Research Article

Genetic diversity and complex structure of the European Roe Deer population at a continental scale

Magdalena Niedziałkowska[1,*], Kamila Plis[1], Barbara Marczuk[1], Johannes Lang[2], Mike Heddergott[3], Juha Tiainen[4,5], Aleksey Danilkin[6], Marina Kholodova[6], Elena Zvychaynaya[6], Nadezhda Kashinina[6], Aleksey Bunevich[7], Ladislav Paule[8], Maryna Shkvyria[9], Nikica Šprem[10], Szilvia Kusza[11], Algimantas Paulauskas[12], Luboš Novák[13], Miroslav Kutal[14], Christine Miller[15], Dimitris Tsaparis[16], Stoyan Stoyanov[17], Boštjan Pokorny[18,19], Katarina Flajšman[19], Vukan Lavadinović[20], Franz Suchentrunk[21], Ana-Maria Krapal[22], Gabriel Dănilă[23], Rauno Veeroja[24], Bogumiła Jędrzejewska[1]

[1]Mammal Research Institute, Polish Academy of Sciences, Stoczek 1, Białowieża 17-230, Poland
[2]Working Group for Wildlife Research at Clinic for Birds, Reptiles, Amphibians and Fish, Justus-Liebig-University Giessen, Frankfurter Straße 108, 35392 Gießen, Germany
[3]Department of Zoology, Musée National d'Histoire Naturelle, rue Münster 25, 2160 Luxembourg, Luxembourg
[4]Natural Resources Institute Finland (Luke), Latokartanonkaari 9, 00790 Helsinki, Finland
[5]Lammi Biological Station, University of Helsinki, Pääjärventie 320, 16900 Lammi, Finland
[6]A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskij prosp. 33, Moscow 119071, Russia
[7]State National Park Belovezhskaya Pushcha, Kamenyuki 225063, Republic of Belarus
[8]Department of Phytology, Technical University in Zvolen, T. G. Masaryka 24, 96001 Zvolen, Slovak Republic
[9]Kyiv Zoological Park of National Importance, Peremohy Ave. 32, Kyiv 04116, Ukraine
[10]Faculty of Agriculture, Department of Fisheries, Apiculture, Wildlife Management and Special Zoology, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia
[11]Centre for Agricultural Genomics and Biotechnology, University of Debrecen, Egyetem tér 1, 4032 Debrecen, Hungary
[12]Department of Biology, Vytautas Magnus University, Vileikos g. 8-802, 44404 Kaunas, Lithuania
[13]Department of Forest Protection and Wildlife Management, Mendel University in Brno, Zemědělská 3, 61300 Brno, Czech Republic
[14]Department of Forest Ecology, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 3, 61300 Brno, Czech Republic
[15]Bureau of Wildlife Biology Bavaria, Haslau 21, 83700 Rottach-Egern, Germany
[16]Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research, Gournes Pediados, P.O. Box 2214, 71003 Heraklion, Crete, Greece
[17]Wildlife Management Department, University of Forestry, 10 Kliment Ohridski Blvd., 1797 Sofia, Bulgaria
[18]Environmental Protection College, Trg Mladosti 7, 3320 Velenje, Slovenia
[19]Department of Forest Ecology, Slovenian Forestry Institute, Večna pot 2, 1000 Ljubljana, Slovenia
[20]Faculty of Forestry, University of Belgrade, Kneza Višeslava 1, 11030 Belgrade, Serbia
[21]Research Institute of Wildlife Ecology, University of Veterinary Medicine Vienna, Savoyenstraße 1, 1160 Vienna, Austria
[22]"Grigore Antipa" National Museum of Natural History, Șoseaua Pavel D. Kiseleff 1, 011341 Bucharest, Romania
[23]Faculty of Forestry, Stefan cel Mare University of Suceava, Str. Universitatii, 13, 720229 Suceava, Romania
[24]Wildlife Department, Estonian Environment Agency, Mustamäe tee 33, 10616 Tallin, Estonia

*Corresponding author: Mammal Research Institute, Polish Academy of Sciences, Stoczek 1, Białowieża 17-230, Poland. Email: mniedz@ibs.bialowieza.pl
Associate Editor was Michael McGowen.

Abstract

Although the European Roe Deer (*Capreolus capreolus*) is one of the most common and widespread ungulate species in Europe and inhabiting a variety of habitats, few studies have addressed its population structure at a large spatial scale using nuclear genetic data. The aims of our study were to: (i) investigate genetic diversity, level of admixture, and genetic structure across European Roe Deer populations; (ii) identify barriers to gene flow; and (iii) reveal factors that have impacted the observed pattern of population genetic structure. Using 12 microsatellite loci, we analyzed 920 European Roe Deer samples from 16 study sites from northern, southern, central, and eastern Europe. The highest genetic diversity was found in central and eastern sites, and lowest in the northern and southern sites. There were 2 main groups of genetically related populations in the study area—one inhabiting mainly Fennoscandia, and the second in the continental part of Europe. This second population was further divided into 3 to 5 spatially distributed genetic clusters. European Roe Deer belonging to the Siberian mitochondrial DNA clade, inhabiting large parts of eastern Europe, were not identified as a separate population in the analysis of microsatellite loci. No isolation by distance (IBD) was detected between roe deer from the fennoscandian and the continental study sites, but the Baltic Sea was inferred to be the main barrier to gene flow. Only weak IBD was revealed within the continental population. Three lower-level genetic barriers were detected in the western, southern, and eastern parts of the study area. The main factors inferred as shaping the observed genetic diversity and population structure of

European Roe Deer were postglacial recolonization, admixture of different populations of the species originating from several Last Glacial Maximum refugial areas, and isolation of several study sites.

Key words: *Capreolus capreolus*, *C. pygargus*, Europe, Fennoscandia, Geneland, genetic diversity, microsatellite loci, nuclear DNA, postglacial recolonization, ungulates

The European Roe Deer (*Capreolus capreolus*) is one of the most abundant wild ungulate species inhabiting large areas of Europe—from the Iberian Peninsula to the Caucasus Mountains in the South, to Fennoscandia in the North, and western Russia in the East (Lovari et al. 2016). In the Volga–Don Rivers region in western Russia, the range of the species overlaps with its sister species, the Siberian Roe Deer (*C. pygargus*; Danilkin 1996, 2014). European Roe Deer is an ecologically flexible species that inhabits forests as well as a mosaic of woodlots, meadows, arable lands, and suburban areas, and it prefers mixed habitats consisting of both woodlands and open areas (Lovari et al. 2016 and references therein).

Not many studies have investigated the genetic diversity of European Roe Deer at a continental scale, and all have focused on maternally inherited mitochondrial DNA (mtDNA; Lorenzini et al. 2014; Plis et al. 2022a, 2022b). To date, nuclear DNA (such as microsatellite DNA) of the species was studied only at the local or regional level (e.g. Mucci et al. 2012; Olano-Marin et al. 2014; Steinbach et al. 2018; Świsłocka et al. 2019; Bužan et al. 2020). Plis et al. (2022a, 2022b) found the highest mtDNA genetic diversity of the species in central and eastern parts of Europe, and the lowest in the peripheral areas of the species range in Fennoscandia and the southernmost regions of the continent. Three clades and several haplogroups of mtDNA were revealed in Europe (Randi et al. 2004; Lorenzini et al. 2014; Plis et al. 2022a). The main factors that shaped mtDNA genetic diversity and population structure of the species were the Quaternary climatic oscillations, postglacial recolonization, and hybridization with the Siberian Roe Deer (Matosiuk et al. 2014; Plis et al. 2022a, 2022b). Through hybridization during the Younger Dryas (10,800 to 10,000 BP)—when their ranges overlapped naturally over larger areas (Matosiuk et al. 2014)—as well as through translocations of Siberian Roe Deer into the range of *C. capreolus* by humans in the 19th and 20th centuries (Danilkin 1996, 2014; Scandura et al. 2014; Plis 2023), many *C. capreolus* individuals inhabiting eastern and central Europe (mainly eastern Poland, Belarus, Baltic States, Slovakia, Hungary, Ukraine, and the European part of Russia) possess mtDNA of *C. pygargus* (Plis et al. 2022a, 2022b).

In contrast to mtDNA studies, there are little data concerning the introgression of *C. pygargus* nuclear genes into the *C. capreolus*. The studies of Olano-Marin et al. (2014) and Świsłocka et al. (2019) conducted in Poland did not reveal genetic differences in microsatellite DNA between roe deer specimens with *C. capreolus* mtDNA and those with *C. pygargus* introgressed mtDNA. On the other hand, Plakhina et al. (2014) were able to distinguish European–Siberian roe deer hybrids in Moscow and Volgograd regions (Russia) using a selected set of 21 microsatellite loci.

