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Application of microsatellite DNA primers for the analysis of the genetic variability of Lithuanian native goose breeds

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The aim of our study was to assess the use of waterfowl species specific primers in order to detect polymorphism in Lithuanian native geese breeds (Vištinės, Skarulės and Vištinės-Skarulės hybrids – Native Mixed). Also, the White-fronted geese species was investigated for comparison. The microsatellite DNA analysis was carried out using 11 microsatellite primers from which only 4 gave a positive PCR product: Sfimu1 (some wild waterfowl species specific marker), TTUCG-1, TTUCG-2, TTUCG-4 (Canada geese specific marker). According to our data, it is possible to use wild waterfowl specific microsatellite DNA markers for a comparative microsatellite DNA analysis of the White-fronted geese species and 2 Lithuanian breeds (Vištinės and Skarulės), and their hybrids (Native Mixed) were obtained by interbreeding these breeds. We found that the TTUCG-1 primer, due to monomorphic PCR products, was not suitable for the population analysis of Skarulės and the Vištinės geese breeds. Due to the absence of the amplified product, the TTUCG-4 primer is not suitable for hybrid geese microsatellite DNA analysis.

Key words: geese, microsatellite DNA, microsatellite primer

INTRODUCTION

Currently many methods of molecular biology are used to investigate the biological variety. The evaluation of genetic changes is very important in breeding research and organized breeding programs of various domestic birds and animals. The evaluation requires an objective assessment of candidates for selection, the mating design and the way to validate the design through a genetic improvement [1]. Geese are one of the oldest domestic poultry species now grown for commercial purposes. Native fowl breeds take a very important place. These breeds are better adapted to local conditions and have greater possibilities to survive in extreme conditions [2, 3]. The preservation of the local fowl breed genome is one of the priorities in goose breeding. The loss of the genetic variety will eliminate unique genetic traits providing fewer chances for the improvement of goose breeds in the future. To achieve this aim it is essential to perform the DNA research and to create specific markers for a separate breed. Nowadays there is a lack of goose genetic markers, as well as of other molecular tools. The aim of our study was to assess the use of waterfowl species specific primers for detecting polymorphism in Lithuanian native goose breeds (Vištinės, Skarulės and Vištinės-Skarulės hybrids – Native Mixed). Also, White-fronted geese species have been investigated for comparison.

MATERIAL AND METHODS

Venous blood from White-fronted goose species (25 individuals), Vištinės goose breed (25 individuals), Skarulės goose breed (20 individuals) and hybrid geese (20 individuals) was collected. For the microsatellite DNA analysis blood samples (2 ml) were collected in EDTA tubes and frozen at –20 ˚C till used. DNA was extracted from blood by the method described by Aljanabi and Martinez [4] using an additional chloroform extraction step and LiCl instead of NaCl [5], dissolved in water and stored at –20 °C.

The microsatellite DNA analysis was carried out using 11 microsatellite primers from which only 4 gave a positive PCR product (Table 1): Sfimu1 [6], TTUCG-1, TTUCG-2, TTUCG-4 [7]. No PCR product was detected using Aalmu1, Aalmu2, Sfimu4, Sfimu5, APH13, TTUCG-3 and TTUCG-5 primers. PCR with the Canada geese specific primers TTUCG-1, TTUCG-2, and TTUCG-4 were carried

Table 1. **Data of primer identification**

Primer	Forward	Reverse	Gene Bank ID
Sfimu1	CAC AAG GAA GCA TGA CCT CAG AA	CTC ATG CCT CCT GTT AGT CAT CT	U63681
TTUCG-1	CCC TGC TGG TAT ACC TGA	GTG TCT ACA CAA CAG C	U66089
TTUCG-2	GAG AGC GTT ACT CAG CAA A	TCA CTC TGA GCT GCT ACA ACA	U66090
TTUCG-4	GGT GTA CTC TGC TGA GTG TC	TTA GAA CTA GTG GAT CTC TC	U66092

Table 2. **Number and range of DNA fragment length (bp) of microsatellite DNA fragments amplified with various microsatellite primers**

Marker	Species / Breeds and hybrids	Range of DNA fragments lenght (bp)	Number of DNA fragments
Sfimu1	White-fronted	190–320	4
	Vištinės	190–320	4
	Skarulės	190–320	4
	Native Mixed	190–320	4
TTUCG-1	White-fronted	30–170	4
	Vištinės	30	1
	Skarulės	30–110	2
	Native Mixed	30–110	2
TTUCG-2	White-fronted	50–150	4
	Vištinės	50–240	7
	Skarulės	50–130	4
	Native Mixed	50–130	3
TTUCG-4	White-fronted	45–60	3
	Vištinės	35–360	9
	Skarulės	45–160	4
	Native Mixed	Not amplified	Not amplified

out according to Cathey et al. [7] with an additional prolonged elongation phase. PCR with some wild waterfowl species specific primer Sfimu-1 was carried out using the touch-down technique [8] with the annealing temperature changing within the range 68–53 °C. The statistical analysis was made using Mega 3.1 software [9]. Using this computer program the computation of genetic distance (p-distance) [10] was made. This distance is the proportion (p) of DNA fragments at which two DNA fragments being compared are different. It is obtained by dividing the number of DNA fragment differences by the total number of DNA fragments compared.

