated in greylag goose (*Anser anser*), TTUCG5 and Bcaμ1 were designed for Canada goose (*Branta canadensis* L.), Aalμ1 and CKW21 were isolated in swan (*Anser cygnoides* L.) and white fronted (*Anser albifrons)* goose respectively (Table 1).

Genotyping in total population of 50 individuals for 6 polymorphic microsatellite loci revealed 31 different alleles. The mean number of alleles was 5.17, with the range extending from 3.67 (Slovak) to 4.0 (Suchovska, Table 2). The number of effective alleles ranged from 2.01 (Suchovska) to 2.16 (Slovak), with a mean number of effective alleles 2.09. A total of 16 private (breed-specific) alleles were detected. The number of private alleles with a frequency < 0.1% was 10, from 3 (Slovak) to 7 (Suchovska), where the most private alleles were detected in loci TTUCG (5) and Ans2 (4).

The average expected heterozgosity over all loci ranged from 0.38 in Suchovska to 0.45 in Slovak, while observed heterozygosity varied from 0.33 in Suchovska to 0.39 in Slovak. Mean expected and observed heterozygosity over all loci and group were 0.43 and 0.56 respectively (Table 2). There was a large deficiency of heterozygotes compared with HWE expectations across the multilocus dataset over groups of breeds (P >0.01). The number of locus-pairs with significant LD was 3 (Slovak) and 7 (Suchovska). In a total population, significant LD was found in 6 out of 15 pairs of loci (Table 2).

Table 2 **Genetic diversity**

Group of breeds	Sample size	Mean No. of allele	Mean No. of effect. allele	No of private allele[1]	Mean expect. heterozyg.	Mean observed heterozyg.	LD
Suchovska	32	4.00	2.01	9/7	0.38	0.33	7
Slovak	18	3.67	2.16	7/3	0.45	0.39	3
Total	50	5.17	2.09	16/10	0.43	0.56	6
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							

In a total population, the number of alleles per locus ranged from 3.0 (Aalμ1) to10 (TTUCG5). Major allele frequencies differed notably in their distribution in the different loci, ranging from 0.22 (TTUCG5) to 0.87 (CKW21, Ans2), with an average value 0.70 per locus. The number of observed genotype varied widely from 6 (Aalμ1, Ans25, Ans2) to 22 (TTUCG5), with an average value 8.83 per locus. The observed heterozygosity is similar or lower to expectation (gene diversity) in all estimated loci. The lowest and the greatest heterozygosity per locus was 0.32 (Bcaμ1) and 0.69 (TTUCG5) respectively, with an average value 0.35. The estimated PIC ranged from 0.22 (CKW21) to 0.81 (TTUCG5), with a mean PIC value 0.40 (Table 3).

Table 3 **Genetic diversity per loci in the total population**

Locus	No. of obs.	Genotype No	Major Allele Frequency	Allele No	Gene Diversity	Heterozyg.	PIC
TTUCG5	49	22	0.22	10	0.84	0.69	0.81
CKW21	47	5	0.87	4	0.23	0.12	0.22
Aalμ1	50	6	0.77	3	0.38	0.34	0.35
Ans25	49	6	0.72	4	0.43	0.43	0.38
Ans2	50	6	0.87	5	0.24	0.14	0.23
Bcaμ1	50	8	0.73	5	0.44	0.32	0.41
Mean	49.2	8.83	0.70	5.17	0.43	0.35	0.40

Mutual comparison of genetic diversity within breeds Suchovska and Slovak showed, that the number of allele per locus ranges from 2 to 9 in Suchovska and from 3 to 6 in Slovak, with higher mean number of alleles (4.00) in Suchovska goose. Major allele frequency ranges from 0.22 (Slovak) to 0.92 (Suchovska), with higher mean major allele frequency (0.74) in Suchovska goose (Table 4). Major allele differs only in TTUCG5 locus, where other 4 private alleles were detected. In locus CKW21 only major allele was common. Private alleles occurred in all remaining loci, from 5 (TTUCG5) to 1 (Aalμ1, Ans25).

The observed heterozygosity was higher than expected (gene diversity) in TTUCG5, CKW21 (Suchovska) and in Ans25 (Slovak) locus. The higher mean heterozygosity was in Slovak goose (0.39). Polymorphic information content ranges from 0.11 (Suchovska) to 0.75, with higher mean PIC value (0.41) in Slovak goose (Table 4).