During the Quaternary glaciations, the range of many European mammals decreased as they survived in areas localized in the southern part of the continent (Taberlet et al. 1998; Hewitt 1999), the Carpathians, and in eastern Europe (e.g. Sommer and Nadachowski 2006; Sommer et al. 2009; Niedziałkowska 2017; Niedziałkowska et al. 2021a). In the time of the Last Glacial Maximum (LGM; 19 to 26,000 BP; Clark et al. 2009), roe deer survived in a large refugial area stretching from the Iberian Peninsula

and present-day northern Italy, through the Carpathians, the Balkans, the northern coast of the Black Sea to the Caucasus Mountains, and in a small area in northeastern Europe on the Belarus–Russian borderland (Sommer et al. 2009; Lorenzini et al. 2014; Plis et al. 2022a). In the Holocene, roe deer recolonized nearly all of Europe through range expansion from different refugial areas, which is reflected in the phylogeographical pattern of the species today. Contact zones of different mtDNA clades and haplogroups of *C. capreolus* exist in central and eastern Europe (Plis et al. 2022a). Yet few data are available to elucidate the role of postglacial migration waves in shaping the present-day nuclear DNA diversity and population structure of the species.

In this paper, we present results of the most comprehensive population genetic analyses of European Roe Deer nuclear DNA at a large spatial scale covering the northern, central, southern, and eastern parts of its range. The aims of the study were to: (i) estimate genetic diversity and level of admixture among study sites; (ii) reveal nuclear DNA population genetic structure; and (iii) infer barriers to gene flow. We also inferred factors that have an impact on the observed genetic pattern. The study was performed by the analysis of 12 microsatellite loci. We hypothesized that the population genetic diversity pattern obtained in the analyses of nuclear DNA is at least partly concordant with that found in the mtDNA studies conducted across the same populations (Plis et al. 2022a, 2022b). We predicted that there would be 2 main genetic populations of roe deer—one inhabiting Fennoscandia, and a second panmixed population occurring on the mainland of Europe. We further predicted that the Baltic Sea would be the main barrier to gene flow in the study area. We also expected that the effect of hybridization between *C. capreolus* and *C. pygargus* would be less visible in the nuclear DNA than in the mtDNA due to differences in the inheritance mechanism of these markers. We finally predicted that the population genetic structure of nuclear DNA had been shaped by a combination of postglacial migration waves, the presence of geographic barriers, and locally by translocations of roe deer by humans.

Materials and methods

Sampling.

We genotyped 920 roe deer (skin or tissue) samples collected in 21 European countries (Supplementary Data SD1). The study area ranged from Germany (6°19′E) in the West to the European part of Russia (47°12′E) in the East, and from Finland in the North (67°52′) to Greece in the South (38°42′N). The analyzed samples were divided (according to their geographic localization) into 16 study sites (Fig. 1; Supplementary Data SD2). The samples were legally collected from hunted animals, georeferenced according to information on culling location provided by hunters, and then stored as described by Plis et al. (2022a). The research was done ethically and in compliance with relevant local, national, or international regulations regarding the collection, care, and use of animals.
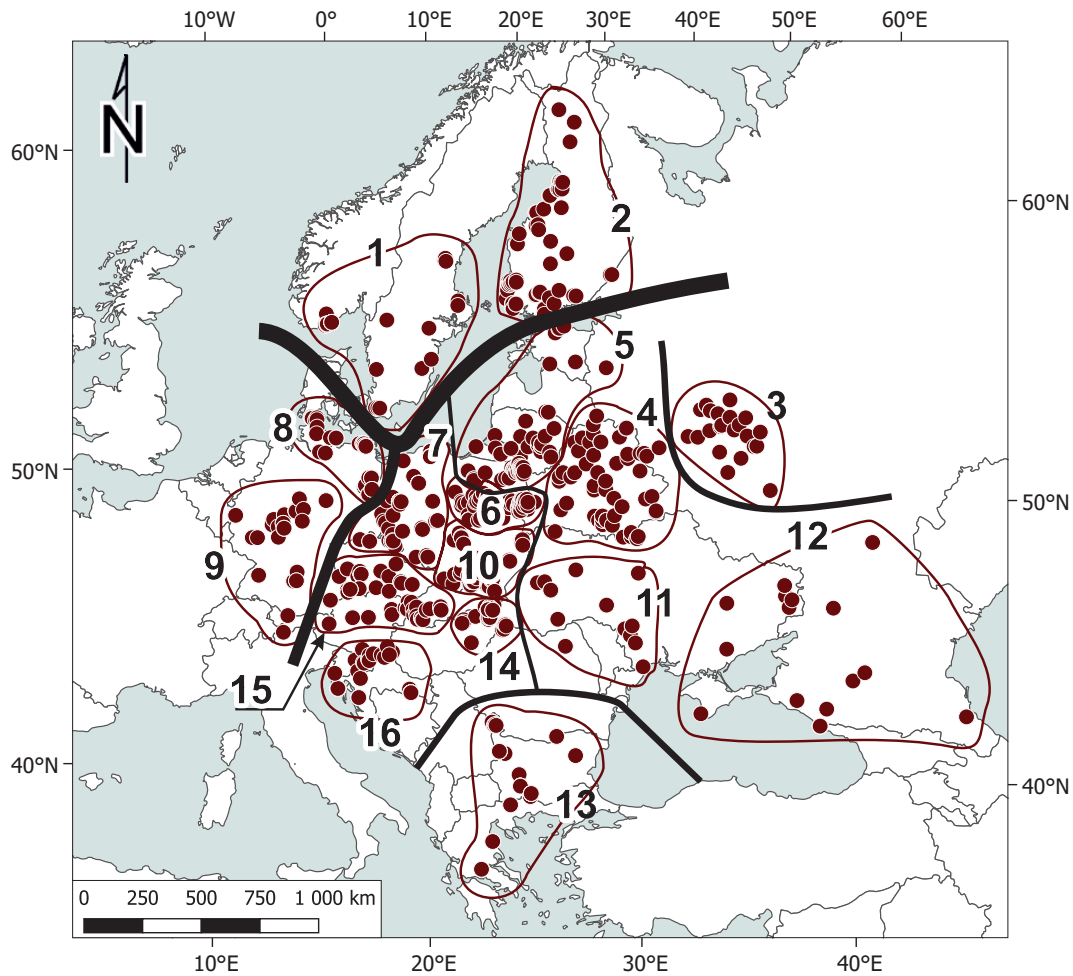
Fig. 1. Distribution of analyzed samples (dots) and 16 study sites of European Roe Deer (Capreolus capreolus). Black lines = barriers to gene flow indicated by the BARRIER software; the width of the lines corresponds to the significance of the barrier.

Laboratory analyses.

DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Valencia, California) following the manufacturer's guidelines. All samples were genotyped at 16 microsatellite loci: BM757, BM1818, BMS119, CSSM66, ETH225, MAF70, MCM64, NVHRT16, NVHRT21, NVHRT24, NVHRT48, NVHRT71, NVHRT73, Roe1, Roe8, RT1 (Røed 1998; Røed and Midthjell 1998; Poetsch et al. 2001; Vial et al. 2003). Microsatellite fragments were amplified in 4 multiplexed polymerase chain reactions (PCRs) using HotStarTaq Master Mix Kit (Qiagen). For more details concerning PCR reactions and protocols, see Olano-Marin et al. (2014). The PCR products were then analyzed on an ABI 3130xl Genetic Analyzer with the GeneScan 400HD ROX Size Standard (Applied Biosystems). Alleles were scored with GeneMarker software (Softgenetics). All laboratory analyses were performed in the Mammal Research Institute Polish Academy of Sciences in Białowieża, Poland.

Preliminary analyses of microsatellite data.

One locus (BMS119) was monomorphic, and there were difficulties with amplification of RT1. Two other loci (BM1818 and NVHRT73) were discarded from further analyses due to stuttering problems and high frequency of null alleles (>5%) indicated by Microchecker software (van Oosterhout et al. 2004). Further analyses were performed using 12 loci (Supplementary Data SD3) that showed no evidence of scoring error due to stuttering, and

no signs for large allele dropout or null alleles (their frequency was <5%) as assessed in Microchecker analyses. Pairwise linkage disequilibrium (LD) between loci, including a Bonferroni correction for multiple testing (1,320 permutations), was assessed with FSTAT 2.9.4. (Goudet 1995). We did not find any signs of genotypic disequilibrium among pairs of analyzed loci with adjusted P-value for 5% nominal level below 0.000757. For 6 of 66 pairs of loci (BM757 × ETH225, BM757 × NVHRT7, NVHRT7 × ETH225, NVHRT48 × Roe1, NVHRT48 × BM757, Roe7 × ETH225) the LD P-values equaled 0.00076.

Estimation of genetic diversity.

Deviation from Hardy–Weinberg equilibrium (HWE) for 16 sites and across all populations was tested in GenePop 4.7.5 (Raymond and Rousset 1995; Rousset 2008) using the globaltest option assessing heterozygote deficiency. Basic parameters of genetic diversity (n_a = mean number of alleles, n_e = mean number of effective alleles, P_A = number of private alleles, H_o = observed heterozygosity, H_e = expected heterozygosity, F = fixation index ($H_e − H_o)/H_e$) for populations at 16 sites were calculated using GeneAlEx ver. 6.5 (Peakall and Smouse 2006). Using 2 mutation models (T.P.M. and S.M.M., default values) and 2 statistical tests (sign and Wilcoxon tests) implemented in the software Bottleneck 1.202 (Piry et al. 1999), we also evaluated the possibility of mutation–drift equilibrium from each of the 16 sites. Mean

allelic richness (A_R), which considers the number of samples in each site, was calculated using FSTAT and genetic differentiation F_{st} using Arlequin (Excoffier and Lischer 2010). Isolation by distance (IBD) tests (regression of linear F_{st} and spatial distance) for 2 groups of sites (among pairs of continental and fennoscandian–continental sites) were calculated using SPAGeDi (Hardy and Vekemans 2002).