RESULTS

Electrophoretic gels of the amplified PCR products obtained by using the TTUCG-1 primer are presented in Fig. 1, and electrophoretic gels of the amplified PCR products obtained by using TTUCG-2 primer are shown in Fig. 2. The Sfimu1 primer was monomorphic in all investigated species and breeds with the DNA fragment number 4 (Table 2). The largest number of DNA fragments (9 bands) was detected in the Vištinės breed geese obtained with the TTUCG-4 primer (DNA fraction length range 35–360 bp), and the smallest number was obtained in Vištinės geese with the TTUCG-1 primer (1 band with 30 bp fraction). There was no PCR product detected after amplification with the TTUCG-4 primer in the hybrids (Native Mixed). Monomorphic DNA fragments were detected only in the Vištinės and Skarulės breeds with the TTUCG-1 primer with the length range 30 and 30–110, respectively.

In order to determine the genetic variability among the geese species and breeds studied, the genetic distance (p-distance) was calculated (Table 3). The largest genetic distance was detected between the White-fronted and Vištinės geese after amplification with the TTUCG-1 primer (0.438). The White-fronted geese have the largest genetic distance with the Vištinės breed as compared with the Skarulės breed and Native Mixed geese. The smallest genetic distance was detected between the Native Mixed geese and the Skarulės breed (TTUCG-1 – 0.167 and TTUCG-2 – 0.138). The genetic distance between the Vištinės and hybrids was greater after amplification with the TTUCG-1 primer (0.417) than with the TTUCG-2 primer (0.295).

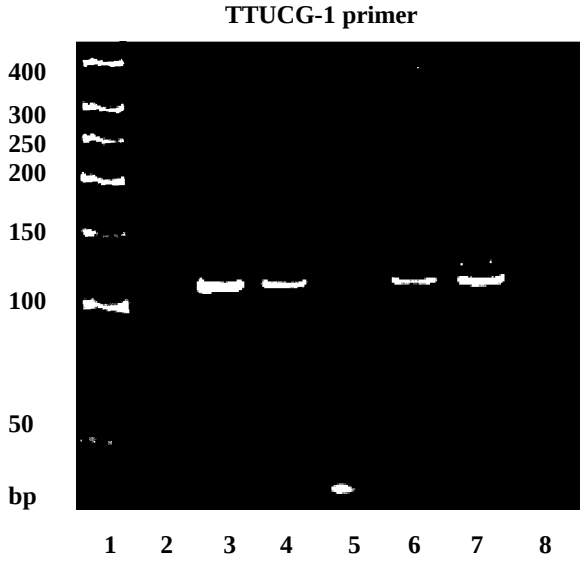

Fig. 1. Electrophoretic gel after amplification with TTUCG-1 primer: molecular weight marker – 1; White-fronted goose – 7, 8; Skarulės – 5; Native Mixed – 3, 4, 6

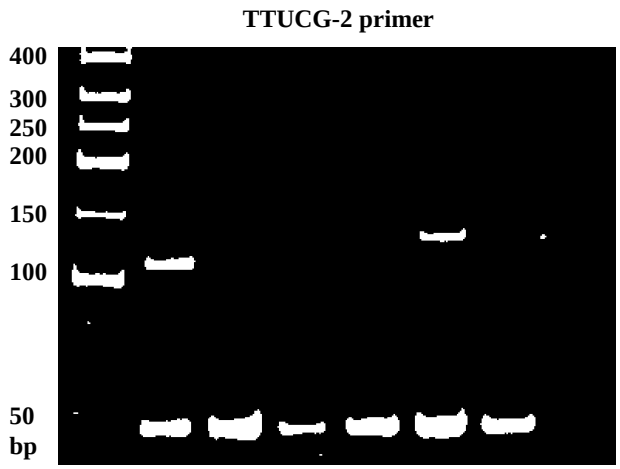

Fig. 2. Electrophoretic gel after amplification with TTUCG-2 primer: molecular weight marker – 1; White-fronted goose – 2; Skarulės – 4, 7; Vištinės – 3, 5, 6, 8

DISCUSSION

The increasing number of available markers provided a powerful tool for a better understanding of the population structure related to the species and breeds of geese. Detection of the genetic variability among and within the species and breeds is very important. Breeds or species that lack a genetic variation may have a reduced fertility and fitness due to inbreeding depression.

