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HAPLOTYPIC VARIABILITY AND POPULATION GENETIC STRUCTURE OF GARGANEY *Anas querquedula* AND COMMON POCHARD *Aythyla ferina* IN THE WESTERN PALEARCTIC

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The mitochondrial DNA (D-loop of mtDNA) sequencing method was used for investigation of various populations of Garganey Anas querquedula *and Common Pochard* Aythya ferina *in the Western Palearctic. Reconstructed phylogenetic relationships based on comparison of D-loop sequences enabled to identify genetic similarity on the level of individual birds and to designate populations with different genetic structure. Identified haplotypic variability indicated the existence of two populations of Garganey with a different genetic structure in Europe. Some genetic variability was also observed among populations of Common Pochard of different origin. Haplotypic variability at different levels was found in both investigated species, despite a considerable overlap in wintering sites used by Garganeys and Common Pochards breeding in various regions.*

Key words: *mtDNA, sequencing, haplotypes, Belarus, Latvia, Lithuania, Russia.*

INTRODUCTION

Garganey *Anas querquedula* and Common Pochard *Aythya ferina* are common duck species with wide distributions in temperate latitudes of the Palearctic across Europe and Asia (Cramp and Simmons, 1977).

Garganey breeds in a huge territory from the Atlantic to Pacific Ocean. It is a typical long-distance migrant. Birds breeding in Europe winter almost exclusively in Africa south of the Sahara, with only small flocks wintering in the Mediterranean region. Wintering areas of birds breeding in the eastern part of the distribution range are located in Eastern Africa, Hindustan and Indochina (Cramp and Simmons, 1977; Scott and Rose, 1996). The European population of Garganey is estimated at about 2–3 million individuals (Delany and Scott, 2002). Garganey is a common breeding duck species in the Baltic States, with about 2,500 pairs estimated in Lithuania and about 1,500 pairs in Latvia (Mednis *et al*., 2003; Stanevicius *et al*., 2003).

Common Pochard is a species characteristic of the Eurasian steppe zone, but during the 19th–20th centuries it markedly expanded the breeding range, at present covering the temperate zones of Western and Northern Europe. Its recent breeding range extends from the Atlantic coast to Lake Baikal in Siberia. Breeding populations of Common Pochard in the Western and Southern Europe are partially migratory, and the main wintering areas of populations of Central and Northern Europe origin are located along coasts of the North and Mediterranean Seas and in Africa (Cramp and Simmons, 1977; Scott and Rose, 1996). The NW and NE Europe population of Common Pochard is estimated at about 350,000 individuals (Delany and Scott, 2002). It is a common and abundant breeding species in Lithuania and Latvia, with about 3,500 pairs estimated in Lithuania and about 1,000–1,500 pairs in Latvia (Strazds *et al*., 1994; Strazds, 1999; Kurlavicius, 2006).

Most pairs of Garganey are formed in their wintering grounds, while pair formation of Common Pochard takes place mostly in breeding sites (Bauer and Glutz von Blotzheim, 1968; 1969; Snow and Perrins, 1998).

A considerable number of recoveries of ringed Garganeys and Common Pochards have been investigated by several authors. Up till now, differences in migration routes and in wintering grounds allowed to identify four different biogeographic populations both in Garganey (Кац и др., 1997) and in Common Pochard (Блумс и др., 1989). Only one European population was identified for both species (Блумс и др., 1989; Кац и др., 1997). Some differences were found in migration patterns and in preferred wintering sites of birds nesting in different parts of Europe, but they were considered as insufficient for designation of different biogeo-

graphic populations. Therefore, it is interesting to investigate the genetic structure of different European populations of Garganey and Common Pochard.

The aim of this study was to determine the genetic variability of population structure of Garganey and Common Pochard in the Western Palearctic by means of DNA analysis. The mitochondrial DNA (D-loop of mtDNA) sequencing method was used for determination of haplotypic variability to identify populations of Garganey and Common Pochard with different genetic structure.

