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MtDNA D-loop genetic diversity of common quail (*Coturnix coturnix*) migrating through Ukraine and Spain

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Abstract: Populations of common quail (*Coturnix coturnix*) have recently been declining in Europe. This is the first study of common quail in eastern Europe using molecular markers. The mtDNA D-loop genetic diversity of common quails migrating through Ukraine was analyzed and compared with that of birds migrating via northern Spain in 2017–2018. In samples from 77 birds, 32 polymorphic sites and 30 haplotypes (H1–H30) were identified, only 4 of which were common for birds sampled in both Ukraine and Spain. Phylogenetic analysis indicated 2 well-supported clades. Found were 3 haplotypes clustered in 1 phylogenetic clade only in common quails sampled in Ukraine. The results can be used for the identification of different flyways, particularly for common quails of eastern European origin.

Key words: *Coturnix coturnix*, D-loop haplotypes, phylogeny, flyways

The common quail (*Coturnix coturnix*) is an abundant and widespread Palearctic bird species (Cramp and Simmons, 1980). The total European population is estimated at 6,600,000–13,400,000 mature individuals (BirdLife International, 2018). The common quail is listed in Annex II/2 of the EU Birds Directive and is one of the most important game species in Europe. The designation of the main flyways of the species (connecting their breeding and wintering sites) is necessary for effective conservation and management (Perennou, 2009). Traditionally, the migratory routes of the common quail have been mainly designated using available ringing recoveries. This technique has allowed for the designation of 3 main flyways in Europe, comprising the Atlantic (northwestern Europe–Iberia–Morocco–West Africa), central Mediterranean (central Europe–Italy–Tunisia), and eastern Mediterranean (eastern Europe–Turkey–Middle East–East Africa) (Perennou, 2009). However, this method is restricted by meager recovery rates in eastern Europe (with less than 1% of all available ringing recoveries in Europe). As an alternative method, genetic analysis specifically based on mtDNA sequencing data has been increasingly used to designate the migratory routes of various bird species (Kraus et al., 2011; Wilson et al., 2018; Butkauskas et al., 2019).

Sequencing data can also be used to identify the population genetic structure of the species grounding different management priorities that are applicable for populations with variable genetic structure in different regions. More than 50% of individuals of the total European population of the common quail breed are in eastern and central Europe (BirdLife International, 2018); however, their flyways and population structure have been poorly investigated (Yanenko and Serebryakov, 2015). The aim of this study is to identify the D-loop haplotype variability and population genetic structure of common quail of eastern European origin to identify different conservation and management priorities in various regions.

Samples from 77 adult individuals, representing different common quail migratory flyways, were used for the genetic analysis. In September 2017–2018, 57 samples were collected from common quail hunted in central (n = 32) and western (n = 25) Ukraine, and in October 2017, 20 birds were hunted in northern Spain. Among common quails sampled during autumn migration in Ukraine, there were possibly both local breeders and migrants from other parts of eastern Europe. Birds sampled in northern Spain in autumn probably represented a mix of local breeders and migrants using the Atlantic flyway. All applicable

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national and institutional guidelines for the use of wild birds were followed in this research.

Genomic DNA was extracted from ethanol-preserved tissues following the universal and rapid salt-extraction method (Aljanabi and Martinez, 1997), and dissolved in 300 µL of nuclease-free water. Partial 569 bp-long mtDNA D-loop sequences were amplified using the Cot D-F (5′-GGCATTACATATTGTCCCCATT-3′) and Cot D-R (5′- ACGCAAACCGTATCATCGAG -3′) primer pair designed in the present study. Each PCR mixture consisted of 25 µL, containing 12.5 µL of Dream-Taq PCR master mix (Thermo Fisher Scientific Baltics, Vilnius, Lithuania), 1 µL (µM) of forward and reverse primers, 2 µL (0.05 µg) of template DNA, and nuclease-free water. Amplification was performed using an initial denaturation at 95 °C for 5 min, followed by 35 cycles at 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 60 s, and a final extension at 72 °C for 7 min. The PCR products were run on 1.5% agarose gel electrophoresis and visualized. Purification of the PCR products and direct sequencing were conducted as described previously (Butkauskas et al., 2019).

