

Genetic diversity of Tesedik Goose

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Genetic diversity study as a tool for knowledge of the within- and among-breed diversity is particularly important in the case of endangered breeds as well as for reconstructing the history of livestock. The characterization of the genetic diversity and structure were assessed in Tesedik Goose based on 6 microsatellite (Aalμ1, Bcaμ1, CKW21, TTUCG5, Ans2, Ans25) loci analysis in a total population of 50 geese. A total of 28 alleles were found across population with a mean number of 4.67 alleles per locus. An average allele frequency was 0.67 per locus. The mean observed heterozygosity was 0.43. The degree of inbreeding calculated as a mean FIS was 0.07.

Keywords: genetic diversity, microsatellite, goose, geese

1. Introduction

During the last decades, development of and increased focus on more efficient selection programmes have accelerated genetic improvement in a number of breeds. As a result, highly productive breeds have replaced local ones across the world. This development has led to growing concerns about the erosion of genetic resources (Groeneveld et al., 2010). Geese are one of the oldest species of domestic poultry. There are 181 recognize breeds of domestic geese with 158 local populations located 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).

The genetic diversity of animals represented by differences among the individual breed populations is evaluated by many types of genetic markers. 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* (Weiß 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). 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 (Aliczki, 2007) and Zatorska (Andres and Kapkowska, 2011) geese breeds.

The aim of this study was assessing the genetic diversity of Tesedik Goose, based on 6 microsatellite loci analysis.

2. Materials and methods

Tседik Goose was created by crossing of the Slovak, Czech and Hungary geese breeds and recognized in 2002.

Samples for analysis were collected from pedigree breeding in 2005. Blood samples from 50 birds (10 gander, 40 goose) were used to isolated genomic DNA followed the protocol of Wizard Genomic DNA Purification kit (Promega). For study of genetic diversity were used microsatellite markers Aalμ1, Bcaμ1, CKW21, TTUCG5, Ans2 and Ans25, specific microsatellite markers of geese. Primers for PCR amplification for loci Ans2 and Ans25 (Weiß et al., 2008) were designed in greylag goose (*Anser anser*), TTUCG5 (Cathey et al., 1998) and Bcaμ1 (Buchholz, 1998) were designed for Canada goose (*Branta canadensis* L.), Aalμ1 (Fields, 1997) and CKW21 (Liu et al., 2006), were designed in swan (*Anser cygnoides* L.) and white fronted (*Anser albifrons*) goose respectively. Six pairs of primers were amplified in one multiplex PCR reaction (AmpliTaqGold). PCR amplification was performed on a thermal cycler MJ Research (annealing 59 °C/60 s, 35 cycles). Amplified PCR products were electrophoresed on sequencer ABI 310 (Applied Biosystems). The size of the analyzed DNA was determined in base pairs using computer package GeneScan v.3.7 (Applied Biosystems).

Genetic diversity within breed was quantified via: the total number of alleles, the number of alleles per locus,

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major allele frequencies, expected heterozygosity (gene diversity), observed heterozygosity and polymorphic information content (PIC) of microsatellite loci (Botstein et al., 1980) were estimated using PoweMarker 3.25 (Liu and Muse, 2005), number of effective alleles was calculated by GenAlEx software (Peakall and Smouse, 2006). Tests for deviations from Hardy-Weinberg equilibrium (HWE) across all loci were performed by estimation of exact P-value by the Markov chain method using GENEPOP 4.2 software (Rousset, 2008). Weir and Cockerham's extension of Wright's within-population inbreeding coefficient (F_{IS}) was calculated by GENEPOP 4.2 software (Rousset, 2008).

3. Results and discussion

The investigated loci were chosen from species-specific microsatellite markers isolated and evaluated in the Anatidae species, in accordance with previously published papers, used in diversity studies of wild ancestor Greylag Goose and some European domestic breeds as Embdem, Hungarian and Zatorska.

Genotyping in total population of 50 individuals for 6 polymorphic microsatellite loci revealed 28 different alleles. The mean number of alleles was 4.67, with the range extending from 2 (Aal μ 1) to 8 (TTUCG5, Table 1). Major allele frequencies ranging from 0.35 (TTUCG5) to 0.92 (Aal μ 1), with an average value 0.67 per locus. Number of effective alleles across all loci range from 1.17(Aal μ 1) to 4.46 (TTUCG5), with a mean number of effective alleles 2.21. The number of observed genotype varied widely from 3 (Aal μ 1) to 17 (TTUCG5), with an average value 8 per locus.