Estimation of the level of admixture and inference of barriers to gene flow.

To estimate the level of admixture and the direction of gene exchange among 16 sites, the assignment and first-generation migrants tests were performed using GeneClass (Piry et al. 2004). The analyses were conducted using the following parameters—(i) for the assignment test: Bayesian methods for computation (Rannala and Mountain 1997), simulation algorithm (after Paetkau et al. 2004), number of simulated individuals = 10,000, and default values of other parameters; (ii) for assessment of first-generation migrants: likelihood ratio L_home/L_max, Bayesian methods for computation (Rannala and Mountain 1997), number of simulated individuals = 10,000 using a simulation algorithm (Paetkau et al. 2004), threshold P-value = 0.01. Barriers to gene flow among 16 sites were indicated using the Monmonier's algorithm implemented in the software BARRIER v 2.2 (Manni et al. 2004) and linear F_{st} calculated in GenAlEx.

Identification of genetic structure.

To reveal the population genetic structure of the roe deer populations across Europe and to have confidence in the results obtained, we used several methods (with different underlying assumptions, including spatial and aspatial Bayesian clustering and principal component discriminant analysis)—followed by a check for consistency of results.

In the Bayesian clustering approach implemented in software STRUCTURE ver. 2.3.4 (Pritchard et al. 2000), the analyses were performed using an admixture model with default settings and Markov Chain Monte Carlo (MCMC) of 100,000 iterations (NUMREPS) after a burn-in (length of burn-in period before the start of data collection) of 100,000. To check for consistency among results, analyses were repeated 5 times for each value of possible number of genetic clusters K (K from 1 to 16). The obtained results were further processed to indicate the most probable number of genetic clusters using the Evanno method (Evanno et al. 2005) implemented in STRUCTURE HARVESTER (Earl and von Holdt 2012). In the next step, to reveal lower-level structure within genetic populations, the hierarchical analysis in STRUCTURE (Vähä et al. 2007) was performed by taking each distinct genetic population indicated by the first analysis and subjecting it to further STRUCTURE runs (in the first case using default parameters and then performing analyses using an allele frequency-independent model, prior ALPHA = 0.17) and STRUCTURE HARVESTER processing. In both cases we assigned individuals to clusters for the run with the highest posterior probability value. Both kinds of STRUCTURE analyses (for the whole data set and only for the continental samples) were repeated with MCMC for 500,000 iterations, at which point the obtained results were consistent and showed the same genetic division of roe deer populations.

Spatial genetic structure was analyzed in Geneland (Guillot et al. 2005), which (except for genotypes) also includes the geographic coordinates of the studied individuals. The analyses consisted of 20 independent MCMC runs with the following parameter settings: number of clusters K from 1 to 16; number

of MCMC iterations = 200,000; thinning = 200, spatial uncertainty fixed to 0.5; spatial model with uncorrelated allele frequencies and null alleles; and discarding the first 200 iterations (thinning) in the post-processing. The optimal value of K was determined based on the likelihoods of all runs.

In the post-processing, individuals were assigned to genetic populations in the highest likelihood run. Similar analyses with the same parameters and 10 MCMC runs were repeated for a data set containing only roe deer samples from the continental part of Europe (without Fennoscandia).

We also performed a discriminant analysis of principal component (DAPC, using adegenet 2.1.5; Jombart 2008) in R, which does not assume HWE or LD. This analysis maximizes differentiation among populations while minimizing within-population variation. The analyses were performed for the whole data set and separately for the continental sites (sites 3 to 16). We also repeated DAPC grouping for the entire data set according to the population genetic structure indicated by Geneland.

Results

Genetic diversity and barriers to gene flow in European Roe Deer.

Results of a global test for all loci and sites showed that populations of European Roe Deer in 11 of 16 study sites are in HWE, whereas 5 (3, 8, 12, 13, 15) deviated from HWE ($P < 0.05$). The parameters of genetic diversity across all roe deer populations were relatively high (Supplementary Data SD2). The highest observed heterozygosity ($H_o > 0.87$) was detected in sites 4, 6, 7, and 10 and the lowest ($H_o = 0.70$) in site 13 (Supplementary Data SD2). The highest mean number of alleles was recorded in site 15 but the highest effective number of alleles, mean allelic richness, and the number of private alleles were indicated in site 12 (Supplementary Data SD2). The lowest mean number of alleles was detected in sites 1 and 2, and the lowest mean effective number of alleles and mean allelic richness were found in sites 1, 2, and 13. The number of private alleles positively correlated with longitude (Supplementary Data SD4). Fixation index (F) had the lowest (negative) values in sites 1, 2, 4, 6, 7, 10, and 16—thereby indicating some excess of heterozygosity—and the highest (positive) values in sites 3, 9, 11, 12, and 13 (Supplementary Data SD2). F-values were not substantially positive in any of the studied sites, indicating no traces of inbreeding. The results of mutation–drift equilibrium analyses obtained for different mutation models and statistical tests for 16 sites were inconsistent (data not shown), so no clear signs of bottleneck were detected.

The genetic differentiation (F_{st}) values between pairs of the study sites were low or moderate, but statistically significant in all cases, ranging from 0.003 between sites 6 and 7 to 0.133 between sites 2 and 13, respectively (Table 1). The assignment and first generations tests indicated that there was no gene exchange between the fennoscandian and continental parts of the study area, but there was gene flow between sites 1 and 2, and among sites on the European mainland (Table 2, Supplementary Data SD5). According to the assignment test, the most isolated sites on the mainland were sites 8 and 13 (the mean probability of individuals being assigned above 0.3 was indicated only for the sites where they occurred; Supplementary Data SD5). Among 31 first-generation migrants detected, 28 were found in the mainland of Europe (Table 2). One emigrant from site 13 found in site 1 could be an effect of translocation performed by humans rather than natural migration. On the continent, the highest number of

Table 1. F_{st} values between pairs of 16 study sites (see Fig. 1) of the European Roe Deer (*Capreolus capreolus*). For all pairs $P < 0.0001$.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	0.013														
3	0.077	0.088													
4	0.066	0.080	0.013												
5	0.064	0.072	0.008	0.006											
6	0.067	0.075	0.021	0.009	0.011										
7	0.070	0.080	0.026	0.013	0.018	0.003									
8	0.051	0.065	0.045	0.029	0.032	0.027	0.037								
9	0.051	0.061	0.036	0.027	0.026	0.016	0.024	0.009							
10	0.076	0.092	0.024	0.010	0.019	0.008	0.011	0.039	0.033						
11	0.070	0.081	0.025	0.010	0.011	0.015	0.020	0.034	0.033	0.012					
12	0.068	0.083	0.023	0.012	0.017	0.010	0.019	0.027	0.028	0.014	0.016				
13	0.129	0.133	0.049	0.041	0.043	0.039	0.045	0.080	0.070	0.032	0.029	0.037			
14	0.086	0.090	0.026	0.019	0.023	0.012	0.019	0.053	0.038	0.008	0.017	0.016	0.029		
15	0.070	0.077	0.027	0.012	0.014	0.008	0.010	0.036	0.025	0.010	0.016	0.017	0.026	0.014	
16	0.093	0.101	0.030	0.019	0.017	0.022	0.029	0.047	0.041	0.016	0.022	0.021	0.028	0.012	0.016

immigrants were detected in sites 5 to 7 and 15; and emigrants from sites 7, 9, and 10. No immigrants were detected in site 9 and no emigrants from site 12 (Table 2).

The largest barrier to gene flow inferred with the software BARRIER based on the linear F_{st} was the Baltic Sea (0.094; Fig. 1). The second most important barrier was found on the European mainland, dividing sites 8 and 9 from the rest of the continental range of roe deer (0.042; Fig. 1). Less significant genetic barriers separated sites 13 (0.028) and 3 (0.023) from the neighboring sites (Fig. 1). Limited gene flow was also observed among sites located in eastern and central-western parts of the continental Europe (linear F_{st} = 0.018; Fig. 1).

Genetic structure among roe deer populations in Europe.

Results of analyses performed in STRUCTURE indicated that there were 2 separate genetic populations of *C. capreolus* in Europe (Supplementary Data SD6)—one inhabiting Fennoscandia and consisting of sites 1 and 2, and a second including all continental sites (Fig. 2). Results of DAPC showed a similar division of the populations (Supplementary Data SD7). Such genetic structure was also supported by higher values of genetic differentiation (F_{st}, linear F_{st}) between pairs of fennoscandian–continental sites than among pairs of continental sites (Table 1, Fig. 3). There was a slight increase in linear F_{st} with spatial distance, which was statistically significant only among continental sites (Fig. 3).

Further hierarchical analysis performed for continental sites showed a lower-level genetic structure for roe deer population inhabiting mainland Europe, resulting in 5 clusters (Fig. 4, Supplementary Data SD6) but with sites differing in percentage of these clusters shared. The most genetically distinct from all other were site 13—located in the southernmost part of the study area (eastern part of the Balkan Peninsula)—and the westernmost sites 8 and 9, in which over 50% of individuals belonged to 1 cluster (black and yellow, respectively; Fig. 4). Some distinctiveness of sites 8 and 9 was also visible in results of DAPC (Supplementary Data SD7 and SD8). Moreover, Sites 3 to 5—occurring in the northeastern part of the study area, and site 16—inhabiting Croatia and Slovenia, differed from others by the higher proportion of the cluster marked red in Fig. 4. In the remaining sites, the proportion

of different clusters shared were similar and formed a panmixed population (Fig. 4). In the fennoscandian genetic population no further structuring was revealed by the hierarchical analyses.

