Evaluation of the genetic structure of the breeding Common Tern (Sterna hirundo) population by means of microsatellite markers

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INTRODUCTION

The Common Tern is a colonial breeding species. Almost half of the Common Tern population (46%) breeding in Lithuania is concentrated in five largest (with more than 100 breeding pairs) colonies: the Nemunas delta (Šilutė district), Kalviai (Klaipėda district), Lazdijai (Lazdijai district), Kretuonas (Švenčioniai district) and Zarasai (Zarasai district). A significant decrease in the Common Tern population is currently observed in most of their habitats in Europe. A similar decrease in the population of this species has also been observed in Lithuania. In 2002, the Common Tern population was estimated to comprise 1500–2500 breeding pairs [1]. This is by 500 pairs less than the number given in the latest review of the populations of birds breeding in Lithuania [2]. It is important to find out whether the decrease in the Common Tern population has a negative influence on the genetic variability of the species. Assessment of the condition of the population of the Common Tern breeding in Lithuania could be of use in creating the species protection strategy. Investigations into the intraspecific genetic diversity are fragmentary in most bird species. This applies to Common Terns too, although their biology, ecology, behavior, taxonomy and morphology are described in several scientific publications [3, 4]. The entire population of Common Terns breeding in Lithuania was considered as an integral unit. However, the first investigations of the past years, carried out by means of protein and isoenzyme electrophoretic analysis, showed that separate colonies of Common Terns, particularly those located in different regions of the country, were genetically differentiated [5]. In order to evaluate the differentiation and genetic diversity of Common Terns, it is necessary to use markers suitable for reconstruction of phylogenetic relationships at the population level. Microsatellite markers are successfully applied in examining the genetic structure of bird populations [6]. A widespread use of the microsatellite loci in the population genetics, phylogenetics, conservation genetic researches is mainly determined by the microsatellite features: their
abundance in the genome, high degree of polymorphism, co-dominant inheritance [7]. The aim of our study was to evaluate, using microsatellite markers, the genetic structure and phylogenetic relationships of the population of Common Terns breeding in different districts of Lithuania.

MATERIALS AND METHODS

Erythrocytes and liver homogenates were taken from Common Terns belonging to seven different colonies located in Lithuania: Kalviai, Kietaviškės (Kaišiadorys district), Lazdijai, the Nemunas delta, Kretuonas, Ignalina (Ignalina district) and Zarasai, which are distributed in the basins of the River Nemunas and the River Daugava (Fig. 1).

Fig. 1. Geographical distribution of Common Tern population colonies studied. 1 – Kalviai, 2 – Nemunas delta, 3 – Lazdijai, 4 – Kietaviškės, 5 – Kretuonas, 6 – Ignalina, 7 – Zarasai

Samples of liver tissues were taken from embryos after incubation of eggs in the laboratory. Additional blood samples were collected from two-three-week aged tern fledglings, from a wing vein into heparinized tubes. Approximately 100–150 µl of blood, which was centrifuged for 10 min at 3000 rpm was taken from each bird. Erythrocytes were separated from plasma and later were used for DNA extraction. Genomic DNA was extracted by means of the universal method of DNA extraction from different tissues [8]. Microsatellite primers were chosen using the data bank (http://tomato.bio.trinity.edu/home.html). The polymerase chain reaction was carried out in a 25 µl volume containing 2.5 µl 10 × PCR buffer, 2.5 µl, 2 mM dNTP, 0.1 µM of each primer, 0.5 µl of Taq polymerase, 2.5 µl MgCl₂, 0.2 µg of genomic DNA, and the remaining volume was water. Amplification was done by an Eppendorf Mastercycler gradient thermocycler: 3 min at 94 °C; 30 cycles of 1 min at 94 °C, then increasing the annealing temperature by one degree from 43 °C to 57 °C (from 2 to 15 cycles) and continuing amplification for the other 15 cycles at 57 °C for annealing, followed by 5 min of a final elongation step at 72 °C. PCR products were fractionated using 10% polyacrilamide gels and the Tris-EDTA-borat buffer electrophoresis analysis (200 V). After electrophoresis the gels were stained with ethidium bromide. The amplification products were evaluated in UV using an image multimedia detecting system. Relative sizes of alleles were determined with the help of TotalLab v1.10 software.