MATERIAL AND METHODS

Specimens (blood/heart/liver derived from living or harvested birds) of two duck species (79 Garganeys and 90 Common Pochards, including migratory and breeding individuals) were collected in Lithuania, Latvia, Kaliningrad Region of Russia, Novgorod Region of Russia, and Western and Southern Belarus. Thirty-eight Garganey individuals wintering in Senegal were also sampled.

Extraction of DNA was carried out according to Aljanabi and Martinez (1997). About 50–100 mg of tissue preserved in alcohol was homogenised in 400 µl homogenysing buffer (0.4 M NaCl, 10 mM Tris-HCl, pH 8.0, and 2 mM EDTA, pH 8.0). Digestion of solution with Proteinase K (20 mg/ml) was followed by incubation at 55–65 ^{o}C at least for an hour and 30 min centrifugation with 300 µl 6M NaCl. After precipitation with isopropanol and washing in alcohol, the samples were dissolved in 300–500 µl of dH_2O and stored at –20 ^{o}C dH_2O until amplification.

Extracted DNA samples were used for amplification of homological fragments of D-loop of mitochondrial DNA of Garganey and Common Pochard. Amplification was carried out at the Laboratory of Population Genetics of the Institute of Ecology of Vilnius University using a Mastercycler Gradien amplifier manufactured by the Eppendorf Company. For amplification of mtDNA fragments of both duck species the following primers were used: C1F1 5–-GTT ATT TGG TTA TGC ATA TCG TG-3– and C1R1 5–-AAA ATG TGA GGA GGG CGA GG-3–. The primers C1F1 and C1R1, specific for *Anas americana, Aythya ferina, Aythya valisineria* and *Aythya collaris*, enabled amplification of the mtDNA fragment of D-loop, which consisted of 392 bases (Sorenson and Fleischer, 1996).

Amplification of DNA fragments was carried out under following conditions: initial denaturation at 96 ^{o}C for 5 minutes, then 30 cycles at 96 ^{o}C for 1 min, annealing at 52 ^{o}C for 1 min, followed by the elongation at 72 ^{o}C for 1 min and finishing with the final elongation step at the 72 ^{o}C for 5 minutes.

The PCR product was checked in 1.5% agarosis gel and purified by Calf Intestine Alkaline Phosphatase (CALF) and Exonuclease I, *E. coli* (Exo I) nucleases (CALF, Exo I and reagents for PCR manufactured by "Fermentas", Vilnius, Lithuania) for 15 min at 37 ^{o}C and then for 15 min at 85 ^{o}C. Sequencing reactions were prepared using a BigDye Terminator v3.1 Cycle Sequencing Kit, carried out at the Sequencing Centre of the Institute of Biotechnology, Vilnius, Lithuania. DNA sequences were determined by the ABI Prism 377 automatic sequencer using the same primers C1F1 and C1R1 as for PCR.

The computer programme CLC Free Workbench version 4.0.1 was used for sequence alignment and for constructing phylogenetic trees.

RESULTS

Garganey *Anas querquedula*

A total of 38 individuals of wintering Garganeys from Senegal (AQ-*) and individuals of breeding or migratory Garganeys from Western Belarus (DK-*), Southern Belarus (FS-*), Kaliningrad Region of Russia (KG-*) and in Latvia (QE-*) were sampled to establish the initial haplotype database. Partial D-loop sequences for each individual were obtained after sequencing the mtDNA fragment consisting of 338 bases including 5–-end fragment of the control region. The 32 variable positions of the partial D-loop fragments of 264 bases revealed high haplotypic variability in the wintering Garganey population.