According to our data, it is possible to use wild waterfowl specific microsatellite DNA markers for a comparative microsatellite DNA analysis of the White-fronted goose species, two Lithuanian breeds (Vištinės and Skarulės) and the Native Mixed geese. Since we did not detect any PCR product in the hybrids after amplification with the TTUCG-4 primer, we can conclude that the Native Mixed geese have a unique microsatellite DNA allele set and this primer is not suitable for the microsatellite DNA analysis of the hybrids. We detected that the TTUCG-1 primer, due to monomorphic PCR products, is not suitable for the

Table 3. **Genetic distance (p-distance) among investigated geese species, domestic breeds and hybrids after amplification with 3 primers**

		TTUCG-1		
Species / Breed and hybrids	White-fronted	Vištinės	Skarulės	Native Mixed
White-fronted	0			
Vištinės	0.438	0		
Skarulės	0.313	0.250	0	
Native Mixed	0.396	0.417	0.167	0
		TTUCG-2		
Species / Breed and hybrids	White-fronted	Vištinės	Skarulės	Native Mixed
White-fronted	0			
Vištinės	0.238	0		
Skarulės	0.225	0.262	0	
Native Mixed	0.178	0.295	0.138	0
		TTUCG-4		
Species / Breed and hybrids	White-fronted	Vištinės	Skarulės	Native Mixed
White-fronted	0			
Vištinės	0.367	0		
Skarulės	0.278	0.333	0	
Native Mixed	–	–	–	–

population analysis of the Skarulės and Vištinės goose breeds.

On the basis of the genetic distance analysis we conclude that wild White-fronted goose are more distant than Lithuanian geese breeds among themselves. The greatest genetic similarity exists between the Native Mixed geese and the Skarulės breed, and the Vištinės breed is more distant from them. Despite the fact that the genetic markers detected by us have an unknown function and most probably have no influence on trait violation, they can be used in combining information from a number of samples and characterization of a breed or species.

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References

1. Slavėnaitė S, Butkauskas D, Sruoga A, Mozalienė E. Veterinarija ir Zootechnika 2004; 26(48): 89–92.
2. Mačiulaitis A. Žąsys. Kaunas, Ūkininko patarėjas. 2001: 2–34.
3. Juknevičius St, Pėtelis K, Žilinskienė A. Paukščiai netradiciniuose ūkiuose. III dalis. Vandens paukščiai. Kaunas, Lietuvos žemės ūkio universitetas. 2004: 2–80.
4. Aljanabi SM, Martinez I. Nucleic Acids Research 1997; 25(22): 4692–3.
5. Gemmell NJ. TIG (Technical tips) 1996; 12(9): 338–9.
6. Fields RL, Scribner KT. Molecular Ecology 1997; 6: 199–202.
7. Cathey JC, Dewoody JA, Smith LM. Journal of Heredity 1998; 89: 173–5.
8. McPherson MJ, Moller SG. PCR. UK, The Cromwell Press, Trowbridge, 2000: 80–90.
9. Kumar S, Tamura K, Nei M. Briefings in Bioinformatics 2004; 5: 150–63.
10. Nei M, Kumar S. Molecular Evolution and Phylogenetics. Oxford University Press, New York. 2000: 32–3.

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MIKROSATELITINIŲ PRADMENŲ PANAUDOJIMAS LIETUVIŠKŲ VIETINIŲ ŽĄSŲ VEISLIŲ GENETINĖS ĮVAIROVĖS ANALIZEI

Santrauka

Darbo tikslas buvo įvertinti specifinių mikrosatelitinių pradmenų panaudojimo laukiniams vandens paukščiams galimybę, siekiant nustatyti lietuviškų vietinių žąsų veislių (Vištinių ir Skarulių) bei jų hibridų (Vietinių mišrūnų) genetinę įvairovę. Palyginimui į analizę buvo įtraukta ir laukinių baltakakčių žąsų rūšis. Mikrosatelitinės DNR analizė buvo atlikta panaudojant 11 mikrosatelitinių pradmenų, iš kurių keturi buvo informatyvūs: Sfimu1 (kai kurioms laukinėms vandens paukščių rūšims specifinis pradmuo), TTUCG-1, TTUCG-2 ir TTUCG-4 (Kanadinių berniklių rūšiai specifinis pradmuo). Buvo nustatyta, jog tirtus laukinių vandens paukščių pradmenis galima naudoti baltakakčių žąsų rūšies bei lietuviškų vietinių žąsų veislių (Vištinių ir Skarulių), taip pat hibridų mikrosatelitinės DNR analizei. Taip pat nustatyta kad, dėl PGR produkto monomorfiškumo TTUCG-1 pradmuo netinka viduveislinei Skarulių ir Vištinių žąsų mikrosatelitinės DNR analizei. Nustatyta, kad nesant amplifikacijos produkto TTUCG-4 pradmuo netinka Vietinių mišrūnų mikrosatelitinės DNR analizei.