Genetic differentiation of Common Tern (*Sterna hirundo*) colonies

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For the analysis of genetic variability, samples of Common Tern were taken from five different colonies in Tauragė, Ignalina, Klaipėda, Birštonas and Zarasai districts. Eight polymorphic loci were identified, and their allele frequencies were estimated in the colonies as a result of electrophoretic analysis of enzyme systems (EST, MDH, ME) and a non-specific protein (NP) of liver and heart tissue homogenates, and blood serum. The mean number of alleles per locus in all colonies varied between 2.7 and 2.8. The mean heterozygosity (H_o) was 0.664–0.721. After summarising the allele frequencies in polymorphic loci of liver, heart and blood serum in all the colonies and the calculating coefficients of genetic distance and similarity (Nei 1972), the shortest genetic distance (0.038) was found between the Klaipėda and Tauragė colonies. Their coefficient of genetic similarity was 0.963. The genetic distance (0.112) was greatest between Zarasai and Tauragė colonies. Rather important genetic differences were observed between terns from the Birštonas and Klaipėda colonies (coefficient of genetic similarity was 0.896, while their genetic distance was 0.110). Cluster analysis of the data revealed the Zarasai and Ignalina colonies to form cluster and the Tauragė and Klaipėda colonies to form another one.

Key words: Common Tern (*Sterna hirundo*), electrofophoresis, genetic distance, genetic indentity

INTRODUCTION

A marked genetic variability conditioned by the adaptation of individuals to different living conditions exists in many natural populations. Studies of the genetic variability may provide additional information on changes in the gene pool of natural populations, gene drift, gene exchange among populations, environmental effects on genetic variability [1]. Genetic variability plays an important role in the evolution of populations and their survival in the constantly changing environment [1, 2]. A direct relationship is observed between the genetic variability and environmental heterogeneity. The level of genetic variability increases with increasing environmental variability. Genetic variability is higher in a non-specialised environment, where wild species are described as continental. A specialised environment is inhabited by geographically separated, rare, specialised, isolated populations [3].

It is very important to preserve and evaluate the intra- and inter-specific genetic variability in populations of wild and domestic species. Sudden appearance of diseases in a specific environment or sudden change of environmental conditions poses a serious threat to the survival of homogenous species, since such species lack plasticity.

At present, protein and isozyme genetic polymorphism is one of the widely used criteria for evaluation of genetic variability. Genetic variability of populations of different species of birds, based on protein, antigen, isozyme plymorphic systems, was investigated by a number of authors: in waterfowl [4–6], in Japanese quail [7, 8].

However, studies of intra-specific genetic variability of populations of many bird species have been rather fragmented so far. This also applies to Common Terns, although their biology, behaviour, taxonomy, ecology and morphology have been described in a number of scientific publications [9–12]. The population status of these birds was evaluated by Becker, Sommer [13–15] and others. However, most often only characteristics of populations from certain regions only were investigated, and in studies of inter-population genetic differentiation of Oystercatchers (*Haematopus ostralegus*) molecular DNA markers were used [16]. Therefore, the aim of our study was to evaluate the genetic variability of different colonies of *Sterna* terns and to determine genetic distances between these colonies from different regions of the country.

MATERIALS AND METHODS

Liver and heart tissue and blood serum from five different tern colonies in Lithuania were used in this study: 15 from Zarasai district, 11 from Klaipėda district, 10 from Tauragė district, 11 from Birštonas, and 14 from Ignalina district.

Liver and heart samples were homogenised and the homogenates analysed by polyacrilamide gel electrophoresis (PAAG), following Davis [17] and Brewer [18] with some modifications. The enzymes screened were as follows (isozyme, abbreviation, E. C. number, and the corresponding structural gene loci in parentheses): lactate dehydrogenase (LDH, 1.1.1.1, *Ldh-1*), glucose-6-phosphate dehydrogenase (GPD, 1.1.1.49, *Gpd-1*), non-specific esterase (EST, 3.1.1., *Es-1,-2,-3,-4*), malate dehydrogenase (MDH, 1.1.1.37, *Mdh-1,-2,-3,-4*) and malic enzyme (ME; 1.1.1.40, *Me-1,-2*).

Three enzyme systems and non-specific proteins (NP, *Npr-1, -2, -3, -4, -5*) were resolved using multilayer 5% and 7.5 % PAA gels with Tris-EDTA-borat buffer (pH 8.3–8.4) system. Gels were prepared using a Harris & Hopkinson protocol [19]. The fractionation of isozymes was performed at 110–130 mA (260–380V) depending on a system analysed. Staining of the enzymes was undertaken following Show & Prasad [20]. Allelic variants were resolved by a direct side-by-side comparison of migrating allozymes on the same gels. The genotypes at polymorphic loci were determined in each specimen according to the principles of enzyme electrophoresis [21]. In some individuals, however, genotypes could not be determined for the entire set of loci due to insufficient resolution.