The pairwise F_{ST} value between Suchovska and Slovak was 0.58. The mean genetic differentiation (F_{ST}) estimated over all populations for each locus was 0.093. All breeds had significant positive mean within-population (F_{IS}) estimates, where mean F_{IS} values ranged from 0.13 (Suchovska) to 0.15 (Slovak). Mean F_{IS} value in total population was 0.18 (Fig. 1). A significant deficiency of heterozygotes occurred in Ans25, Bcaμ1 (Suchovska) and TTUCG5, Ans2 (Slovak) locus.

Table 4 **Genetic diversity per loci in Suchovska and Slovak breeds**

Suchovska goose							
Locus	No. of obs.	Genotype No	Major Allele Frequency	Allele No	Gene Diversity	Heterozyg.	PIC
TTUCG5	31	13	0.35	9	0.78	0.80	0.75
CKW21	31	2	0.92	2	0.15	0.16	0.13
Aalμ1	32	3	0.86	2	0.24	0.22	0.21
Ans25	31	6	0.69	4	0.46	0.42	0.41
Ans2	32	3	0.94	2	0.12	0.06	0.11
Bcaμ1	32	8	0.65	5	0.52	0.34	0.47
Mean	31.5	5.83	0.74	4.00	0.38	0.33	0.35
Slovak goose							
Locus	No. of obs.	Genotype No	Major Allele Frequency	Allele No	Gene Diversity	Heterozyg.	PIC
TTUCG5	18	11	0.22	6	0.79	0.50	0.75
CKW21	16	4	0.78	3	0.35	0.31	0.31
Aalμ1	18	5	0.61	3	0.53	0.55	0.45
Ans25	18	3	0.78	3	0.36	0.44	0.33
Ans2	18	4	0.75	4	0.41	0.28	0.38
Bcaμ1	18	3	0.86	3	0.24	0.28	0.22
Mean	17.68	5.00	0.67	3.67	0.45	0.39	0.41
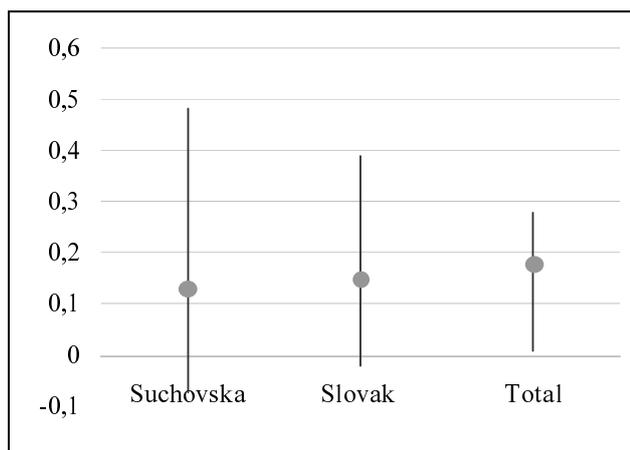
Fig. 1 **Within-population and total inbreeding coefficient (F_{IS})**

Genetic distance was calculated based on allelic frequencies in each breed, after 10 000 permutations (Nei, 1972). The Nei´s genetic distance 0.093 points out small genetic distance between Suchovska and Slovak breeds.

For presentation of geometric relationship among the Suchovska and Slovak breeds, a principal component analysis (PCA) was applied using Tanagra software (Fig. 2). Deviation in the axis 1 showed 19.59% captures of the total observed variability, deviation in the axis 2 is 18.09%.

Discussion

Maintaining genetic variation is an important requirement for future animal breeding strategies. This study was focused on genetic diversity within and between Suchovska and Slovak breeds. All estimated microsatellite loci were polymorphic as was published in previous studies (Aliczki 2007; Wieß *et al*. 2008; Andres & Kapkowska, 2011), with a varying number of alleles and were useful for the genetic study according to the number of detected loci over the population. The number of alleles detected in locus TTUCG5, Aalμ1, Bcaμ1 were similar as in other breeds (Bucholz et. al; 1998, Aliczki 2007; Andres & Kapkowska, 2011). A considerable smaller number of detected alleles was in CKW21, Ans2 and Ans25 locui, compared to other published papers (Liu et al., 2006; Aliczki 2007; Wieß *et al*. 2008; Andres & Kapkowska, 2011).

The mean expected heterozygosity (gene diversity) across all population were similar as in other European geese (Aliczki, et al. 2007; Andres & 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 those of PIC. Over all population, the mean observed heterozygosity within breeds was lower than 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). Consequently, significant deviation from HWE was observed in Suchovska and Slovak breeds. In contrast to results reported here, livestock breeds are primarily homogenous genetic populations that rarely deviate from within-breed HWE proportion (Lawson Handley et al., 2007), where observation of excess heterozygosity are not uncommon in geese (Cathey et al., 2001).