In all estimated loci the observed heterozygosity are similar or lower to their expectation (gene diversity), with an average value 0.45. The lowest and the greatest heterozygosity per locus was 0.12 (Aal μ 1) and 0.74 (TTUCG5) respectively, with an average value 0.43. The estimated PIC ranged from 0.14 (Aal μ 1) to 0.75 (TTUCG5), with a mean PIC value 0.42. In a total population only in Bca μ 1 locus significant deviations from HWE was revealed. This was reflected in the within-population

(F_{IS}) estimated across all loci where Bca μ 1 locus had significant positive F_{IS} value (0.45, $P < 0.001$). Deficiency of homozygotes occurred only in loci CKW21 (-0.04) and Ans25 (-0.01). Mean F_{IS} value in total population was 0.07.

This study was mainly focused on genetic diversity study of Tesedik Goose when diversity study of livestock species is of considerable scientific interest for understanding phenotypic variation (FAO, 2007). Currently, the breed is the unit of species of conservation. However, breeds are also social entities with a role in the national or regional identity, which leaves room for subjective perceptions of their uniqueness. Breed uniqueness is also not immediately obvious from molecular data. These show invariably that most of the variation is shared by the breeds, most of which harbour a considerable part of the total diversity of the species. In other words, most of the genetic diversity is present within a breed and not between breeds (Groeneveld et al., 2010).

All estimated microsatellite loci were polymorphic as was published in previously studies (Aliczki 2007; Weiß et al. 2008; Andres and Kapkowska, 2011), with a varying number of alleles and expect for Aal μ 1 used in diversity study were suitable according to number of detected loci over the population. The number of alleles detected in loci TTUCG5, CKW21, Bca μ 1 was similar as in Zatorska breed (Andres and Kapkowska, 2011). Considerable lower number of detected alleles were in CKW21, Aal μ 1, Ans2 and Ans25 loci, compare to other published papers (Liu et al., 2006; Aliczki 2007; Weiß et al. 2008). The higher number of alleles were detected in locus CKW21 and Ans2 as in the other Slovak breeds as Suchovska and Slovak Goose (Mindek et al., 2014).

The results of the expected heterozygosity (gene diversity) were consistent with that of PIC. The mean expected heterozygosity (gene diversity) across all loci was considerably lower than in Chinese breeds (Tu et al., 2006; Li et al., 2007) and greylag goose (Weiß et al. 2008). The observed heterozygosity over all loci was similar or lower than mean expected. The mean observed heterozygosity in Tesedik Goose was similar

Table 1 Genetic diversity per loci

Locus	No. of obs.	Genotype no.	Major allele frequency	Allele no.	Mean no. of effect. allele	Gene diversity	Heterozygosity	PIC
TTUCG5	50	17	0.35	8	4.46	0.78	0.74	0.75
CKW21	50	8	0.66	5	2.08	0.52	0.56	0.48
Aal μ 1	50	3	0.92	2	1.17	0.15	0.12	0.14
Ans25	50	6	0.51	3	2.53	0.61	0.62	0.53
Ans2	50	8	0.82	6	1.47	0.32	0.32	0.31
Bca μ 1	50	6	0.79	4	1.55	0.36	0.20	0.33
Mean	50	8	0.67	4.67	2.21	0.45	0.43	0.42

as in other European geese (Aliczki et al., 2007; Andres and Kapkowska, 2011; Noreikiene et al., 2012), but higher than in Suchovska and Slovak Goose breeds (Mindek et al., 2014).

4. Conclusions

This work is the study of genetic diversity of Tesedik goose base on microsatellite loci analysis that is important for reconstructing the history of livestock and understanding the genetic relationships between breeds, when Slovak Goose as an endangered national breed served as one of the progenitors of Tesedik goose.