Statistical analysis was performed using GENEPOP (2003), TFPGA (1997), Fstat (2002), STATISTICA (1995) computer programs. The genetic diversity of the population was quantified by the frequency of alleles, the mean number of alleles per locus, the proportion of polymorphic loci (P₀.₉₅; frequency of the most common alleles <0.95), the mean heterozygosity (Hₒ) observed and the expected Hardy–Weinberg heterozygosity (Hₑ) (TFPGA). The non-parametric Wilcoxon test was applied in testing differences in the variability among all pairs of the populations comparing the observed heterozygosity for each locus in all the populations (STATISTICA). Conformity to the Hardy–Weinberg equilibrium was analysed using a single locus test by the Markov chain method (TFPGA). GENEPOP was used to calculate the number of immigrants per generation (Nₑ), and a multi-locus test for heterozygote deficit or excess was performed. Genetic distances among the populations according to Nei [9] (1972) were calculated using TFPGA. A dendrogram based on genetic distances was constructed using the unweighed pair-group arithmetic average (UPGMA) cluster analysis by TFPGA. Population genetic differentiation was investigated using the Raimond and Rousset [10] test. The values of the inbreeding coefficients Fₑ and Rₑ were calculated by Fstat. A correlation between genetic and geographic distances of the population was evaluated using the Mantel [11] test.

RESULTS

By means of 11 microsatellite primer pairs designed for Larus novaehollandiae scopulinus (RBG-13, RBG-18, RBG-29, RBG-39) [12] and Rissa tridactyla (K-16, K-31, K-56) [13] 14 loci were amplified, 11 whereof were polymorphic. Thereinafter, genotypes and allele frequencies were determined (on average 15.4 individuals per sample) for a total of 108 individuals (Table 1).

Five polyallelic loci were detected, in which the number of alleles varied from 3 to 7. Furthermore, two alleles at the locus were detected for the remaining six loci (Table 1). The mean number of the alleles per polymorphic locus was 3.5. Two loci with the primers K-31, RBG-18, RBG-27 were amplified.
Evaluation of the genetic structure of the breeding Common Tern (*Sterna hirundo*) population...

Some loci (K-56, RBG-18(2)) were polymorphic in several but not all colonies. For RBG-39 locus, a unique allele whose relative size was 81 bp was detected only in the Kretuonas colony. A significant deviation from the Hardy–Weinberg equilibrium was found in all colonies except Kretuonas. In five loci – K-56, RBG-13, RBG-18(1), RBG-27(1), RBG-29(1) (Table 1) – the most significant deviation from the Hardy–Weinberg equilibrium was observed in the Nemunas delta colony which is subjected to the highest pressure of natural selection.

The proportion of the polymorphic loci in different colonies ranged from 55% in Ignalina to 82% in Kietaviškės (Table 2). The same degree of polymorphism (73%) in Kalviai, the Nemunas delta and Lazdijai shows a similar level of genetic variability in three colonies belonging to the Nemunas River basin. The mean number of alleles per locus ranged from 2.3 in Kretuonas to 2.8 in the Nemunas delta. The observed heterozygosity in all colonies varied from 0.1809 to 0.4029, the expected heterozygosity ranging from 0.3121 to 0.3795. The value of $H_o$ in Kretuonas was higher than in all other colonies.

### Table 1. Allele frequencies and deviation from Hardy–Weinberg equilibrium in different Common Tern colonies

<table>
<thead>
<tr>
<th>Loci</th>
<th>Alleles</th>
<th>Kalviai n(23)</th>
<th>Kietaviškės n(15)</th>
<th>Nemunas delta n(19)</th>
<th>Lazdijai n(22)</th>
<th>Kretuonas n(7)</th>
<th>Zarasai n(9)</th>
<th>Ignalina n(13)</th>
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<td>K-16</td>
<td>138</td>
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<td>-</td>
<td>0.0455</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>K-31(1)</td>
<td>150</td>
<td>0.7174</td>
<td>0.5357</td>
<td>0.7105</td>
<td>0.7000</td>
<td>1.0000</td>
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<td>0.4643</td>
<td>0.2895</td>
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</table>

* A significant deviation from the Hardy–Weinberg equilibrium (p < 0.05).
that of H_e, but in the other colonies a significant deficit of heterozygosity was detected. The non-parametric Wilcoxon test yielded P values ranging from 0.1891–0.3579 when the colony of the Nemunas delta was compared to all other colonies and 0.8182–1.0000 when all other colonies were compared to each other. These results suggest that only slight differences in the amount of genetic variability exist between seven tern colonies.