The obtained mtDNA D-loop sequences were compared with each other and with other sequences from common quails using the nucleotide BLAST program megablast option . Multiple alignments were obtained and different haplotypes were identified, as described previously (Butkauskas et al., 2019). The median-joining haplotype network was constructed using NETWORK 5.0.1.1 . Selection of the nucleotide substitution model and phylogenetic analysis was conducted with MEGAX (Kumar et al., 2018). The phylogenetic tree was constructed using the neighbor-joining clustering method and the Tamura-Nei model of sequence divergence among haplotypes. The bootstrap test was performed with 1000 replicates. The Φ_{ST} over all populations, pair-wise Φ_{ST}, and the hierarchical analysis of molecular variance (AMOVA) with 10,000 permutations were estimated using Arlequin 3.5.2.2 (Excoffier et al., 2005).

Based on the 492 bp-long mtDNA D-loop sequences of 77 birds, 32 polymorphic sites and 30 haplotypes (H1–H30) were identified. The sequence divergence among the distinguished haplotypes ranged from 0.2% to 2.4%, and the haplotypes differed by 1–12 nucleotide substitutions. Sequences representing all of the obtained haplotypes were deposited in the GenBank database under accession numbers MN807850–MN807879.

The frequency of 13 haplotypes varied from 2 to 7, while 16 haplotypes were rare, and H1 was the most common haplotype identified in 22 birds (Figure 1). The most common haplotype was detected in all 3 sampling areas. Additionally, 3 other haplotypes, H2–H4, were observed in 2 sampling areas, whereas the remaining 26 haplotypes (H5–H30) were detected in 1 area. In the median-joining haplotype network, haplotype H1 had single-mutation step relationships with 12 haplotypes, while 3 haplotypes (H19, H22, and H25) were positioned further from those remaining by several mutational steps. Based on the phylogenetic tree, 2 well-supported clades were distinguished (Figure 2). Observed were 27 haplotypes clustered together, while haplotypes H19, H22, and H25 diverged clearly from the other haplotypes and

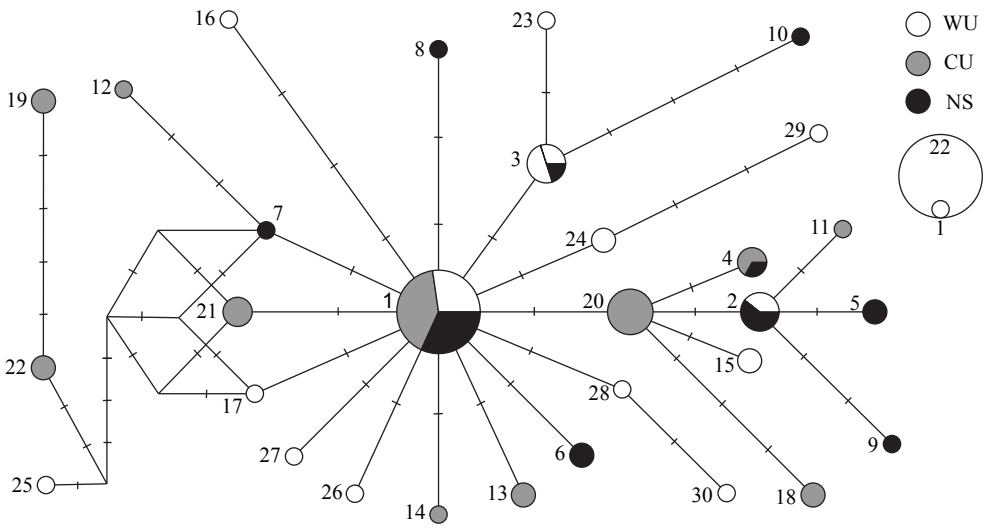

Figure 1. Median-joining network of common quail mtDNA D-loop haplotypes. The circle area is proportional to the haplotype frequency. Dashes indicate mutational steps. Colors and patterns within circles show the relative frequency of sequences from western Ukraine (WU), central Ukraine (CU), and northern Spain (NS).