We perfomed additional STRUCTURE analyses using different parameters (prior for ALPHA = 0.17 and an allele frequency-independent model)—results for the entire data set were consistent with the previous analyses (the most probable $K = 2$, data not shown). However, hierarchical analyses of the continental data set indicated that the most probable number of clusters was 7 (Supplementary Data SD6 and SD9). One additional cluster (marked in pink) was indicated in site 12, and an second additonal cluster (marked in navy blue) was revealed (although with low frequency) in sites 5, 7, 10, 13, 15, and 16 (Supplementary Data SD9).

All Geneland analyses showed 4 genetic populations of roe deer in Europe: one consisting of fennoscandian sites; the second inhabiting the western part of the study area; the third (the largest) comprising central, southern, and eastern parts of the continent; and the fourth including only 6 individuals from the eastern part of the continent (Fig. 5). Such genetic division of roe deer populations was also visible in the results of DAPC, when the analyzed samples were grouped according to these 4 Geneland populations (Supplementary Data SD10). Several genetically distinct individuals in eastern Europe (site 12) were indicated by the STRUCTURE hierarchical analyses performed using an allele frequency-independent model (Supplementary Data SD9).

Parameters of genetic diversity calculated for the 4 Geneland populations were relatively high (Table 3). The largest population (G3) was also the most diverse—having highest observed heterozygosity (H_o), highest mean number of alleles (N_a) and highest number of private alleles (P_A). However, highest number of effective alleles (N_e) was recorded in population G2, inhabiting the western part of the study area, while the highest mean allelic richness was found in the smallest population G4. No signs of genetic inbreeding depression were noticed in any of the populations (Table 3). Genetic differentiation (F_{st}) among pairs of Geneland populations was low or moderate but, in all the cases, statistically significant. The highest F_{st} values were recorded between G4 and all other populations (Table 4).

Table 2. First-generation migrants detected among 16 sites of Roe Deer with probability $P < 0.01$. Site = site where the migrant was found; Site of origin = site from which the migrant most probably came from; n = number of migrating individuals.

Site of origin	Direction	Site	n
1	→	2	2
3	→	5	1
4	→	7	1
4	→	10	1
5	→	8	2
6	→	5	1
6	→	15	1
7	→	5	1
7	→	6	1
7	→	10	1
7	→	11	1
7	→	14	1
8	→	15	1
9	→	4	1
9	→	7	1
9	→	12	1
9	→	15	1
10	→	5	2
10	→	6	1
10	→	11	1
10	→	15	1
10	→	16	1
11	→	6	1
13	→	1	1
13	→	16	2
15	→	7	1
15	→	13	1
Total			31

Further analysis, including only individuals inhabiting continental Europe, showed a similar division in most runs as in the whole data set (Supplementary Data SD11). However, in 30% of the runs, Geneland showed a division of mainland roe deer into 4 populations (Supplementary Data SD11)—one in the western part of the study area, the second in central and eastern Europe, and the third with a disjunct distribution (i.e. occurring in the northeastern and southern parts of the continent). The fourth population consisted of several individuals in the easternmost part of the study area, similar to results for the entire data set analysis (Supplementary Data SD11).

Discussion

The overall population genetic diversity of roe deer in Europe appeared relatively high compared to other large ungulates inhabiting the temperate climatic zone such as Red Deer (*Cervus elaphus*; Niedziałkowska et al. 2012; Zachos et al. 2016) and Moose (*Alces alces*; Niedziałkowska et al. 2016a). Observed heterozygosity of roe deer was higher than in Red Deer and Moose populations, whereas allelic richness was similar to Moose and higher than in Red Deer (Zachos and Hartl 2011; Niedziałkowska et al. 2012; Zachos et al. 2016). We found some excess heterozygosity in all except one studied roe deer site. Such excess has not been commonly observed in other populations of ungulates in Europe (e.g. Niedziałkowska et al. 2012, 2016a; Veličković et al. 2016; Zachos et al. 2016). According to Cornuet and Luikart (1997) heterozygosity excess or deficit can occur after a recent change of the effective population size but also in a situation where heterozygotes have a selective advantage or disadvantage. However, the results of mutation–drift equilibrium analyses performed using different mutation models were not consistent. So further studies including more loci are needed to reveal any signs of recent changes in effective population sizes due to, for example, population bottlenecks in roe deer in Europe, or if other factors could explain such mutation–drift disequilibrium.

The highest genetic diversity of roe deer was recorded in central, eastern, and southeastern parts of the study area. A similar genetic diversity pattern and high-latitude genetic hotspot was detected in mtDNA of the same roe deer populations (Plis et al. 2022a, 2022b). This pattern could be the result of postglacial recolonization of the central European regions by populations originating from different LGM refugia and their admixture in more northern regions. European Roe Deer probably survived the LGM in a large range covering an area from the Iberian Peninsula to the Caucasus Mountains and in 2 northern regions—one in the proximity of the Carpathian Mountains, and one in eastern Europe (present-day Belarussian–Russian border area; Plis et al. 2022a). The presence of contact (suture) zones of different genetic lineages and clades in central and eastern Europe, increasing the genetic diversity in these regions, has also been revealed in the Bank Vole (*Clethrionomys glareolus*) (Wójcik et al. 2010; Tarnowska et al. 2016, 2019; Marková et al. 2020), Moose (Niedziałkowska et al. 2014, 2016a), and Wild Boar (*Sus scrofa*; Niedziałkowska et al. 2021b)—as well as in other rodent, ungulate, and carnivore species (Stojak and Tarnowska 2019).

Among all roe deer populations in Europe, genetic structure indicated by microsatellite loci was partly concordant with the pattern obtained in analyses of mtDNA (Plis et al. 2022a, 2022b). In the central part of the study area, roe deer were highly admixed as indicated by the assignment and first-generation tests—while those inhabiting northern, western, eastern, and southern parts of the European mainland were clearly genetically distinct (as revealed by STRUCTURE hierarchical analyses). Geneland combined most continental sites into 1 genetic population, which is consistent with intensive gene flow among roe deer, as indicated by the very weak IBD relationship and the results of the assignment and first-generation tests in central Europe. However, some Geneland runs that included only continental roe deer divided this large population into 2 genetic groups—one with a disjunct distribution. Such a pattern also corresponds well to the distribution of 1 or 2 of the STRUCTURE clusters (Fig. 4; Supplementary Data SD9) but further studies including more nuclear loci (e.g. single-nucleotide polymorphisms—SNPs) are needed to confirm this division. Most likely, such structure (both spatial and aspatial) resulted from overlap of different recolonization waves from the LGM refugial areas located, for example, in the Balkans, the Carpathians, and western Europe rather than from presence of landscape barriers and IBD limiting gene exchange among different genetic groups. Such a complex phylogeographic pattern in higher-latitude areas was also described for other species, for example, Bank Vole, which recolonized central and northern Europe not only from several different LGM refugia but also by
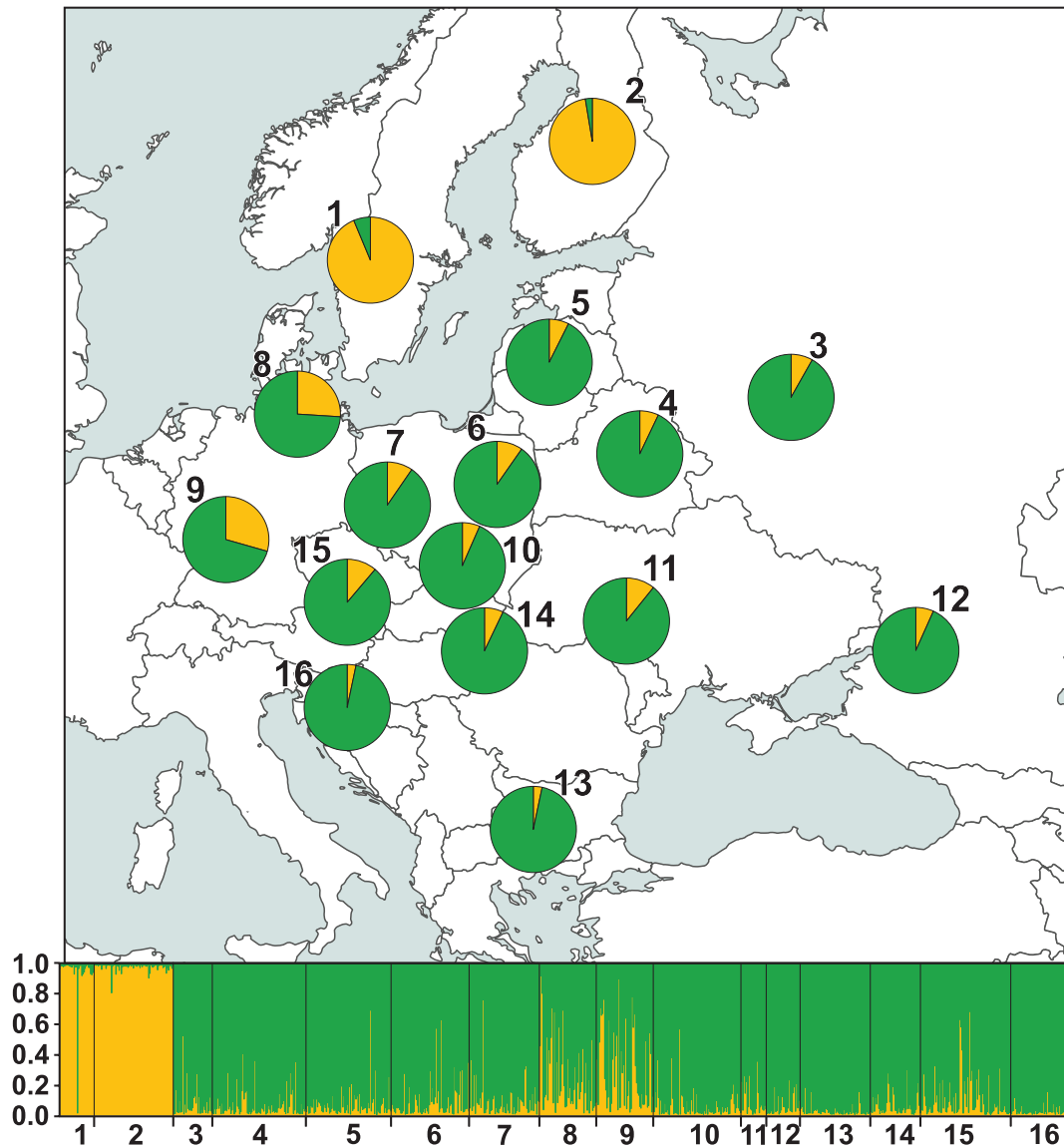
Fig. 2. Two main genetic clusters of the European Roe Deer (Capreolus capreolus) indicated by the aspatial Bayesian clustering analyses performed using algorithm implemented in the software STRUCTURE. Each color represents 1 cluster. Numbers correspond to the 16 study sites (Fig. 1, Supplementary Data SD2). Upper panel = pie charts present the frequencies of clusters in each site. Lower panel = proportions of each cluster in the studied individuals; 1 column represents 1 individual.
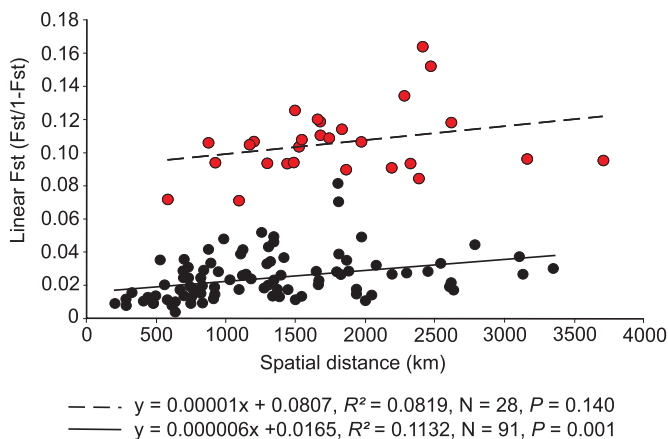

