Intraspecific genetic variability of white-fronted geese *(Anser albifrons)* in springtime migration

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The intraspecific genetic variability of white-fronted geese *(Anser albifrons)* was investigated by applying random amplified polymorphic DNA (RAPD) analysis and protein disk-electrophoresis were performed. Disk-electrophoresis was carried out in a two layer vertical block of polyacrilamide slides (2.5 / 7.5%, 5%). Ten random primers were used for RAPD analysis. Fifteen enzyme systems were investigated and 20 loci were detected, from which 18 were polymorphic. Intraspecific isoensyme analysis showed that genetic identity between two white-fronted goose flocks (1999 March and 2000 April) is only 0.6984, suggesting that populations from different breeding grounds migrate throw Lithuania on different days of a month. Intraspecific RAPD analysis showed what genetic identity between three white-fronted goose flocks, as in isoensyme analysis, depended on the migration date.

Key words: white-fronted goose, RAPD, isoenzymes, polymorphism

INTRODUCTION

White-fronted goose (*Anser albifrons*) is a holarctic goose breeding at high latitudes in northern Europe, Asia and North America, and wintering south to Mexico, southern Europe, the Middle East, China and Japan (Scott and Rose, 1996). Lithuania is located on two white-fronted goose flyways: East Atlantic and Central Europe. Certain Lithuanian areas, located on the East Atlantic flyway are very important for migratory populations of geese (Švažas et al., 1997). The most important Lithuanian staging areas with abundantly available food and free from disturbing factors allow the birds to accumulate rapidly reserves of fat and protein to continue their flight and contribute to egg production, as well as provide energy for incubating bird in early spring when food availability in the breeding grounds of arctic populations is poor. In Lithuania, the numbers of White-fronted geese have enormously increased other the last decades (from almost zero in 1950–1960 up to about 50,000 individuals in 1997). Presently, white-fronted goose is the dominant goose species in Lithuania during a migration period. The total population wintering in NW Europe has increased (Scott and Rose, 1996). There is a suggestion what such an increase is caused by a decrease of wintering populations in Central Europe.

In the present study we examined and evaluated the genetic variability and genetic distances between two flocks of White-fronted geese *(Anser albifrons)* in protein polymorphism and three flocks in RAPD analysis. These flocks migrated through Lithuania in different years and different days of the year.

MATERIALS AND METHODS

Multilocus enzyme analysis. By the protein electrophoresis method we analysed 42 individuals of White-fronted Goose. Samples were obtained during research expeditions organized by the Vytautas Magnus University and Institute of Ecology in Šilutė region in March 23–30 1999 and on April 18 in 2000.

White-fronted goose liver homogenate was used as a study material for protein electrophoresis. Samples were homogenized and stored at –20 °C until use.

Disk-electrophoresis was carried out in a two-layer vertical block of polyacrylamide slides following the methods suggested by Brewer (Brewer, 1970), Orstein (Orstein, 1964) and Murphy (Murphy et al., 1996) with some modifications to increase separation. The acrylamide concentration in gels was 2.5 / 7.5%, 5%, Electrophoresis was performed using gel and electrode Tris-glycine, pH 8.3

(Davis, 1964) and Tris-borate-EDTA (Murphy et al., 1996) buffers. Proteins were stained with Coomassie Blue G-250, the enzymes were stained according to the commonly, accepted methods with some modifications (Короткин и др., 1977; Haris and Hopkinson, 1978; Murphy et al., 1996).

Location of the protein fractions in the electrophoregram was evaluated by the relative electrophoretical mobility Rm, in relation to the migration band of bromphenol blue unit. Genetic interpretation, considering the high evolutionary conservativeness of the quaternary structure of protein, was based on the data of other groups of animals for which genetical determination of analogous markers is known (Короткин и др., 1977). The following factors were used as the parameters of biochemical polymorphism: a) gene frequency, genotype frequency, b) observed (H_o) and expected (H_e) heterozygosity of loci, c) average value of heterozygosity ($-H_o$ and $-H_e$), d) heterozygote deficiency (H_D), e) alleles per locus. Genetic similarity and distance were evaluated according to Nei (1972) and the modified Rogers distance (Wright, 1978). All parameters and distances were calculated using Biosys-1 (Swafford et al., 1981), Biosys-2 and PopGene 1.32.

By electrophoresis 15 protein systems were investigated: macroglobulin (Mc), posttransferin (Ptf), transferin (Tf), pretransferin (Prtf), postalbumin (Pa), albumin (Al), prealbumin (Pr), lactate dehydrogenase (Ldh), malate dehydrogenase (Mdh), esterase (Est), a-glycerophosphate dehydrogenase (a-Gpd), xanthine dehydrogenase (Xdh), glucose-6-phosphatedehydrogenase (G-6-pdg), malicenzyme (Me) and superoxide dismutase (Sod).