On the basis of allele frequencies the genetic distance estimates among the colonies were calculated. The lowest values of the genetic distances (Nei, 1972) were found among the colonies belonging to the basin of the River Nemunas (0.0246–0.0712) (Table 3). Low values of genetic distances (0.0620–0.1746) among the northeast Lithuanian colonies belonging to the basin of the River Daugava were identified too. The highest values of genetic distances were observed between Kretuonas and all other colonies. The values of the Raymond and Rousset test of comparing the colony pairs showed that Common Tern colonies of the Nemunas delta and northeast Lithuania were significantly differentiated. A significant differentiation was not found between the following colony pairs: Kalviai–Kietaviškės, Kietaviškės–Lazdijai, Zarasai–Kretuonas, Zarasai–Ignalina. The investigation revealed a distinct genetic structuring of the Common Tern population breeding in Lithuania. The F_ST (0.1463) and R_ST (0.1545) values show a the general scope of genetic differentiation of the population. When comparing the colonies of the River Nemunas basin (Kalviai, Kietaviškės, the Nemunas delta, Lazdijai) and those of northeast Lithuania of the River Daugava basin (Kretuonas, Zarasai, Ignalina), lower R_ST values were obtained (0.0361 and 0.0958, respectively). The number of immigrants per generation (N_m) was 1.3681 and indicated a significant, though low, gene flow among the Common Tern colonies.

The UPGMA cluster analysis divides four colonies of the River Nemunas basin and three colonies of northeast Lithuania into two clades (Fig. 2). The bunching of the colonies belonging to the River Nemunas in the dendrogram reflects their close geographical distances. Zarasai and Ignalina form a separate clade, and Kretuonas is situated close to this clade but is farther from the other colonies.

The coefficient of correlation between the genetic and geographic distances of the Common Terns, estimated by the Mantel test, was significant and equal to 0.4628 for overall dataset. The correlation coefficients between the genetic and geographic distances were 0.7252 and 0.1899, respectively, when colonies of the River Nemunas basin and the northeast Lithuania colonies of the River Daugava basin were pooled separately. These results show the importance of geographical differences in the formation of the population structure of the Common Tern, but this is not the only factor responsible for the determination of genetic distances among different tern colonies. Nevertheless, a segregation of the Common Tern population into two subpopulations in the breeding area, which is distributed in the basins of two biggest rivers, could be suggested by the results of genetic analysis.

**Table 2. The rate of genetic variability in different Common Tern colonies**

<table>
<thead>
<tr>
<th>Colonies</th>
<th>Mean number of alleles per locus</th>
<th>Polymorphism (%)</th>
<th>The mean of heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Observed (H_o)</td>
</tr>
<tr>
<td>Kalviai</td>
<td>2.7272</td>
<td>72.7273</td>
<td>0.2948</td>
</tr>
<tr>
<td>Kietaviškės</td>
<td>2.3636</td>
<td></td>
<td>0.3120</td>
</tr>
<tr>
<td>Nemunas delta</td>
<td>2.8181</td>
<td>72.7273</td>
<td>0.1809</td>
</tr>
<tr>
<td>Lazdijai</td>
<td>2.7272</td>
<td>72.7273</td>
<td>0.2831</td>
</tr>
<tr>
<td>Kretuonas</td>
<td>2.3000</td>
<td>80.0000</td>
<td>0.4029</td>
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<tr>
<td>Zarasai</td>
<td>2.4545</td>
<td>63.6364</td>
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<tr>
<td>Ignalina</td>
<td>2.7272</td>
<td>54.5455</td>
<td>0.2843</td>
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</table>

**Table 3. Differentiation and genetic distances among Common Tern colonies (above the diagonal: p-values for test of genetic differentiation (Raymond and Rousset, 1995); below the diagonal: Nei’s genetic distance (Nei, 1972) between all pairs of colonies)**