Phylogenetic analysis based on comparison of sequences of haplotypes using the "neighbour joining" method showed 31 different haplotypes among 38 individuals harvested in Senegal. The remaining Garganey revealed differences in variable sites and distribution of haplotypes among wintering and breeding populations. It was found that several individuals collected in Kaliningrad Region of Russia, Latvia and Western Belarus shared the same haplotype as two individuals harvested in Senegal. Five different haplotypes were detected twice among the wintering population and the remaining 26 haplotypes were detected once. Such high haplotypic variability is characteristic of wintering populations of migratory birds due to concentration of individuals arriving from a numerous breeding sites, including geographically more or less isolated breeding sub-populations, into a relatively small wintering area. Such a concentration of genetically different individuals in wintering grounds usually leads to pronounced genetic variability of the wintering population in comparison to more genetically uniform separate breeding populations (Sruoga *et al*., 2005).

Based on the comparison of DNA sequences that consisted of 149 nucleotides a "neighbour joining" tree was derived to determine phylogenetic relationships of the Garganey individuals collected in wintering grounds in Senegal (A-AQ** individuals) and breeding migratory birds from six sampling locations in Western Belarus (A-DK*), Southern Belarus (A-FS* and A-MO*), Kaliningrad Region of Russia (Western Russia), (A-KG*), Novgorod Region of Russia (NW Russia), (A-1M* and A-QNK*) and Latvia (A-QE*) (Fig. 1).