Random amplified polymorphic DNA analysis. For DNA analysis venous blood was collected from 50 birds. Samples were obtained during Institute of Ecology research expeditions in Šilutė region on March 23 in 1995, April 12 in 1997 and April 7 in 1998. Blood samples (400–500 μl) were collected in heparin tubes and frozen at –20 °C till use. DNA was extracted from blood by the method described by Miller et al. (1998) with an additional chloroform extraction step (Miller et al., 1998), dissolved in water and stored at –20 °C.

Ten primers, each of 10 nucleotids pairs (Shanghai Sangon Ltd., China) were used for amplification (Table 1). The PCR and electrophoresis were performed as described by Sruoga et al. (1997). The gels were photographed and saved by the Gel Doc 1000 (Bio Rad, Germany) computer video system. 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).

Table 1. **Composition of primers, number and size (bp) of amplification products**

Primer	Sequence (5^l to 3^l)	Number of band range	Size range
ol-2	CTACGAGACT	15–20	150–2730
ol-3	CTCACCCGTC	20–27	150–2960
ol-4	CAATCGCCGT	19–22	160–2730
ol-5	CAAACGTCGG	15–21	290–2910
ol-6	GTCCACACGG	18–24	220–2640
ol-7	ACGCCGTACG	19–21	170–1860
ol-8	ACGTCGAGCA	19–25	170–2350
ol-9	TCCGCTCTGG	21–23	170–2580
ol-11	GTGAGGCGTC	16–22	200–2590
ol-12	GATGACCGCC	20–23	130–2900

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

$D = 2N_{AB}/(N_A + N_B)$, where N_A and N_B are the total number of fragments possessed by individuals A and B, N_{AB} is the number of DNA fragments common between individuals A and B (Wetton et al., 1987).

RESULTS AND DISCUSSION

Multilocus enzyme analysis. Fifteen enzyme systems were investigated and 20 loci were detected, from which 2 (Sod and Me-1) were monomorphic (Table 2). By White-fronted Goose gene frequency analysis we found that in the 1999 flock it varied from 0.801 (Me-2 A allele) to 0.8421 (Mdh-1 B allele) and in the 2000 flock from 0.100 (Mc B allele) to 0.900 (Mc A allele). In the 1999 flock the G-6-pdg-3 locus no heterozygotes were detected, possibly owing to selection against heterozygotes. Besides, we did not detect the G-6-pdg-2 and G-6-pdg-3 loci in the 2000 population. The highest heterozygosity in the 1999 and 2000 flocks was observed in Pr-2 loci (0.909 and 0.941, respectively). Heterozygosity was also high in the 1999 flock's Tf locus (0.909); also in the 2000 flock the heterozygosity of this locus was high (0.765). The average heterozygosity was higher than expected, possibly because of direct selection or a small sample size. A great deviation from the Hardy–Weinburg law (except G-6-pdg-3 loci in the 1999 flock) was detected in Xdh locus – $H_D = -0.521$ (1999 flock) and in G-6-pdg-1 locus – $H_D = -0.521$ (2000 flock). Such a deficiency can be explained by selection against heterozygotes, inbreeding or Wahlund effect. We have no information on the possibility of the two latter reasons, so we suggest selection against heterozygotes. A great heterozygote excess was found

Table 2. **Genetic variability between two white-fronted (Anser albifrons) goose flocks. Ho – observed heterozygosity, He – expected heterozygosity, HD – heterozygosity deficience**

Locus	Flock of 1999					Flock of 2000				
	Alleles per locus	Ho	He	HD	χ^2	Alleles per locus	Ho	He	HD	χ^2
Ldh	2	0.290	0.448	–0.352	4.240	2	0.588	0.415	0.417	2.609
a - Gpd	2	0.500	0.480	0.042	0.005	–	–	–	–	–
Xdh	2	0.227	0.474	–0.521	6.548	–	–	–	–	–
G-6-pdg - 1	2	0.375	0.469	–0.200	0.872	2	0.214	0.437	–0.509	4.276
G-6-pdg - 2	2	0.462	0.497	–0.071	0.162	–	–	–	–	–
G-6-pdg - 3	2	0.000	0.459	–1.000	15.294	–	–	–	–	–
Mdh - 1	2	0.316	0.266	0.188	0.544	2	0.462	0.497	–0.071	0.162
Mdh - 2	2	0.762	0.490	0.556	5.941	2	0.462	0.473	–0.025	0.056
Me - 2	5	0.645	0.733	–0.120	10.152	5	0.529	0.725	–0.270	13.221
Est	3	0.741	0.621	0.193	2.293	3	0.765	0.614	0.245	7.739
Mc	2	0.364	0.397	–0.083	0.200	2	0.200	0.180	0.111	0.059
Ptf	2	0.455	0.351	0.294	0.735	2	0.667	0.444	0.500	0.333
Tf	4	0.909	0.607	0.497	7.518	4	0.765	0.645	0.185	5.237
Prtf	2	0.455	0.434	0.048	0.000	2	0.471	0.415	0.133	0.186
Pa	2	0.455	0.434	0.048	0.000	2	0.412	0.500	–0.177	0.728
Alb	2	0.546	0.463	0.179	0.192	2	0.588	0.498	0.181	0.387
Pr - 1	2	0.455	0.500	–0.091	0.212	2	0.118	0.457	–0.742	10.286
Pr - 2	2	0.909	0.496	0.833	6.818	2	0.941	0.498	0.889	12.549
Average	2.333	0.443	0.431	0.029	1.406	2.429	0.513	0.486	0.056	1.098