<table>
<thead>
<tr>
<th></th>
<th>Kalviai</th>
<th>Kietaviškės</th>
<th>Nemunas delta</th>
<th>Lazdijai</th>
<th>Kretuonas</th>
<th>Zarasai</th>
<th>Ignalina</th>
</tr>
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<td>0.3067</td>
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<td>0.1263</td>
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<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>Zarasai</td>
<td>0.2460</td>
<td>0.2186</td>
<td>0.2547</td>
<td>0.1532</td>
<td>0.1746</td>
<td>0.0620</td>
<td>***</td>
</tr>
<tr>
<td>Ignalina</td>
<td>0.1921</td>
<td>0.2069</td>
<td>0.1941</td>
<td>0.1532</td>
<td>0.1746</td>
<td>0.0620</td>
<td>***</td>
</tr>
</tbody>
</table>
BY a high inbreeding level, the genetic drift and in the colonies of the Common Terns might be caused to resettlement of the breeding colony which possesses a high genetic diversity. Apparently, the restored colony was formed of the individuals of a different origin, therefore, the total genotype of the colony is characterized as more heterogeneous as compared to stable colonies that have been in existence for a long time. A relatively small amount of heterozygosity as compared to that of all other populations was observed in the Nemunas delta colony. This can be explained by the fact that this colony is young and unstable. Several factors determine the annual fluctuations of the colony size of the Nemunas delta: breeding nests are sometimes destroyed in sandy islands as a result of water rise in the Curonian Lagoon, short floods in spring, especially rainy weather and disturbance by holiday-makers. A as a result, the number of breeding pairs ranged from 10–20 to 150.

In six from seven colonies, a deficit of heterozygosity as compared to the expected Hardy–Weinberg values was observed. The largest deviation from the Hardy–Weinberg equilibrium was found in the Nemunas delta. In our opinion, this is so because the highest pressure of natural selection falls on this colony due to especially difficult breeding conditions. The deviation from the Hardy–Weinberg equilibrium in the colonies of the Common Terns might be caused by a high inbreeding level, the genetic drift and a relatively small number of the sample studied.

The level of inbreeding was assessed in the Common Terns population by estimating the $F_{S_T}$ and $R_{ST}$ coefficients. Recent theoretical analyses have not recommended to apply classical $F$-statistics to the microsatellite data but to use $H_{S_T}$, $R_{ST}$, $G_{ST}$ coefficients instead [14, 15]. The calculated $R_{ST}$ value exceeds 0.15 and shows an intensive differentiation of the Common Tern population. These data are controversial in comparison to observations of Van Treuren et al. [16] when genetic analysis of the population structure of socially organized oystercatchers (Haiatopus ostralegus) by microsatellites revealed a nonsignificant genetic differentiation among colonies of oystercatchers breeding in different parts of the same island. Absence of evidence of genetic differentiation might be caused by relatively small distances (20–50 km) between the colonies, breeding biology of this marine bird species, the specificity of the loci or by other multiple reasons. In our case, a high amount of inbreeding is caused by philopatry of the Common Terns (after migrating in autumn the terns return to their native breeding places). When colonies of the River Nemunas basin and those of northeast Lithuania belonging to the River Daugava basin were pooled separately, the lower values of $R_{ST}$ were obtained and showed a lower genetic differentiation within these groups. Consequently, it can be stated that geographic differences are the main factor determining the genetic differentiation of Common Tern populations.

In our opinion, individuals of the Zarasai, Kietaviškės, the Nemunas delta colonies are mostly involved in an inter-population genetic interchange. Seasonal fluctuations in the abundance of the individuals depending on various environmental factors were observed in these colonies. When the environmental conditions are unfavorable in the native colonies, some part of individuals from Zarasai and Kietaviškės join geographically close colonies. A sudden increase in the colonies of Kietaviškės and Zarasai could be most probably determined only by immigration of the birds ready for breeding. Buddle [17] reported a case when 50% of fledglings of the Common Terns were picked to death by the nestling adults when the distance between the nearest nests was less than 0.5–1.2 m. According to ornithological observations in Lithuania, the minimal distances between the nests were 0.34 m and 0.44 m in the thickest colonies of Kalviai and Lazdijai, respectively. Despite this high density in the colonies, no increased aggression of the adult birds against the fledglings was observed. Thus, a sudden increase in the number of breeding individuals in the Kietaviškės colony was most likely determined by the immigration from dense and numerous colonies of Lazdijai. In a similar way the unstable colony of Zarasai could be joined by the immigrants from Ignalina and Kretuonas. When the conditions are favorable, the population of the Nemunas delta most probably is increased by the immigrants from small and sporadic colonies located between Smalininkai and Kulautuva near the Nemunas River. The breeding conditions are similar in these colonies - unstable sandy islands of the river. Hence, five largest colonies of the Common Terns constituting nearly