$$— — \cdot \quad y = 0.00001x + 0.0807, R^2 = 0.0819, N = 28, P = 0.140$$
$$——— \quad y = 0.000006x + 0.0165, R^2 = 0.1132, N = 91, P = 0.001$$

Fig. 3. Isolation by distance analysis. Relationships between genetic and spatial distances among pairs of European Roe Deer (Capreolus capreolus) sites. Red dots = fennoscandian–continental pairs of study sites; black dots = continental pairs of sites.

several migration waves from the same refugial areas resulting in admixture of different populations (Wójcik et al. 2010; Marková et al. 2020; Niedziałkowska et al. 2023).

In the case of mtDNA, introgression of Siberian Roe Deer mtDNA—which occurred in the past as a result of both natural and human-induced processes—had a major impact on genetic diversity values in the eastern range of the European Roe Deer (Plis et al. 2022a, 2022b). The high occurrence of the Siberian mtDNA clade in the populations in eastern Europe was not detected as a separate genetic population in the analyses of nuclear markers (microsatellites), although we found traces of past hybridization between the 2 species. We showed that a high number of private alleles and a high mean allelic richness in comparison to other study sites characterized the eastern sites 3, 4, 6, and 12. Several individuals from eastern Europe were also separated by Geneland into a distinct genetic population. Similarly, hierarchical STRUCTURE analyses (performed using allele frequency-independent model) indicated an additional genetic cluster consisting
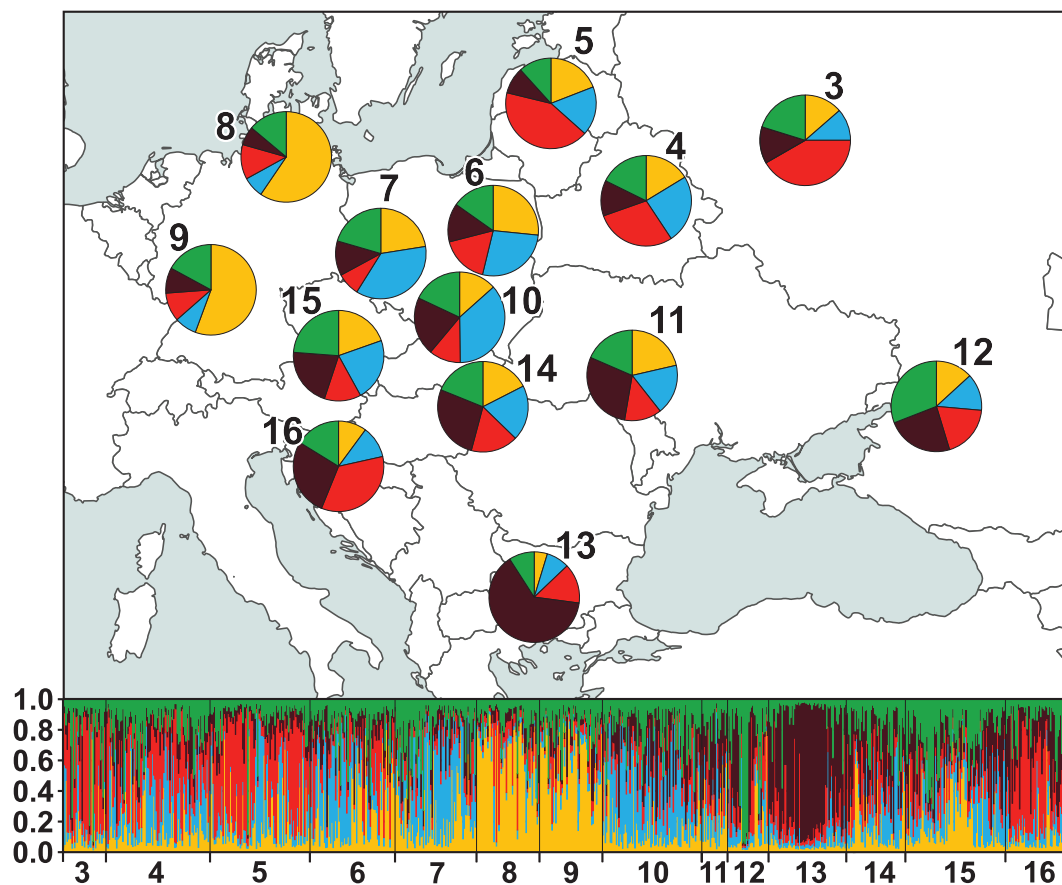
Fig. 4. Five genetic clusters of continental European Roe Deer (Capreolus capreolus) indicated by the aspatial Bayesian clustering analyses performed using algorithm implemented in the computer program STRUCTURE. Each color represents 1 cluster. Numbers correspond to 16 study sites (Fig. 1, Supplementary Data SD1). Upper panel = pie charts present the frequency of each cluster in the sites. Lower panel = proportion of each cluster in the studied individuals; 1 column represents 1 individual.

of several specimens in site 12. Such distinctiveness of eastern European Roe Deer can be traced to past hybridization with the Siberian Roe Deer, which resulted from either overlap of the ranges of those species and/or translocation caused by humans (Danilkin 1996, 2014; Matosiuk et al. 2014; Scandura et al. 2014; Plis 2023). Further genetic studies, including more nuclear markers (e.g. SNPs) and *C. pygargus* samples, are needed to confirm this hypothesis. In the microsatellite analyses, the signal of such introgressions is weaker than for mtDNA due to the different inheritance processes involved (e.g. by gene recombination), so more loci are needed to confirm such hybridization in nuclear genes. The European Roe Deer and Siberian Roe Deer differ in karyotype and morphology, and do not easily crossbreed because the hybrid males are sterile (Danilkin 1996).

