Genetic variability of the subfamily *Anserinae* defined by RAPD analysis

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The interspecific genetic variability of the subfamily *Anserinae* was investigated. To this end, random amplified polymorphic DNA (RAPD) analysis was performed. Ten random primers were used for RAPD analysis. Seven species (*Anser albifrons*, *Branta canadensis*, *Anser anser*, *Anser fabalis*, *Anser erythropus*, *Cygnus cygnus*, *Cygnus olor*) were investigated. Cluster data analysis showed that all species could be separates into two big groups, one of them containing species of the genus *Cygnus*. In the other group White-fronted goose clearly stand out from the other species. This can be explained by a high intraspecific genetic variability of this species. The data of the DNA fragment analysis showed what Ol-5 (studied best) can be used as a specific genetic marker for White-fronted goose (*Anser albifrons*), Ol-2, Ol-3, Ol-6, Ol-7, Ol-9, Ol-11 and Ol-12 for Mute Swan (*Cygnus olor*), Ol-6 (studied best) for Whooper Swan (*Cygnus cygnus*).

Key words: DNA polymorphism, RAPD, *Anserinae*, geese

INTRODUCTION

Certain Lithuanian areas located on the East Atlantic flyway are very important for migratory populations of swans and geese [1]. Very marked changes in the number and distribution of most species of swans and geese have been recorded in recent years [1]. White-fronted Goose is the dominant goose species in Lithuania during migration period. The identification of some *Anserinae* species is problematic. The White-fronted Goose (*A. albiftons*) and Lesser White-fronted Goose (*A. erythropus*) are morphologically very similar. *A. erythropus* is a rare species in Lithuania. There is a need to study the relationship among the species of the subfamily *Anserinae* and to find most informative molecular markers. The only species that has markedly decreased in numbers during the last decades is the Bean Goose (*Anser fabalis*). There is a suggestion that these changes can be caused by a decrease of populations wintering in Central Europe.

Many genetic variation detection methods refer to changes within a specific, deliberately targeted segment of DNA. An alternative method of detecting a variation specific to PCR is to detect the presence or absence of randomly amplified polymorphic DNAs (RAPD) [2]. Even random primers anneal with some probability in any given genome, and by screening a large number of primer pairs it is possible, by chance, to find some that produce useful products [3]. It provides a very useful tool for genome analysis in population studies, where individual isolates can be compared rapidly [4].

In this study we examined and evaluated the genetic variability and genetic distances between seven species of the subfamily *Anserinae.*

MATERIALS AND METHODS

For DNA analysis venous blood was collected from 50 birds. Blood samples (400–500 μl) were collected in

Table 1. **Composition of primers and number of amplification products**

Primer	Sequence (5^{l} to 3^{l})
ol-2	CTACGAGACT
ol-3	CTCACCCGTC
ol-4	CAATCGCCGT
ol-5	CAAACGTCGG
ol-6	GTCCACACGG
ol-7	ACGCCGTACG
ol-8	ACGTCGAGCA
ol-9	TCCGCTCTGG
ol-11	GTGAGGCGTC
ol-12	GATGACCGCC

heparin tubes and frozen at –20 °C till use. DNA was extracted from blood by the method described by Miller et al. with an additional chloroform extraction step [5], dissolved in water and stored at –20 °C.

Ten primers each of 10 nucleotides (Shanghai Sangon Ltd., China) were used for amplification (Table 1). The PCR and electrophoresis were performed as described by Sruoga et al. [6]. The gels were photographed and saved by the Gel Doc 1000 computer video system (Bio Rad, Germany). Analysis was performed using TotalLab v.1.10 (Nonlinear Dynamics Limited, England) software. DNA fragment sizes were assessed by comparison with GeneRuler™ 100 bp DNA Lader Plus (MBI Fermentas, Lithuania).

The level of genetic similarity among the species was calculated as follows:

$D = 2N_{AB} / (N_A + N_B)$, where N_A and N_B are the total number of fragments in individuals A and B, N_{AB} is the number of DNA fragments common for individuals A and B [7].

RESULTS AND DISCUSSION

After amplified DNA product analysis with all 10 primers we found that the average number of DNA fragments was biggest in *Anser albifrons* (26.4), possibly because of a big number of geese migrating through Lithuania from different populations and breeding grounds. The smallest average number of amplified DNA fragments was detected in *Cygnus cygnus* (9.1), the average range of other species DNA fragments varied from 10.2 to 11.3. Besides, the biggest amplified DNA product size range was also detected in *Anser albifrons* (130–2960).

A genetic similarity analysis (Table 2) showed the greatest similarity between *Anser fabalis* and *Anser erythropus* (0.8799), the lowest between *Cygnus olor* and *Anser anser* (0.3104). According to our data, *Anser erythropus* has the greatest genetic similarity with all other species studied, *Anser albifrons* having the least. It can be explained by a large *Anser albifrons* sample number and high intraspecific genetic variability. *Anser albifrons* phenotypically is very similar to *Anser erythropus*; our data confirm this fact because *Anser albifrons* has the greatest genetic similarity with *Anser erythropus* (0.5429) but a different polymorphism which was also investigated by Kholodova et al. [8]. Individuals of the genus *Cygnus* also stand out from the others by a low genetic similarity and also differ from each other (0.4499). This fact also confirms the phenotypical data, because *Cygnus olor* in Lithuania is a breeding species, whereas *Cygnus cygnus* is a migrating one (rarely bred, and in our study only migrating swan samples were used).