Statistical analysis

The genetic diversity of populations was quantified by the mean number of alleles per locus (A), the proportion of polymorphic loci (P) ($P_{0.95}$; frequency of the most common allele <0.95), the mean observed heterozygosity (H_0) and Hardy–Weinberg expected heterozygosity (H_E) [22] for each locus and averaged over all loci using the BIOSYS- software 2 [23]. Deviations from Hardy–Weinberg equilibrium were tested using chi-square analysis [24] with the significance value assigned by Monte Carlo permutation process with 1000 replicates in the TFPGA computer program. Nei's genetic distance (D) was calculated between all pairs of the populations analysed [25]. A dendrogram based on genetic distance was constructed using the un-weighted pair-group arithmetic average (UPGMA) cluster analysis by TFPGA.

RESULTS AND DISCISSION

After investigation of liver homogenates from different colonies by using three enzymes (EST, MDH, ME) and one non-specific protein (NP) system, eight loci which were polymorphic in all investigated colonies were identified: *Es-1, Es-3, Es-4, Es-7, Mdh-1, Me-1, Np-2*, and *Np-3* (Table). Frequencies of alleles from the loci from different tern colonies indicated that the mean heterozygosity (H_o) varied from 0.621 ± 0.061 in the Zarasai colony to 0.756 ± 0.033 in the Tauragė colony. These values exceed the theoretically estimated heterozygosity (H_{ex}) which varied from 0.533 ± 0.023 in the Ignalina colony to 0.572 ± 0.031 in the Klaipėda colony. Analysis of heterozygote surplus and deficit in different colonies revealed that there was a prominent surplus of heterozygotes in all colonies. Namely, the surplus of AB heterozygotes in *Mdh-1* locus was identified in the Zarasai colony (H_D = = 0.309, p = 0.029), surplus of heterozygotes AB in locus *Es-3* was identified in the Ignalina colony (H_D = 0.909, p = 0.003), surplus of heterozygotes AB and AC in *Mdh-1* locus was identified in the Klaipėda colony (H_D = 0.241, p = 0.0102) surplus of heterozygotes AB in locus *Es-3* was identified in the Tauragė colony (H_D = 0.700, p = 0.056), and surplus of heterozygotes AB in locus *Es-4* was identified in the Birštonas colony (H_D = 0.625, p = = 0.0161). An analogous electrophoretic analysis of heart tissue homogenate, using two enzymes (EST and ME) and non-specific protein systems revealed four polymorphic loci in all colonies: *Es-2*, *Es-3*, *Me-1* and *Np-5.* The mean heterozygosity varied from 0.642 ± 0.083 in the Ignalina colony to 0.734 ± 0.1119 in the Birštonas colony. The latter values exceed the theoretically estimated heterozygosity (H_{ex}) which varied from 0.561 ± 0.72 in Birštonas colony to 0.661 ± 0.015 in the Zarasai colony. Analysis of these polymorphic loci of heart homogenate revealed a statistically significant surplus of heterozygotes in four colonies, and only in the Ignalina colony a surplus of homozygote BB in locus *Es-2* was revealed. A statistically significant surplus of heterozygotes was revealed in the following colonies and loci: in the Zarasai colony – heterozygote AB in locus *Es-2*, in the Klaipėda colony – heterozygote BC in locus *Es-2*, in the Tauragė colony – heterozygote BC in locus *Me-1*, and in the Birštonas colony – heterozygote BC in locus *Np-5*. Electrophoretic analysis of blood serum, using two enzymes (EST and ME) and non-specific proteins, showed that the synthesis of these structures is controlled by five polymorphic loci: *Es-3, Es-4, Me-1, No-3*. According to the allele frequencies and the Hardy–Weinberg distribution, the he-

terozygosity (H_o) varied between 0.571 ± 0.066 in the Birštonas colony to 0.768 ± 0.085 in the Tauragė colony. The theoretically estimated heterozygosity (H_{ex}) was lower and varied between 0.611 ± ± 0.049 in the Birštonas colony to 0.667 ± 0.045 in the Ignalina colony. Similarly to liver and heart homogenates, a significant surplus of heterozygotes was identified in most loci, and only in Common Terns from the Birštonas colony a surplus of homozygote BB in locus *Es-3* was identified.

After summarising the allele frequencies in the polymorphic loci of liver, heart and blood serum, and calculating Nei's coefficients of genetic distance and similarity, the shortest genetic distance (0.038) was observed between the Klaipėda and Tauragė colonies. They are genetically most similar. Their coefficient of genetic similarity is 0.963. The longest genetic distance (0.112) was observed between the Zarasai and Tauragė colonies. Their coefficient of genetic similarity is only 0.826. Rather prominent overall genetic differences were observed between terns from the Birštonas and Klaipėda colonies.