The lowest values of genetic diversity were found in roe deer sites from the northern and southernmost peripheral areas of Europe. Also, the study by Plis et al. (2022a, 2022b) showed that the fennoscandian population of roe deer had some of the lowest mtDNA diversity in Europe. Low genetic diversity (of both nuclear and mtDNA loci) of populations inhabiting Fennoscandia has been recorded in several other mammal species, such as Red Deer (Zachos et al. 2016), Moose (Niedziałkowska et al. 2014, 2016a), and Bank Vole (Marková et al. 2020). During the postglacial period, populations of different species recolonized Scandinavia through the land bridge that had connected it with the European mainland between 10 ka and 8 ka years BP, and disappeared after with rising sea level (Björck 1995). Since then, those northern populations

have been isolated by the Baltic Sea (e.g. Niedziałkowska et al. 2016a, 2016b; Marková et al. 2020), supported in the present study.

Furthermore, game species such as European Roe Deer, Red Deer, and Moose have suffered declines in abundance across Europe in the more recent past due to climate change and overhunting, which has also reduced their genetic diversity (Randi et al. 2004; Niedziałkowska et al. 2012, 2014). In isolated populations, genetic diversity remains low, even when overall population abundance has increased. Moreover, although Finland is well connected with the European mainland via northwestern Russia, European Roe Deer went extinct there during the Little Ice Age in the 17th and 18th centuries, while the present-day population has originated both from translocated individuals and those spreading naturally from Sweden (Pulliainen 1980; Helle 1996). This is also the reason behind the grouping of the Fennoscandia roe deer into 1 genetic population as migration from the southeast was too sparse to lead to permanent colonization (Helle 1996). No gene exchange between fennoscandian and continental roe deer was recorded by the assignment and first-generation tests in our study.

There were, however, some traces of previous gene flow between those northern and continental populations. The western roe deer (sites 8 and 9) have a large proportion of alleles commonly found in the fennoscandian population, and were indicated as a distinct genetic cluster or population by STRUCTURE, DAPC, and Geneland. Such patterns indicate that in postglacial times Fennoscandia was recolonized by roe deer originating from western Europe, which is in agreement with the distribution of
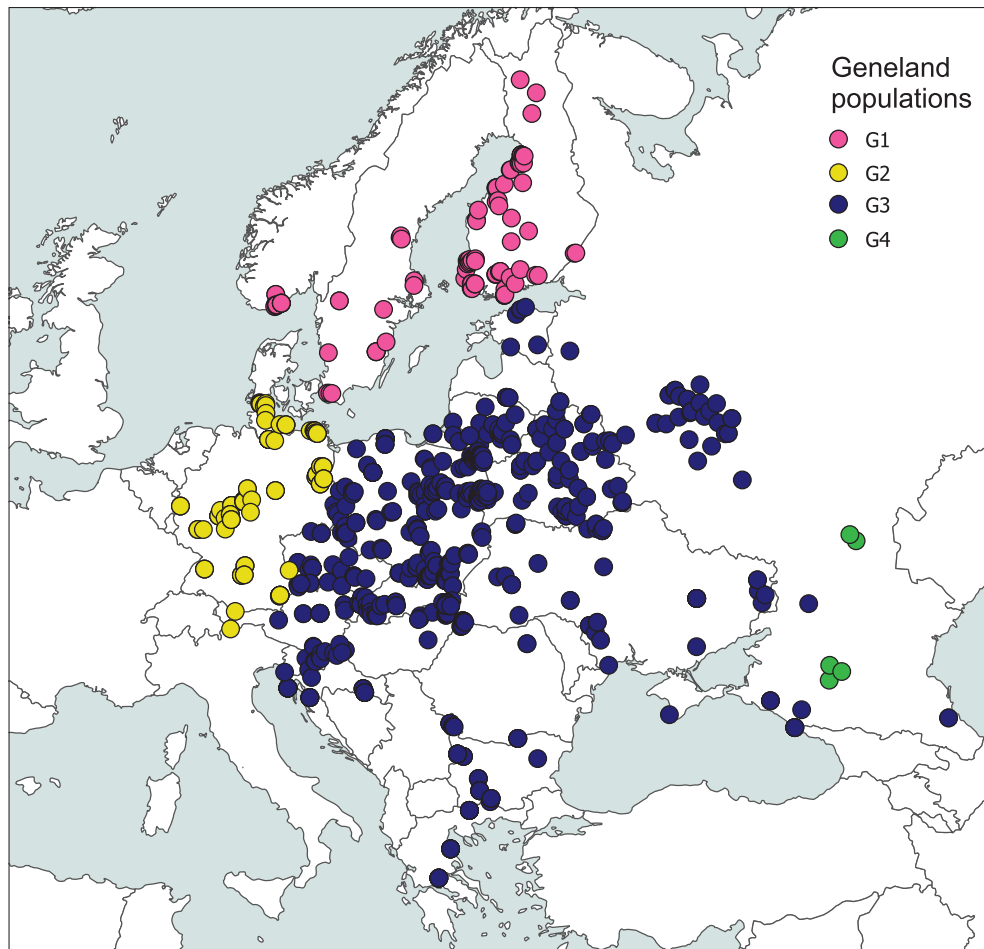
Fig. 5. Four genetic clusters (G1 to G4) of European Roe Deer (Capreolus capreolus) indicated by spatial Bayesian clustering analyses performed using Geneland. One dot represents 1 individual. Colors represent different genetic populations.

Table 3. Parameters of genetic diversity of 4 genetic populations (G1 to G4) of the European Roe Deer (*Capreolus capreolus*) indicated by Geneland (see Fig. 5). Values of standard error (SE) in parentheses. n = number of samples; n_a = mean number of alleles; n_e = mean number of effective alleles; A_R = mean allelic richness; P_A = number of private alleles; H_o = observed heterozygosity; H_e = expected heterozygosity; F = fixation index $(H_e - H_o)/H_e$; mtDNA lineage = mitochondrial DNA lineage of individuals in each genetic population (after Plis et al. 2022a); Eur = lineage of *C. capreolus*; Siber = lineage of *C. pygargus*.

Parameter	G1	G2	G3	G4
n	104	116	694	6
n_a	9.25 (0.85)	13.00 (1.49)	18.17 (1.56)	6.17 (0.63)
n_e	3.48 (0.32)	5.14 (0.62)	4.88 (0.52)	4.84 (0.56)
A_R	3.53 (0.07)	4.45 (0.09)	4.41 (0.03)	5.03 (0.57)
P_A	1	3	42	12
H_o	0.78 (0.03)	0.79 (0.04)	0.82 (0.02)	0.77 (0.07)
H_e	0.69 (0.03)	0.77 (0.03)	0.77 (0.02)	0.75 (0.03)
F	−0.15 (0.05)	−0.04 (0.03)	−0.07 (0.03)	−0.01 (0.08)
mtDNA lineage	Eur	Eur	Eur/Siber	Eur/Siber

Table 4. F_{st} among 4 genetic populations indicated in Geneland analyses. For all pairs $P < 0.0001$.

	G1	G2	G3
G2	0.053		
G3	0.072	0.025	
G4	0.194	0.136	0.138

1998), Hedgehog (*Erinaceus europaeus/concolor*; Hewitt 1999), and Bank Vole (Marková et al. 2020).

The lower genetic diversity and endemicity of southern European Roe Deer may be a consequence of their continued isolation in former LGM refugia, as has been demonstrated for several mammalian species such as the Bank Vole (Marková et al. 2020), Grey Wolf (*Canis lupus*; Stronen et al. 2013), Wild Boar (Niedziałkowska et al. 2021b), and Red Deer (Zachos et al. 2016; Doan et al. 2017; Doan et al. 2022). Low genetic diversity and/or genetic distinctiveness of the southern European Roe Deer have earlier been documented by studies on populations in Spain, Italy, and Greece (Mucci et al. 2012; Tsaparis et al. 2019; Barros et al. 2020; Plis et al. 2022a, 2022b). Furthermore, in our study area, limited gene flow among the southernmost site 13 and other sites was confirmed by the detection of a barrier dividing the southern Balkan site from the remaining sites. STRUCTURE analyses and the assignment test also revealed limited gene flow between the southernmost site 13

mtDNA clades (Plis et al. 2022a, 2022b). Similar recolonization routes have been suggested for Red Deer (Doan et al. 2022), Moose (Niedziałkowska 2017), Brown Bear (*Ursus arctos*; Taberlet et al.

and its neighboring regions. The southern populations have been isolated by geographic barriers such as the Alps (Taberlet et al. 1998) or blocked by more northern populations expanding from other LGM refugial areas, as has also been revealed in mtDNA studies of Red Deer (Borowski et al. 2016; Doan et al. 2022).

Supplementary data

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—Number of European Roe Deer (*Capreolus capreolus*) samples collected in each of the country and analyzed in this study.

Supplementary Data SD2.—Parameters of genetic diversity of 16 sites of the European Roe Deer (*Capreolus capreolus*).

Supplementary Data SD3.—Number of alleles and of allele size ranges of 12 microsatellite loci used in the study.

Supplementary Data SD4.—Correlation between the number of private alleles and longitude in 16 study sites of European Roe Deer (*Capreolus capreolus*).

Supplementary Data SD5.—Mean probability of assignment of individuals from 16 study sites to each of them.

Supplementary Data SD6.—The most probable number of clusters of European Roe Deer (*Capreolus capreolus*) indicated by STRUCTURE HARVESTER based on the results of aspatial Bayesian clustering analyses performed using STRUCTURE software.

Supplementary Data SD7.—Genetic structure of European Roe Deer (*Capreolus capreolus*) indicated by discriminant analysis of principal component (DAPC) performed in adegenet.