Data of the DNA fragment analysis showed that all 10 primers can be used to identify *Anser albifrons*, but Ol-5 gives the best divergence from other species. Ol-2, Ol-3, Ol-6, Ol-7, Ol-9, Ol-11 and Ol-12 primers can be used as specific markers for Mute Swan (*Cygnus olor*) and Ol-7, Ol-11, Ol-6 (studied best) for Whooper Swan (*Cygnus cygnus*).

Based on genetic similarity data, the dendrogram was built applying a cluster analysis method (Figu-

Table 2. **Average genetic similarity of 7 species from the subfamily *Anserinae***

Specie	*A. albifroons*	*B. canadiensis*	*A. anser*	*A. erythropus*	*A. fabalis*	*C. olor*	*C. cygnus*
A. albifroons	1	0.5082	0.5268	0.5429	0.5132	0.3396	0.3526
B. canadiensis		1	0.8391	0.8126	0.7949	0.4304	0.3512
A. anser			1	0.8057	0.7541	0.4403	0.3104
A. erythropus				1	0.8799	0.5067	0.3691
A. fabalis					1	0.4312	0.3252
C. olor						1	0.4499
C. cygnus							1

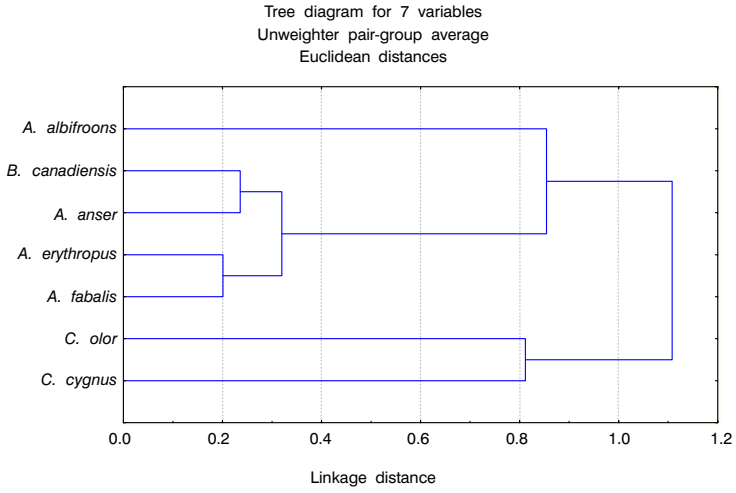

Figure. Dendrogram of the genetic distances in 7 species from the subfamily *Anserinae*

re). All species in the dendrogram are divided into two large groups, in one of them divides genera *Cygnus* species. *Anser albifrons* clearly differs from the others because of its great intraspecific genetic variability (our experimental data not published yet). *BrantaI* individuals are in one cluster with *Anser anser.* It is possible that *Branta canadensis* DNA fragments have an other nucleotide substitution, only the fragment size (bp) is similar. However, to prove it hybridization or sequencing must be performed.

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ŽĄSINIŲ POŠEIMIO RŪŠIŲ GENETINIO PANAŠUMO ANALIZĖ

S a n t r a u k a

Buvo atlikta 7-ių žąsinių pošeimio rūšių (baltakaktės žąsies (*Anser albifrons*), kanadinės berniklės (*Branta canadensis*), pilkosios žąsies (*Anser anser*), želmeninės žąsies (*Anser fabalis*), mažosios žąsies (*Anser erythropus*), gulbės giesminin-kės (*Cygnus cygnus*), gulbės nebylės (*Cygnus olor*)) atsitiktinai amplifikuotos polimorfinės DNR analizė (AAPD). Tam buvo panaudota 10 skirtingų kompozicijų atsitiktinių pradmenų. Remiantis tarprūšinės AAPD analizės genetinio panašumo duomenimis, buvo atlikta klasterinė analizė ir sudarytos dendrogramos. Visos tirtos rūšys dendrogramoje pasiskirsto į dvi dideles grupes; vienoje iš jų atsiskiria gulbių genties atstovai. Baltakaktės žąsys aiškiai išsiskiria iš kitų tirtų rūšių ir tai galima paaiškinti didele jų vidurūšine įvairove. Berniklių genties atstovės, kanadinės berniklės dendrogramoje neatsiskiria ir patenka į vieną klasterį su pilkosiomis žąsimis. Nustatyta, kad baltakakčių žąsų rūšiai specifiniu genetiniu žymeniu geriausiai tinka Ol-5 pradmuo. Ol-2, Ol-3, Ol-6, Ol-7, Ol-9, Ol-11, Ol-12 pradmenys yra specifiniai gulbių nebylių rūšiai, Ol-6 pradmuo – labiausiai specifinis gulbių giesmininkių rūšiai.