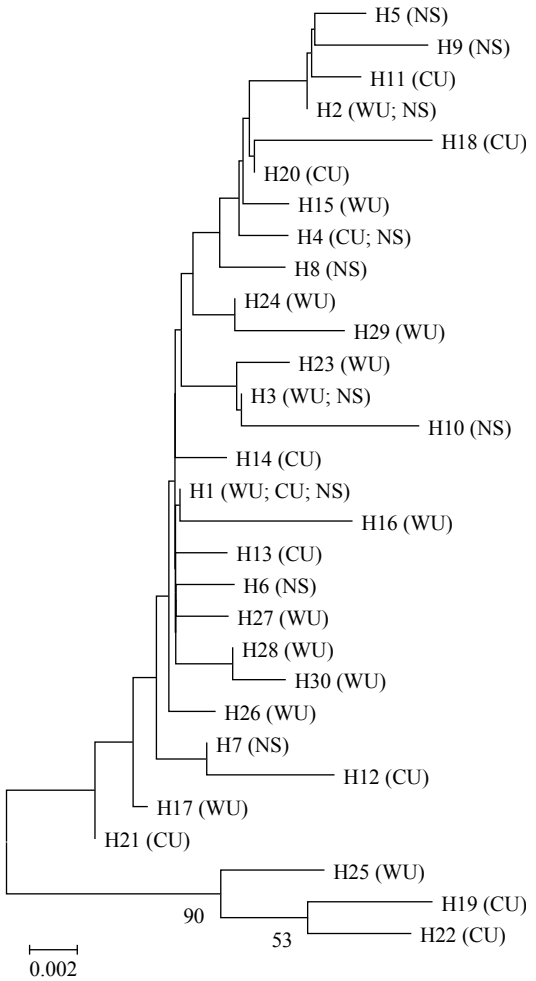

Figure 2. Midpoint rooted neighbor-joining phylogenetic tree of common quail based on the D-loop haplotypes (H1–H30) identified in this study. Figures indicate bootstrap support values higher than 50%. Abbreviations WU, CU, and NS represent the same sampling areas as in Figure 1.

formed a separate clade. These 3 clearly distinguishing haplotypes, shared by 5 individuals, were found only in common quails sampled in Ukraine.

The overall Φ_{ST} showing population differentiation was low (0.039), though significant ($P < 0.01$). The pair-wise Φ_{ST} value between the Ukrainian samples was 0.040 ($P = 0.03$). Similar Φ_{ST} values were calculated between the birds sampled in northern Spain and western Ukraine (0.021; $P = 0.120$), as well as in northern Spain and central Ukraine (0.049, $P = 0.031$). The genetic differentiation between common quails sampled in Spain and Ukraine was not confirmed by AMOVA ($\Phi_{CT} = -0.010$, $P = 0.676$).

To date, population genetic studies of common quails using mtDNA sequence analysis and microsatellite markers have focused on birds migrating via the Atlantic flyway (Sanchez-Donoso et al., 2012, 2014). These studies have not been performed in eastern Europe. This study revealed certain differences in the composition of haplotypes of bird populations migrating via Spain and Ukraine. The analysis based on the mtDNA D-loop sequence indicated the existence of 2 distinct clades in phylogenetic trees, as identified in earlier investigations (Sanchez-Donoso et al., 2014). Within the D-loop, these 2 distinct genetic clades were possibly the result of previous long-term reproductive isolation at the major glacial refugia, including Iberia, Italy, the Balkans-Greece, and the Caspian/Caucasus regions (Hewitt 2001).

The results of this study can be used for a further assessment of possible hybridization between *Coturnix coturnix coturnix* and *C. c. japonica* in eastern Europe, as was earlier reported in western and southern Europe (Chazara et al., 2010; Sanchez-Donoso et al., 2012, 2014). Rearing common quails in captivity for restocking instead of domestic Japanese quail or hybrids helps to overcome the risk of hybridization between the 2 *Coturnix* species in western Europe (Smith et al., 2018); however, possible hybridization between Japanese and common quail in eastern Europe has thus far not been investigated.

The Atlantic and central Mediterranean flyways of the species have been well identified (Perennou, 2009); however, there are very few ringing recoveries available from birds using the eastern Mediterranean flyway. Those that are available have indicated that common quails breeding in Ukraine and other countries of eastern Europe migrate mainly to East Africa via Turkey and the Middle East (Nankinov, 1982; Yanenko and Serebryakov, 2015). Specific haplotype distribution patterns characteristic of common quails of eastern European origin defined in the current study can also be used for the designation of their migratory routes. New studies of mtDNA genetic variability and other available genetic markers (Morris et al., 2020), as well as new ringing schemes and the use of GPS/GMS loggers, are necessary for the designation of flyways of common quail breeding in eastern Europe.

Conflicts of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this paper.

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