The estimated number of alleles and the level of heterozygosity as available parameters to assess genetic diversity within breeds refers to low genetic diversity of Suchovska and Slovak breeds. These results point out to inbreeding caused by small number of individuals and

farmers as well as genetic drift, frequent in the small population that may have led to the fixation of some alleles at the expense of others. In addition, the bottleneck effect after dramatic reduction of the population size was the likely cause of reduction of diversity within breeds of Suchovska and Slovak.


(X1) PCA_1_Axis_2 vs. (X2) PCA_1_Axis_1 by (Y) Pop

● Suchovska ▲ Slovak
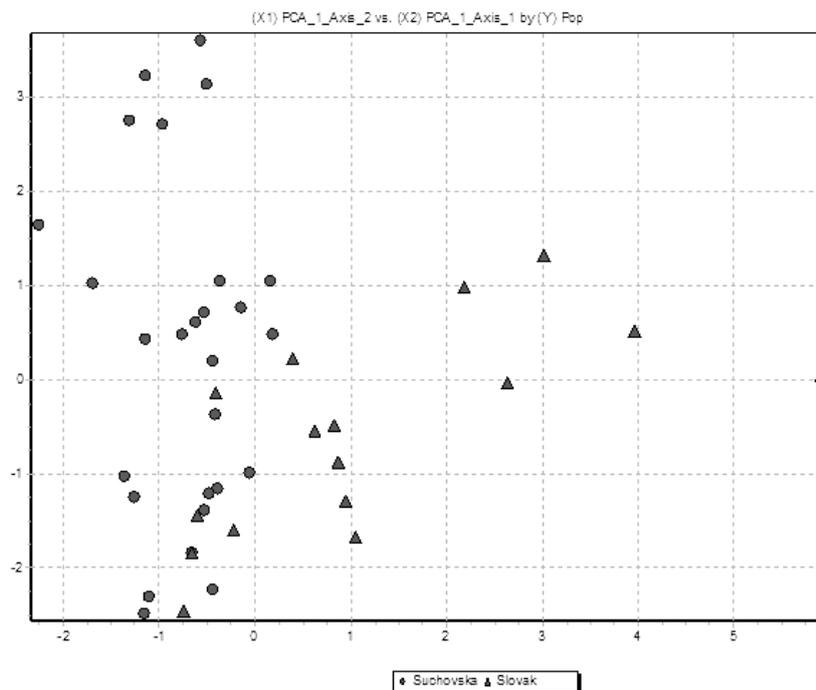
Fig. 2 PCA analysis

The genetic differentiation (F_{ST}) amongst the groups of breeds 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). All calculated population fixation index (F_{ST}) range near the value 0.01 to 0.1. These results show a generally low level of genetic differentiation, with small differences across groups of breeds. Compared to Chinese goose breeds (Tu et al., 2006; Li et al., 2007), the genetic distance between Suchovska and Slovak goose is very small. Geometric relationship among the Suchovska and Slovak breeds, presented by PCA analysis, also showed close relationship between breeds. The positions of individuals are widely dispersed, where some individuals from both breeds are clustered in one mixed group. On the other hand, some individuals of Suchovska and Slovak goose made a relatively independent cluster.

Although, Suchovska and Slovak goose are phenotypically different (wildly coloured yellow, heavy Suchovska vs. white, medium weight Slovak), between breeds the diversity is considerably lower. Close relationship among the populations had an obvious association with their historical relations and geographical distribution (Li et al., 2007). The majority of goose breeds in Europe originated from *Anser anser* (Tu et al., 2006). In addition, Suchovska and Slovak shared a common local indigenous ancestry. On the other hand, the high number of private (breed-specific) alleles occurring over all estimated groups of breeds proved genetic uniqueness of Suchovska and Slovak breeds.

The application of populaltion genetics to breed conservation essentially encompasses two different components: identifying high breed genetic diversity (measured by average number of alleles, expected heterozygosity) and high breed genetic uniqueness (measured by F_{ST}, genetic distance). These tools can be used to identify genetically robust breeds as well as those of potential conservation concern. Genetically vulnerable breeds are those that possess both low genetic diversity and uniqueness (Wilkinson et al., 2012). Suchovska and Slovak breeds satisfied these criteria. These results coupled with the low population size confirm that these breeds are critically endangered, where consideration is required for the conservation and preservation of Suchovska and Slovak breeds. In addition, these breeds are carriers of the gene pool of the indigenous local yellow fathering geese that no longer exist.

Conclusions

This work is the preliminary study of genetic diversity and structure between Suchovska and Slovak goose base on microsatellite loci analysis. For the future, a higher number of tested microsatellite markers, as a suitable tool for genetic diversity study, and comparison of genetic diversity with other breeds, that participated on breeding process, are necessary.

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