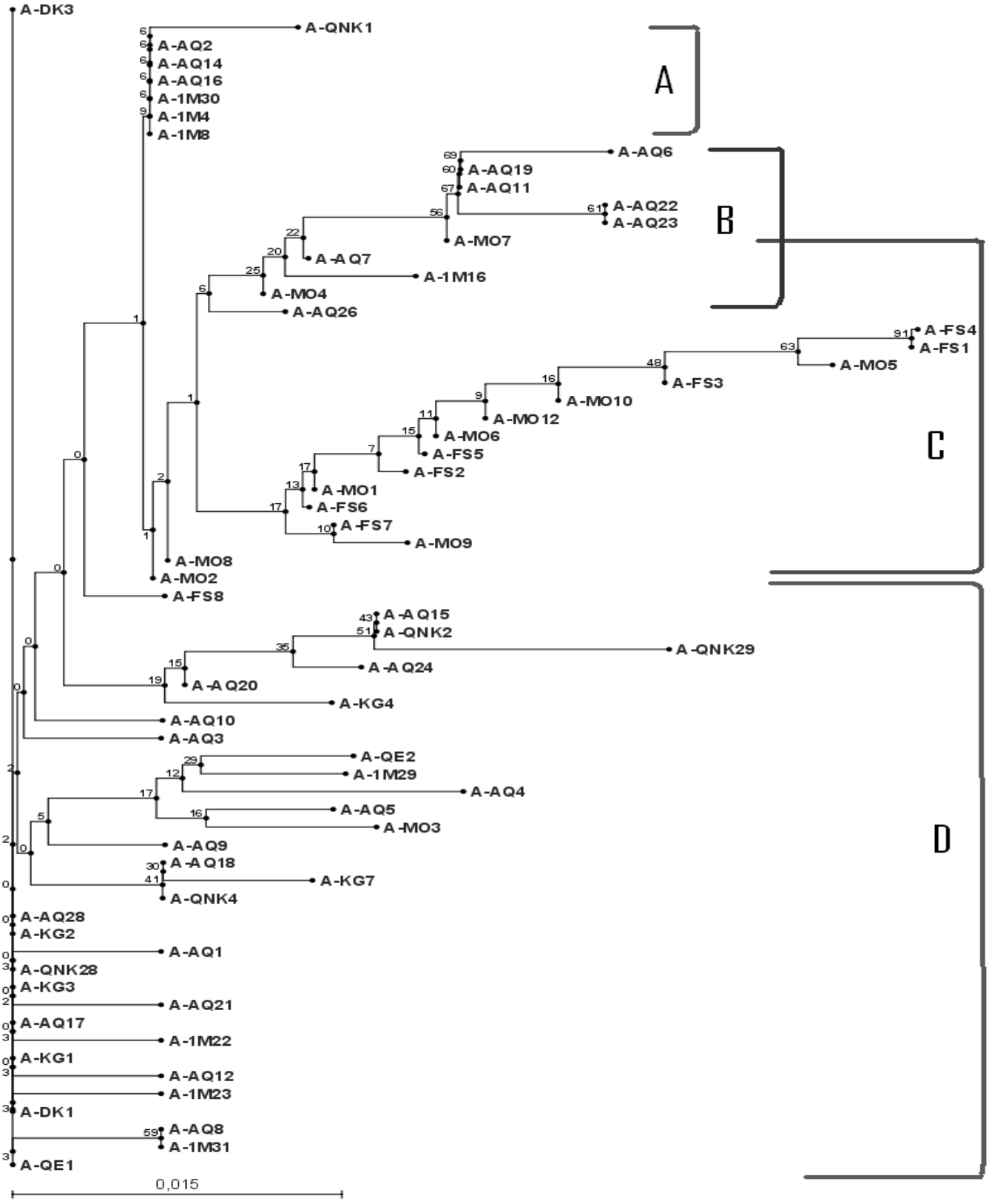

Fig. 1. "Neighbour joining" tree based on comparison of homological fragments of D-loop of mtDNA reflects phylogenetic relationships of Garganeys collected in Senegal (A-AQ*), Western Belarus (A-DK*), Southern Belarus (A-FS* and A-MO*), Kaliningrad Region of Russia (A-KG*), Novgorod Region of Russia (A-1M* and A-QNK*) and Latvia (A-QE*). Scale bar shows exchange of bases per 100 nucleotides.

Four different clusters (A, B, C and D) can be designated according to the pattern of haplotypic distribution in the "neighbour joining "tree.

Cluster A includes six individuals sharing the same haplotype. Three individuals (A-AQ2, A-AQ14 and A-AQ16) are wintering Garganeys in Senegal and the others (A-1M30,

A-1M4 and A-1M8) represent a population of breeding ducks from NW Russia (Novgorod Region). Considering that the wintering population of Garganey is highly variable according to distribution of haplotypes among individuals such an unusually high frequency of the same haplotype among wintering and breeding individuals clearly indicates that wintering sites of Garganeys of the NW Russia breeding population are located in Western Africa.

Cluster B also includes individuals wintering in Senegal grouped with some Garganey individuals from NW Russia and Belarus. These haplotypes are more genetically distant in comparison to individuals in cluster A. Unlike cluster A, cluster B includes individuals from Southern Belarus in addition to individuals collected in Senegal and NW Russia. Thus, cluster B represents a group of individuals sharing an evolutionary more distant ancestor.

Cluster C includes the most interesting group of individuals distinguished in the phylogenetic tree. All Garganeys grouped into cluster C were collected during the breeding season or in August – early September in the Prypiat River region of Southern Belarus (samples A-FS* and A-MO*). Among individuals sampled in Southern Belarus (cluster C) was found the closest phylogenetic relationship and their genetic structure was very different from all other Garganeys collected in Senegal, NW Russia, Western Russia and the Baltic States.

Despite the relative geographical isolation of distant breeding sites, mixing between different populations of Garganey occurs in their wintering grounds. This was illustrated by mixed haplotypes in the cluster B. Moreover, cluster D includes individuals from all studied populations of Garganey. According to reconstruction of phylogenetic relationships in the "neighbour joining" tree the most frequent haplotype can be considered as one of the most ancestral, from which the others haplotypes evolved. This most ancestral haplotype was identified in representatives from most collection areas including Senegal (ind. A-AQ28, A-AQ17), Western Russia (ind. A-KG1, A-KG2, A-KG3), Western Belarus (ind. A-DK1, A-DK3), Latvia (ind. A-QE1) and NW Russia (A-QNK28).