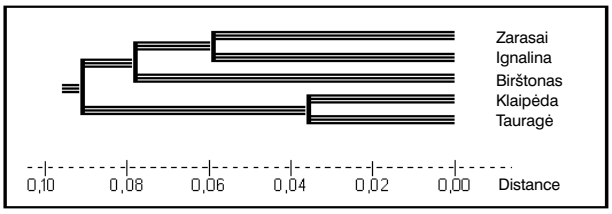

Figure. Clusters of genetic distances (Nei, 1972) of different colonies of Common Tern

Their coefficient of genetic similarity was 0.896, while their genetic distance was 0.110.

Cluster analysis of the obtained data revealed that the Zarasai and Ignalina colonies form one cluster, while the Tauragė and Klaipėda colonies form another one. Cluster analysis showed that the Birštonas tern colony was closer to colonies from the first cluster, i.e. Zarasai and Ignalina (Figure).

Thus, the electrophoretic analysis of enzymes (EST, MDH, ME) and a non-specific protein (NP) of liver and heart tissue, and blood serum allowed to identify eight polymorphic loci, to estimate allele frequencies of these loci, the mean number of alleles per locus, surplus and deficit of heterozygotes in certain loci and colonies. It was established that a surplus of heterozygotes prevailed in most colonies. Therefore it can be stated that intense intra-specific panmixia takes place within the colonies. Finally, genetic differences between the colonies were evaluated by calculating the coefficients of genetic similarity and distance. The values of the coefficients of genetic similarity and distance depend on the colony age and on the geographical distance between the colonies. The larger the distance between colonies, the more marked their genetic differentiation.

Table. **Allele frequencies of eight loci of enzymes and non-specific proteins in five different colonies of Common Tern**

Loci	Alleles	Colonies of Common Tern				
		Zarasai	Ignalina	Klaipėda	Tauragė	Birštonas
ES-1	A	0.143	0.077	0.250	0.150	0.125
	B	0.500	0.500	0.333	0.450	0.563
	C	0.357	0.423	0.417	0.400	0.313
ES-3	A	0.591	0.500	0.583	0.444	0.400
	B	0.409	0.500	0.417	0.556	0.600
	HW	0.554	0.003	1.000	0.056	1.000
ES-4	A	0.625	0.364	0.462	0.500	0.429
	B	0.375	0.636	0.538	0.500	0.571
ES-7	A	0.625	0.722	0.643	0.643	0.688
	B	0.375	0.278	0.357	0.357	0.313
Mdh-1	A	0.400	0.107	0.357	0.318	0.227
	B	0.400	0.500	0.321	0.364	0.364
	C	0.200	0.393	0.321	0.318	0.409
Me-1	A	0.067	0.107	0.107	0.091	0.227
	B	0.467	0.464	0.500	0.500	0.318
	C	0.467	0.429	0.393	0.409	0.455
Np-2	A	0.357	0.455	0.536	0.563	0.455
	B	0.643	0.545	0.464	0.438	0.545
Np-3	A	0.458	0.571	0.571	0.667	0.727
	B	0.542	0.429	0.429	0.333	0.273

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UPINIŲ ŽUVĖDRŲ (*STERNA HIRUNDO*) KOLONIJŲ GENETINĖ DIFERENCIACIJA

S a n t r a u k a

Genetinio kintamumo tyrimams upinių žuvėdrų (*Sterna hirundo*) pavyzdžiai paimti iš Tauragės, Ignalinos, Klaipėdos, Birštono ir Zarasų regionuose esančių kolonijų. Atlikus širdies, kepenų homogenatų bei kraujo serumo fermentinių sistemų (EST, MDH, ME*)* ir nespecifinio baltymo (NP) elektroforetinius tyrimus nustatyti 8 polimorfiniai lokusai ir įvertintas šių lokusų alelių dažnis. Vidutinis stebimas heterozigotiškumas (H_o) svyravo 0,664–0,721 ribose ir šios reikšmės didesnės už tikėtinas (H_{ex}) – 0,577–0,607. Apibendrinus polimorfinių lokusų alelių dažnius visose kolonijose bei apskaičiavus genetinių distancijų ir genetinio panašumo koeficientus (Nei, 1972) mažiausia genetinė distancija (0,038) nustatyta tarp Klaipėdos ir Tauragės rajono kolonijų. Jų tarpusavio genetinio panašumo koeficientas sudaro 0,963. Didžiausia genetinė distancija (0,112) nustatyta tarp Zarasų ir Tauragės rajonų kolonijų. Jų genetinio panašumo koeficientas tesudaro 0,826. Gana ryškūs suminiai genetiniai skirtumai aptikti tarp Birštono kolonijos ir Klaipėdos rajono kolonijos žuvėdrų. Jų genetinio panašumo koeficientas sudaro 0,896, o genetinė distancija siekė 0,110. Klasterinė gautų duomenų analizė parodė, kad Zarasų bei Ignalinos rajonų kolonijos sudaro viena klasterį, o Tauragės bei Klaipėdos – kitą. Remiantis klasterine analize, galima teigti, kad Birštono upinių žuvėdrų kolonija artimesnė pirmajam klasteriui, t. y. Zarasų bei Ignalinos kolonijų paukščiams.