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**DISCUSSION**

The levels of genetic variability expressed as the number of polymorphic loci, the mean number of alleles per loci and heterozygosity were determined in the Common Terns. The highest genetic diversity was assessed in Kretuonas colony. The reconstruction of favorable breeding conditions after the scrubs on the island of Lake Kretuonas had been cut might have led to resettlement of the breeding colony which possesses a high genetic diversity. Apparent, the restored colony was formed of the individuals of a different origin, therefore, the total genotype of the colony is characterized as more heterogeneous as compared to stable colonies that have been in existence for a long time. A relatively small amount of heterozygosity as compared to that of all other populations was observed in the Nemunas delta colony. This can be explained by the fact that this colony is young and unstable. Several factors determine the annual fluctuations of the colony size of the Nemunas delta: breeding nests are sometimes destroyed in sandy islands as a result of water rise in the Curonian Lagoon, short floods in spring, especially rainy weather and disturbance by holiday-makers. As a result, the number of breeding pairs ranged from 10–20 to 150.

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half of the national population are genetically differentiated, therefore it is important to ensure a conservation of all five basic colonies thus preserving the intra-specific variability in Lithuania. Taking into consideration the results of this investigation, the strategy of protecting the species should be created, focusing attention on all largest differentiated colonies rather than preserving separate most successful ones.

The colonies of Kretuonas, Ignalina and Zarasai, attributed to the subpopulation of the Common Tern breeding in the Daugava river, form a single cluster in the dendrogram. A nother big cluster is composed of the Nemunas basin subpopulation including the Kalviai, Kietaviškės, Nemunas delta and Lazdijai colonies. The Mantel test shows a fairly high correlation between genetic and geographic distances of the tern subpopulation belonging to the basin of the Nemunas River. Zarasai and Ignalina form one cluster in the dendrogram, though the colonies of Ignalina and Kretuonas are geographically closer. The results of the investigations allow to conclude that differences in the genetic structure of the Common Tern colonies are influenced by the geographical distribution of large rivers, the origin and life span of a colony. Stable and earlier established colonies are more original from the genetic point of view. Young and unstable colonies are influenced by immigrations which increase the genetic variability of the breeding population. In order to investigate the various mechanisms of natural selection and evaluate fluctuations in the size of the breeding Common Tern population it is necessary to analyze data of genetic studies, field observations and ringing. The stability of population structure is possibly based on the returning of mature birds to native breeding colonies; thus, maintenance of areas suitable for breeding is one of the keystones ensuring the stability of the Common Tern population and preserving it from a critical decrease.

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References


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UPINIŲ ŽUVĖDRŲ (STERNA HIRUNDO) PERINČIOS POPULACIJOS GENETINĖS STRUKTUROS ĮVERTINIMAS PANAUDOJANT MIKROSATELITINIUS ŽYMENIS

Santrauka

Upinių žuvėdrų (Sterna hirundo) populacijos genetinės įvairovės tyrimams audinių pavyzdžiai surinkti iš Lietuvos teritorijoje (Nemuno ir Dauguvos upių baseinuose ties Kalviais, Kietaviškėmis, Nemuno delta, Lazdijai, Kretuono ežero sąs., Zarasai bei Ignalina) įsitikinimo kolonijose perinčios paukščių. Panaudojus 11 pradmenų porų, sukurtytų mikrosatelitinių sekių analizė alko alkūnų įvairovės 0,1809–0,4029 ribose. Ršikų genetinio variabilumo skirtumų tarp tirtų upių žuvėdrų kolonijų nenustatyta. Tačiau Nemuno deltos kolonijoje nustatytas mažesnis alelių skaičius lokusui, žemesnes polimorfinio atskiros kolonijose įvairovė 0,1809–0,4029 ribose.

Visos populacijos mastu nustatytas aukštas vidupopuliacinis genetinės diferenciacijos lygis (RST = 0,1545). Nuokrypis nuo Hardžio-Vainbergo pusiausvyros, pasireiškės heterozigotų deficitu, nustatytas šešiose iš septynių žuvėdrų kolonijų, kurį sąlygoja imbydingas bei genų dreifas. Tirtosios upių žuvėdrų kolonijos UPGMA dendrogramoje formuoja atskiras sugrupuotų kolonijų atsakas sudarydamos dvi subpopuliacijas, priskiriamas Nemuno bei Dauguvos upių baseinams, ir tų atspindžiai populacijos genetinės struktūros formavimą priklausomai nuo divydių upių baseinių.