Common Pochard *Aythya farina*

The homological sequence of Common Pochard was amplified using the same primer pair C1F1 and C1R1 used for amplification of a partial sequence of mtDNA of Garganey. The fragment consisting of 266 bases of mtDNA control region was sequenced using DNA extracted from Common Pochard individuals sampled in Novgorod Region of Russia (A-2M*), Kaliningrad Region of Russia (A-KP*), Southern Belarus (A-RA*) and two sampling sites in Latvia (A-AF* and A-FE*).

The most frequent haplotype (ind. A-FE1, A-AF7, A-2M20 and A-2M17) was observed among the individuals collected in Novgorod Region and in Latvian sampling sites (Fig. 2). Another three haplotypes (A-AF*, A-2M* and A-KP*) were detected twice in sampling sites. The most phylogenetically distinct haplotypes of Common Pochard were observed in the sampling site located in Southern Belarus. Since the individuals A-RA-1, A-RA3 and A-RA4 were collected in late September it is likely that these birds arrived from distant breeding sites located in northern Russia and were harvested in their stop-over area.

The most similar genetic structure was observed among Common Pochards collected at Lake Ilmen (NW Russia), in Latvia and in the Kaliningrad Region of Russia. The migratory flyway of this population stretches mainly along the Baltic and North Sea. A different genetic structure was characteristic of Common Pochards sampled in Southern Belarus, although haplotypic variability among different populations of Common Pochard was significantly smaller than among different populations of Garganey.

DISCUSSION

The genetic analysis clearly indicates the existence of a uniform genetic structure, characteristic of Garganeys breeding in Southern Belarus, which is very different from that of populations in the NW Russia/Baltic region (Fig. 3), although most Garganeys from NW Russia, Baltic region and Southern Belarus recently use the same migration routes crossing Balkans and winter in the same areas in Africa.

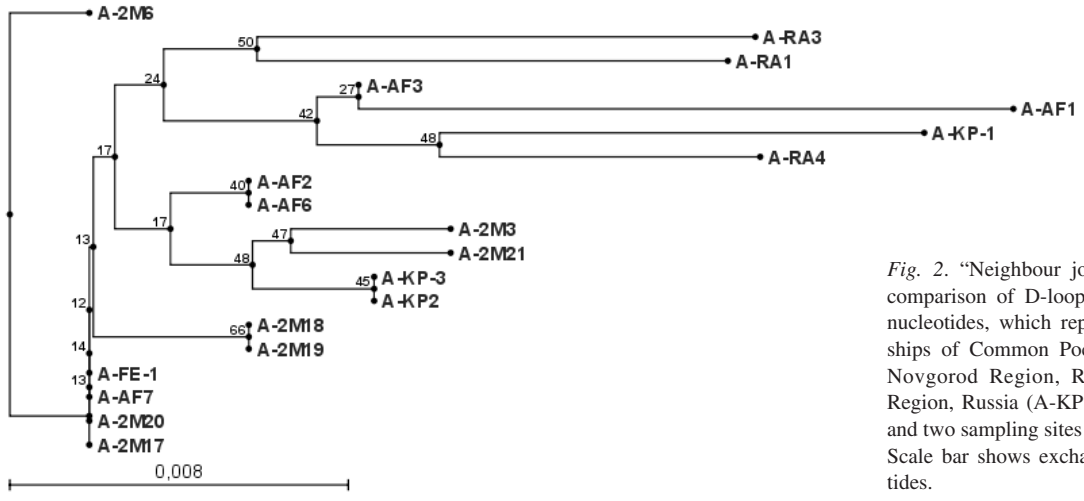

Fig. 2. "Neighbour joining" tree derived from the comparison of D-loop sequences consisting of 266 nucleotides, which represents phylogenetic relationships of Common Pochard individuals collected in Novgorod Region, Russia (A-2M*), Kaliningrad Region, Russia (A-KP*), Southern Belarus (A-RA*) and two sampling sites in Latvia (A-AF* and A-FE*). Scale bar shows exchange of bases per 100 nucleotides.