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6. References

- ALICZKI, K. K. (2007) *Examining genetic variability in ancient hungarian goose population with microsatellite*. PhD Diss. Debrecen: University Of Debrecen, Center of Agric. Sci. 150 p.
- ANDRES, K. and KAPKOWSKA, E. (2011) Applicability of anatic and galliform microsatellite markers to the genetic diversity studies of domestic geese (*Anser anser domesticus*): Short report. In *BMC Research Notes*, vol. 4, no. 65, 10 p. DOI: <http://dx.doi.org/10.1186/1756-0500-4-65>.
- BOTSTEIN, D. et al. (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphism. In *American Journal of Human Genetics*, vol. 32, no. 3, pp. 314–331.
- BUCHHOLZ, W. G. et al. (1998) Dinucleotide repeat polymorphisms in waterfowl (family *Anatidae*): characterization of a sexlinked (Z-specific) and 14 autosomal loci. In *Animal Genetics*, vol. 29, no. 4, pp. 323–325.
- CATHEY, J. C., DEWOODY, J. A. And SMITH, L. M. (1998) Microsatellite markers in Canada geese (*Branta canadensis*). In *Journal of Heredity*, vol. 89, no. 2, pp. 173–175. DOI: <http://dx.doi.org/10.1093/jhered/89.2.173>.
- FAO (2007) *The state of the World's Animal Genetic Resources for Food and Agriculture*. FAO: Rome, 38 p.
- FIELDS, R. L. and SCRIBNER, K. T. (1997) Isolation and characterization of novel waterfowl microsatellite loci: cross-species comparisons and research applications. In *Molecular Ecology*, vol. 6, no. 2, pp. 199–202. DOI: <http://dx.doi.org/10.1046/j.1365-294X.1997.d01-123.x>.
- GROENEVELD, L. F. et al. (2010) Genetic diversity in farm animals – review. In *Animal Genetics*, vol. 41, Suppl.1, pp. 6–31. DOI: <http://dx.doi.org/10.1111/j.1365-2052.2010.02038.x>
- LI, H. F. et al. (2007) Evaluation of genetic diversity of Chinese native geese revealed by microsatellite markers. In *World's Poultry Science Journal*, vol. 63, no. 3, pp. 381–390. DOI: <http://dx.doi.org/10.1017/S0043933907001511>.
- LIU, K. and MUSE, S. V. (2005) PowerMarker: Integrated analysis environment for genetic marker data. In *Bioinformatics*, vol. 21, no. 9, pp. 2128–2129. DOI: <http://dx.doi.org/10.1093/bioinformatics/bti282>.
- LIU, S. et al. (2006) Analysis of genetic variations in different goose breeds using microsatellite markers. In *Yi Chuan*, vol. 28, pp. 1389–1395.
- MINDEK, S. et al. (2014). Genetic diversity and structure of Slovak domestic goose breeds. In *Veterinarija ir Zootechnika*, vol. 67, pp. 80–86.
- NOREIKIENE, K. et al. (2012) Isolation and characterization of 55 novel microsatellite markers for the pink-footed goose (*Anser brachyrhynchus*). In *Conservation Genetics Resources*, vol. 2, no. 2, pp. 423–428. DOI: <http://dx.doi.org/10.1007/s12686-011-9565-6>.
- PEAKALL, R. and SMOUSE, P. E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. In *Molecular Ecology Notes*, vol. 6, no. 1, pp. 288–295. DOI: <http://dx.doi.org/10.1111/j.1471-8286.2005.01155.x>.
- ROUSSET, F. (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. In *Molecular Ecology Resources*, vol. 8, no. 1, pp. 103–106. DOI: <http://dx.doi.org/10.1111/j.1471-8286.2007.01931.x>.
- SIMIÄNER, H. (2006) Use of molecular markers and other information for sampling germplasm to create an animal gene bank. In RUANE, J. and SONNINO, A. (eds.) *The role of biotechnology in exploring and protecting agricultural genetic resources*. Rome: FAO, pp. 81–96.
- TU, Y. J. et al. (2006) Genetic diversity of 14 indigenous grey goose breeds in China based on microsatellite markers. In *Asian-Australasian Journal of Animal Sciences*, vol.19, no. 1, pp. 1–6. DOI: <http://dx.doi.org/10.5713/ajas.2006.1>
- WEIR, B. S. (1996) *Genetic Data Analysis II: Methods for Discrete Population Genetic Data*. Sunderland: Sinauer Associates, Inc., 445 p.
- WEIß, B. M. et al. (2008) Isolation and characterization of microsatellite marker loci in the greylag goose (*Anser anser*). In *Molecular Ecology Resources*, vol. 8, no. 6, pp. 1411–1413. DOI: <http://dx.doi.org/10.1111/j.1755-0998.2008.02339.x>.