in Pr-2 locus in both flocks (1999 – 0.833, 2000 – 0.889). Deviation from the Hardy–Weinburg law shows the unstable state of natural population. However, the average heterozygote deficiency does not deviate from the Hardy–Weinburg equilibrium (1999 – 0.029, 2000 – 0.056).

Intraspecific genetic similarity analysis according to Nei (1978) showed that the genetic identity between two white-fronted goose flocks (March 1999 and April 2000) was only 0.6984. It is possible that the geese whose breeding grounds are farther to North migrate through Lithuania earlier, *i. e*. that populations from different breeding grounds migrate through Lithuania on different days of the month. The sampling dates differ by a month, so there is quite a lot of time for completely different populations to migrate.

Random amplified polymorphic DNA analysis. In similarity analysis of three white-fronted goose flocks (1995, 1997 and 1998) the genetic distances in different primers differ, but the average genetic distances is rather great (Table 3). From third table we can see the greatest genetic identity is between the 1997 and 1998 flocks and the largest genetic distance between the 1995 and 1997 flocks.

The obtained data were processed by cluster analysis and the diagrams were drawn (Figure). The figure shows that the 1997 and 1998 flock belong to the same cluster. The 1995 flock was migrating through Lithuania two weeks earlier than the 1998 flock and 3 weeks earlier than the 1997 flock. Distances in the diagram show a correspondence with migration dates. In RAPD analysis, as in isoensyme analysis, we hypothesize that the genetic identity among the three white-fronted goose flocks depends on the migration date. There is a suggestion that a

Table 3. **Average genetic similarity among three white-fronted goose flocks. BZ95 – flock of 1995, BZ97 – flock of 1997, BZ98 – flock of 1998**

Flock	BZ95	BZ97	BZ98
BZ95	1	0.8232	0.8257
BZ97		1	0.8348
BZ98			1

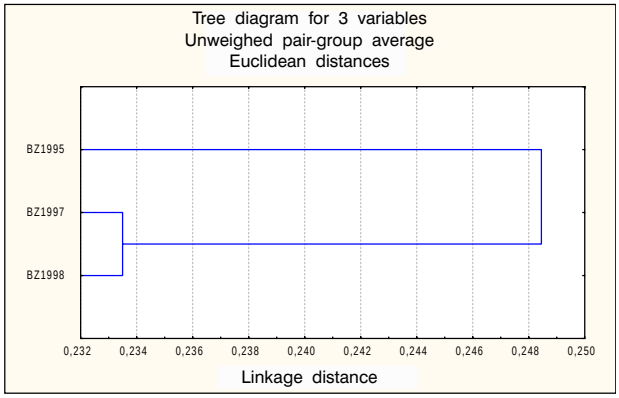

Figure. Dendrogram of the genetic distances in three white-fronted goose flocks. BZ – white-fronted goose flock (depending on the year)

higher number of White-fronted geese wintering in NW Europe is related to a decrease of populations wintering in Central Europe.

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BALTAKAKČIŲ ŽĄSŲ *(ANSER ALBIFRONS)* VIDURŪŠINĖ GENETI NĖ ĮVAIROVĖ PAVASARINĖS MIGRACIJOS METU

S a n t r a u k a

Per Lietuvą migruojančių baltakakčių žąsų skaičius labai stipriai išaugo (nuo beveik nulio 1950-1960 m. iki 50000 1997 m.). Mūsų tyrimuose buvo įvertinta skirtingais metais ir skirtingomis metų dienomis per Lietuvą migruojančių žąsų genetinė įvairovė pagal baltymų polimorfizmą ir 10 Atsitiktinai Amplifikuotos Polimorfinės DNR (AAPD) pradmenų.

Tiriant baltymų polimorfizmą buvo išanalizuota 15 fermentinių sistemų ir nustatyta 20 lokusų, iš kurių 18 buvo polimorfiniai. Vidurūšinė baltymų polimorfizmo analizė parodė, kad skirtingais metais migruojančių baltakakčių žąsų (1999 m. kovas ir 2000 m. balandis) genetinis panašumas yra tik 0,6984. Tai parodo, kad žąsys iš skirtingų perėjimo vietovių migruoja per Lietuvą skirtingomis metų dienomis. AAPD analizė taip pat parodė labai didelę vidurūšinę baltakakčių žąsų genetinę įvairovę bei priklausomybę nuo migracijos datos.

Raktažodžiai: baltakaktės žąsys, AAPD, izofermentai, polimorfizmas