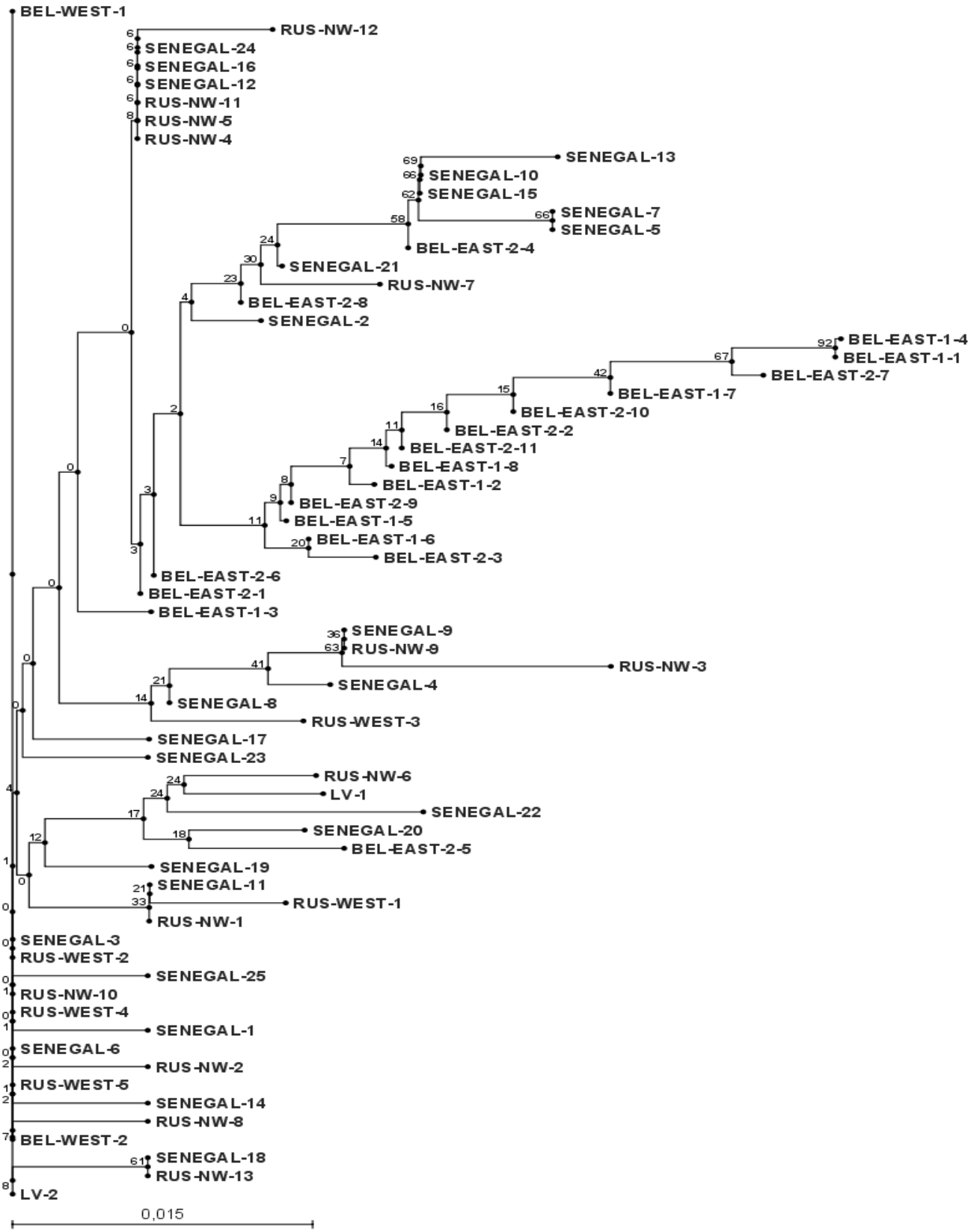

Fig. 3. "Neighbour joining" phylogenetic tree of Garganey haplotypes: samples grouped geographically, indicating countries where samples were collected.