Supplementary Data SD8.—Genetic structure of European Roe Deer (*Capreolus capreolus*) population in the mainland of Europe (Fennoscandian samples excluded) indicated by discriminant analysis of principal component (DAPC) performed in adegenet.

Supplementary Data SD9.—Seven genetic clusters of the continental European Roe Deer (*Capreolus capreolus*) population indicated by the aspatial Bayesian clustering analyses performed using algorithm implemented in the software STRUCTURE (continental data set only, Fennoscandia excluded). STRUCTURE analyses performed using allele frequency-independent model, prior ALPHA = 0.17.

Supplementary Data SD10.—Genetic structure of European Roe Deer (*Capreolus capreolus*) indicated by discriminant analysis of principal component (DAPC) performed in adegenet, and sample grouping according to 4 genetic populations indicated in the Geneland analyses.

Supplementary Data SD11.—Results of spatial Bayesian clustering analyses performed using algorithm implemented in the software Geneland indicating the genetic structure of European Roe Deer (*Capreolus capreolus*) population in the mainland of Europe.

Acknowledgments

The authors thank to the Polish Hunting Association, J. Žák, K. Turek, M. Mati, V. E. Sidorovich, I. Dykiy, D. Vishnevsky, M. Kolesnikov, and V. Lobkov and numerous hunters across Europe for their help in collecting samples.

Author contributions

MN conceived the ideas, designed methodology, and led the writing of the manuscript; KP, JL, MH, JT, AD, MK, EZ, NK, AB, LP, MS, NŠ, SzK, AP, LN, MK, ChM, DT, SS, BP, KF, VL, FS, A-MK, GD, and RV collected the data; MN, BM, and KP analyzed the data; BJ conceived the ideas and applied for the financial support; all authors contributed critically to the drafts and gave final approval for publication.

Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

The data that support the findings of this study will be openly available in the Open Forest Data Repository https://openforest-data.pl/ after acceptance of the paper for publication.

References

Barros T, Ferreira E, Rocha RG, Brotas G, Carranza J, Fonseca C, Torres RT. 2020. The multiple origins of roe deer populations in Western Iberia and their relevance for conservation. Animals 10(12):2419. https://doi.org/10.3390/ani10122419.

Björck S. 1995. A review of the history of the Baltic Sea, 13.0–8.0 ka BP. Quaternary International 27:19–40. https://doi.org/10.1016/1040-6182(94)00057-C.

Borowski Z, Świsłocka M, Matosiuk M, Mirski P, Krysiuk K, Czajkowska M, Borkowska A, Ratkiewicz M. 2016. Purifying selection, density blocking and unnoticed mitochondrial DNA diversity in the Red Deer, *Cervus elaphus*. PLoS One 11:e0163191. https://doi.org/10.1371/journal.pone.0163191.

Bužan E, Gerič U, Potušek S, Flajšman K, Pokorny B. 2020. First insights into the population genetic structure and heterozygosity–fitness relationship in roe deer inhabiting the area between the Alps and Dinaric Mountains. Animals 10:2276. https://doi.org/10.3390/ani10122276.

Clark PU, Dyke AS, Shakun JD, Carlson AE, Clark J, Wohlfarth B, Mitrovica JX, Hostetler SW, McCabe AM. 2009. The Last Glacial Maximum. Science 325:710–714. https://doi.org/10.1126/science.1172873.

Cornuet JM, Luikart G. 1997. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014. https://doi.org/10.1093/genetics/144.4.2001.

Danilkin A. 1996. Behavioural ecology of Siberian and European Roe Deer. Wildlife ecology and behaviour series. London (UK): Chapman & Hall.

Danilkin AA. 2014. Roe deer (biological basis of resource management). Moscow: KMK Scientific Press (in Russian).

Doan K, Niedziałkowska M, Stefaniak K, Sykut M, Jędrzejewska B, Ratajczak-Skrzatek U, Piotrowska N, Ridush B, Zachos FE, Popović D, *et al.* 2022. Phylogenetics and phylogeography of red deer mtDNA lineages during the last 50 000 years in Eurasia. Zoological Journal of the Linnean Society 194:431–456. https://doi.org/10.1093/zoolinnean/zlab025.

Doan K, Zachos FE, Wilkens B, Vigne JD, Piotrowska N, Stanković A, Jędrzejewska B, Stefaniak K, Niedziałkowska M. 2017. Phylogeography of the Tyrrhenian Red Deer (*Cervus elaphus corsicanus*) resolved using ancient DNA of radiocarbon-dated

subfossils. Scientific Reports 7:2331. https://doi.org/10.1038/s41598-017-02359-y.

Earl DA, VonHoldt BM. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4:359–361. https://doi.org/10.1007/s12686-011-9548-7.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology 14:2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x.

Goudet J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. Journal of Heredity 86:485–486. https://doi.org/10.1093/oxfordjournals.jhered.a111627.

Guillot G, Mortier F, Estoup A. 2005. GENELAND: a computer package for landscape genetics. Molecular Ecology Notes 5:712–715. https://doi.org/10.1111/j.1471-8286.2005.01031.x.

Hardy OJ, Vekemans X. 2002. Spagedi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2:618–620. https://doi.org/10.1046/j.1471-8286.2002.00305.x.

Helle P. 1996. Metsäkauris. In: Lindén H., Hario M., Wikman M., editors. Riistan jäljille. Helsinki (Finland): Finnish Game and Fisheries Research Institute, Edita; p. 100–102.

Hewitt GM. 1999. Post-glacial re-colonization of European biota. Biological Journal of the Linnean Society 68:87–112. https://doi.org/10.1111/j.1095-8312.1999.tb01160.x.

Jombart T. 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405. https://doi.org/10.1093/bioinformatics/btn129.

Lorenzini R, Garofalo L, Qin XB, Voloshina I, Lovari S. 2014. Global phylogeography of the genus *Capreolus* (Artiodactyla: Cervidae), a Palaearctic meso-mammal. Zoological Journal of the Linnean Society 170:209–221. https://doi.org/10.1111/zoj.12091.

Lovari S, Herrero J, Masseti M, Ambarli H, Lorenzini R, Giannatos G. 2016. *Capreolus capreolus*. The IUCN Red List of Threatened Species 2016: e.T42395A22161386. [accessed 2022 Sept 9]. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T42395A22161386.en.

Manni F, Guérard E, Heyer E. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. Human Biology 76:173–190. https://doi.org/10.1353/hub.2004.0034.

Marková S, Horníková M, Lanier HC, Henttonen H, Searle JB, Weider LJ, Kotlík P. 2020. High genomic diversity in the bank vole at the northern apex of a range expansion: the role of multiple colonizations and end-glacial refugia. Molecular Ecology 29:1730–1744. https://doi.org/10.1111/mec.15427.

Matosiuk M, Borkowska A, Świsłocka M, Mirski P, Borowski Z, Krysiuk K, Danilkin AA, Zvychaynaya EY, Saveljev AP, Ratkiewicz M. 2014. Unexpected population genetic structure of European Roe Deer in Poland: an invasion of the mtDNA genome from Siberian Roe Deer. Molecular Ecology 23:2559–2572. https://doi.org/10.1111/mec.12745.

Mucci N, Mattucci F, Randi E. 2012. Conservation of threatened local gene pools: landscape genetics of the Italian roe deer (*Capreolus c. italicus*) populations. Evolutionary Ecology Research 14:897–920.

Niedziałkowska M. 2017. Phylogeography of European Moose (*Alces alces*) based on contemporary mtDNA data and archaeological records. Mammalian Biology 84:35–43. https://doi.org/10.1016/j.mambio.2017.01.004.

Niedziałkowska M, Doan K, Górny M, Sykut M, Stefaniak K, Piotrowska N, Jędrzejewska B, Ridush B, Pawełczyk S, Mackiewicz P, *et al.* 2021a. Winter temperature and forest cover have shaped red deer distribution in Europe and the Ural Mountains since the Late Pleistocene. Journal of Biogeography 48:147–159. https://doi.org/10.1111/jbi.13989.

Niedziałkowska M, Hundertmark KJ, Jędrzejewska B, Niedziałkowski K, Sidorovich VE, Górny M, Veeroja R, Solberg EJ, Laaksonen S, Sand H, *et al.* 2014. Spatial structure in European Moose (*Alces alces*): genetic data reveal a complex population history. Journal of Biogeography 41:2173–2184. https://doi.org/10.1111/jbi.12362.

Niedziałkowska M, Hundertmark KJ, Jędrzejewska B, Sidorovich VE, Zalewska H, Veeroja R, Solberg EJ, Laaksonen S, Sand H, Solovyev VA, *et al.* 2016a. The contemporary genetic pattern of European Moose is shaped by postglacial recolonization, bottlenecks, and the geographical barrier of the Baltic Sea. Biological Journal of the Linnean Society 117:879–894. https://doi.org/10.1111/bij.12713.

Niedziałkowska M, Jędrzejewska B, Danyłow J, Niedziałkowski K. 2016b. Diverse rates of gene flow and long-distance migration in two Moose *Alces alces* subpopulations in Europe. Mammal Research 61:171–178. https://doi.org/10.1007/s13364-016-0274-0.