This study showed the existence of two populations of Garganey with a different genetic structure in Europe. Two genetically different populations of Garganey were formed long ago, probably after the last ice-age. It is interesting that marked differences in genetic population structure were found despite a large-scale mixing of Garganeys of different origin in their wintering sites located in Western and Central Africa (Кац и др., 1997). The results of genetic analysis

coincide with the ringing recover data. Garganeys breeding in different regions of Europe and in Siberia in winter concentrate in Western and Central Africa. Pair formation takes place in wintering sites, change migration routes to very distant places (Кац и др., 1997). For example, one male of Latvian origin was recovered in Siberia near Lake Baikal.

Available ringing recover data of Common Pochard indicate a considerable mixture of individuals from different populations in the main wintering sites located along the North Sea and in the Mediterranean region (Блумс и др., 1989; Svazas *et al.*, 2001). The analysis of ringing recoveries does not allow to identify more than one biogeographical population of this species in Europe (Блумс и др., 1989). Monval and Pirot (1989) also concluded that ringing recoveries are insufficient for designation of different populations of European Common Pochards. However, ringing recoveries indicate that birds hatched close to the northern border of their breeding range in Eastern Europe prefer to winter in the North Sea region, while those hatched in the southern part of the distribution more often winter in the Mediterranean region.

The considerable overlap in the breeding areas of Common Pochards wintering along the Atlantic coast and in the Mediterranean region (Блумс и др., 1989) can be partly caused by recent overall westward expansion of this former typical steppe zone species. The species has been recorded in Northwest Europe only since the mid-19th century (Cramp and Simmons, 1977). It was established in Lithuania, Sweden and Finland during the late 19th century (Bauer and Glutz, 1969; Zalakevicius, 1995), while in Estonia and in the St. Petersburg Region of Russia range expansion was observed in the 1930s (Мальчевский и Пукинский, 1983; Renno, 1993). In the 1960s–1970s, large breeding colonies of Common Pochard were recorded in Latvia, with up to 1,800 pairs counted on Lake Engure (Viksne, 1997).

Some genetic variability of different populations of Common Pochard was observed, although differences in their genetic structure were smaller in comparison with those identified for Garganey. It can be partly caused by the relatively short period since their establishment in NW Europe.

As mentioned above, Common Pochard has established as a breeding species in NW Europe only during the past 150 years. The newly formed Baltic/NW Russia populations are relatively young, with generally similar genetic structure.

In conclusion, we found that the mitochondrial DNA (D-loop of mtDNA) sequencing method enabled the identification of genetically different populations of Garganey and Common Pochard in Europe and evaluation of genetic similarity on the level of individual birds. The genetic analysis combined with traditional ringing data can be used as an effective tool for identification of bird populations, particularly in conditions of global climate change affecting the distribution of different populations and in view of the recent spread of the pandemic of avian influenza in the Western Palearctic region.

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RIETUMU PALEARKTIKAS PRĪKŠĶES *Anas querquedula* UN BRŪNKAKĻA *Aythyla ferina* HAPLOTIPU MAINĪBA UN POPULĀCIJU ĢENĒTISKĀ STRUKTŪRA

Lai pētītu dažādas prīkšķes *Anas querquedula* un brūnkakļa *Aythya ferina* Rietumu Palearktikas populācijas, tika izmantota mitohondriālās DNS D-cilpas sekvenēšana. Identificētā haplotipu variabilitāte norāda uz divu prīkšķes ģenētiski atšķirīgu populāciju eksistenci Eiropā. Konstatēta arī ģenētiskā variabilitāte starp dažādas izcelsmes brūnkakļa populācijām. Haplotipu variabilitāte abām sugām ir atrasta, neraugoties uz to, ka dažādos reģionos ligzdojošās prīkšķes un brūnkakļa ziemošanas vietas būtiski pārklājas.