Niedziałkowska M, Jędrzejewska B, Wójcik JM, Goodman SJ. 2012. Genetic structure of Red Deer population in northeastern Poland in relation to the history of human interventions. Journal of Wildlife Management 76:1264–1276. https://doi.org/10.1002/jwmg.367.

Niedziałkowska M, Tarnowska E, Babik W, Konczal M, Gharbi K, Cezard T, Jędrzejewska B. 2023. Different waves of postglacial recolonisation and genomic structure of bank vole populations in NE Poland. Heredity 130:269–277. https://doi.org/10.1038/s41437-023-00600-1.

Niedziałkowska M, Tarnowska E, Ligmanowska J, Jędrzejewska B, Podgórski T, Radziszewska A, Ratajczyk I, Kusza S, Bunevich AN, Danila G, *et al.* 2021b. Clear phylogeographic pattern and genetic structure of Wild Boar *Sus scrofa* population in Central and Eastern Europe. Scientific Reports 11:9680. https://doi.org/10.1038/s41598-021-88991-1.

Olano-Marin J, Plis K, Sönnichsen L, Borowik T, Niedziałkowska M, Jędrzejewska B. 2014. Weak population structure in European Roe Deer (*Capreolus capreolus*) and evidence of introgressive hybridization with Siberian Roe Deer (*C. pygargus*) in Northeastern Poland. PLoS One 9:e109147. https://doi.org/10.1371/journal.pone.0109147.

Paetkau D, Slade R, Burden M, Estoup A. 2004. Direct, real-time estimation of migration rate using assignment methods: a simulation-based exploration of accuracy and power. Molecular Ecology 13:55–65. https://doi.org/10.1046/j.1365-294x.2004.02008.x.

Peakall ROD, Smouse PE. 2006. Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x.

Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A. 2004. GeneClass2: a software for genetic assignment and first-generation migrant detection. The Journal of Heredity 95:536–539.

Piry S, Luikart G, Cornuet J-M. 1999. Computer note. BOTTLENECK: a computer program for detecting recent reductions in the effective size using allele frequency data. Journal of Heredity 90(4):502–503. https://doi.org/10.1093/jhered/esh074.

Plakhina D, Zvychaynaya EY, Kholodova M, Danilkin A. 2014. Identification of European (*Capreolus capreolus* L.) and Siberian

(*C. pygargus* Pall.) roe deer hybrids by microsatellite marker analysis. Russian Journal of Genetics 50:757–762. https://doi.org/10.1134/S1022795414070151.

Plis K. 2023. Phylogeography of European Roe Deer (*Capreolus capreolus*) in Central and Eastern Europe [Dissertation]. [Warsaw (Poland)]: University of Warsaw.

Plis K, Niedziałkowska M, Borowik T, Lang J, Heddergott M, Tiainen J, Bunevich A, Šprem N, Paule L, Danilkin A, *et al.* 2022a. Pan-European phylogeography of the European Roe Deer (*Capreolus capreolus*). Ecology and Evolution 12:e8931. https://doi.org/10.1002/ece3.8931.

Plis K, Niedziałkowska M, Borowik T, Lang J, Heddergott M, Tiainen J, Bunevich A, Šprem N, Paule L, Danilkin A, *et al.* 2022b. Mitochondrial DNA diversity and the population genetic structure of contemporary roe deer (*Capreolus capreolus*) in Europe. Mammalian Biology 102:1743–1754. https://doi.org/10.1007/s42991-022-00274-y

Poetsch M, Seefeldt S, Maschke M, Lignitz E. 2001. Analysis of microsatellite polymorphism in red deer, roe deer, and fallow deer—possible employment in forensic applications. Forensic Science International 116:1–8. https://doi.org/10.1016/s0379-0738(00)00337-6.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959. https://doi.org/10.1093/genetics/155.2.945.

Pulliainen E. 1980. Occurrence and spread of the roe deer (*Capreolus capreolus* L.) in eastern Fennoscandia since 1970. Memoranda Societatis pro Fauna et Flora Fennica 56:28–32.

Randi E, Alves P, Carranza J, Milošević-Zlatanović S, Sfougaris A, Mucci N. 2004. Phylogeography of roe deer (*Capreolus capreolus*) populations: the effects of historical genetic subdivisions and recent nonequilibrium dynamics. Molecular Ecology 13:3071–3083. https://doi.org/10.1111/j.1365-294X.2004.02279.x.

Rannala B, Mountain JL. 1997. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences of the United States of America 94:9197–9201. https://doi.org/10.1073/pnas.94.17.9197.

Raymond M, Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249. https://doi.org/10.1093/oxfordjournals.jhered.a111573.

Røed KH. 1998. Microsatellite variation in Scandinavian Cervidae using primers derived from Bovidae. Hereditas 129:19–25. https://doi.org/10.1111/j.1601-5223.1998.00019.x.

Røed KH, Midthjell L. 1998. Microsatellites in Reindeer, *Rangifer tarandus*, and their use in other cervids. Molecular Ecology 7:1773–1776. https://doi.org/10.1046/j.1365-294x.1998.00514.x.

Rousset F. 2008. Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources 8:103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x.

Scandura M, Apollonio M, Šprem N. 2014. Reintroductions as a management tool for European ungulates. In: Putman R, Apollonio M, editors. Behaviour and management of European ungulates. Dunbeath: Whittles Publishing; p. 46–77.

Sommer R, Fahlke J, Schmölcke U, Benecke N, Zachos F. 2009. Quaternary history of the European Roe Deer *Capreolus capreolus*. Mammal Review 39:1–16.

Sommer RS, Nadachowski A. 2006. Glacial refugia of mammals in Europe: evidence from fossil records. Mammal Review 36:251–265. https://doi.org/10.1111/j.1365-2907.2008.00137.x.

Steinbach P, Heddergott M, Weigand H, Weigand AM, Wilwert E, Stubbe M, Helm B, Campbell RE, Stubbe A, Frantz AC. 2018. Rare migrants suffice to maintain high genetic diversity in an introduced island population of roe deer (*Capreolus capreolus*):

evidence from molecular data and simulations. Mammalian Biology 88:64–71. https://doi.org/10.1016/j.mambio.2017.11.009.

Stojak J, Tarnowska E. 2019. Polish suture zone as the goblet of truth in post-glacial history of mammals in Europe. Mammal Research 64:477–477. https://doi.org/10.1007/s13364-019-00433-6.

Stronen AV, Jędrzejewska B, Pertoldi C, Demontis D, Randi E, Niedziałkowska M, Pilot M, Sidorovich VE, Dykyy I, Kusak J, *et al.* 2013. North-south differentiation and a region of high diversity in European wolves (*Canis lupus*). PLoS One 8:e76454. https://doi.org/10.1371/journal.pone.0076454.

Świsłocka M, Czajkowska M, Matosiuk M, Saveljev AP, Ratkiewicz M, Borkowska A. 2019. No evidence for recent introgressive hybridization between the European and Siberian roe deer in Poland. Mammalian Biology 97:59–63. https://doi.org/10.1016/j.mambio.2019.04.008.

Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF. 1998. Comparative phylogeography and postglacial colonization routes in Europe. Molecular Ecology 7:453–464. https://doi.org/10.1046/j.1365-294x.1998.00289.x.

Tarnowska E, Niedziałkowska M, Gerc J, Korbut Z, Górny M, Jędrzejewska B. 2016. Spatial distribution of the Carpathian and Eastern mtDNA lineages of the Bank Vole in their contact zone relates to environmental conditions. Biological Journal of the Linnean Society 119:732–744. https://doi.org/10.1111/bij.12764.

Tarnowska E, Niedziałkowska M, Jędrzejewska B. 2019. Genetic structure of Bank Vole populations in the contact zone of two lineages in north-eastern Poland. Mammalian Biology 96:93–101. https://doi.org/10.1016/j.mambio.2018.10.011.

Tsaparis D, Sotiropoulos K, Legakis A, Kotoulas G, Kasapidis P. 2019. New phylogeographic insights support the distinctiveness and conservation value of the little-known Greek roe deer populations. Mammalian Biology 96:23–27. https://doi.org/10.1016/j.mambio.2019.03.010.

Vähä J-P, Erkinaro J, Niemelä E, Primmer CR. 2007. Life-history and habitat features influence the within-river genetic structure of Atlantic Salmon. Molecular Ecology 16:2638–2654. https://doi.org/10.1111/j.1365-294X.2007.03329.x.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x.

Veličković N, Ferreira E, Djan M, Ernst M, Obreht Vidaković D, Monaco A, Fonseca C. 2016. Demographic history, current expansion and future management challenges of Wild Boar populations in the Balkans and Europe. Heredity 117:348–357. https://doi.org/10.1038/hdy.2016.53.

Vial L, Maudet C, Luikart G. 2003. Thirty-four polymorphic microsatellites for European Roe Deer. Molecular Ecology Notes 3:523–527. https://doi.org/10.1046/j.1471-8286.2003.00499.x.

Wójcik JM, Kawałko A, Marková S, Searle JB, Kotlik P. 2010. Phylogeographic signatures of northward post-glacial colonization from high-latitude refugia: a case study of bank voles using museum specimens. Journal of Zoology 281:249–262. https://doi.org/10.1111/j.1469-7998.2010.00699.x.

Zachos FE, Frantz AC, Kuehn R, Bertouille S, Colyn M, Niedziałkowska M, Pérez-González J, Skog A, Sprěm N, Flamand M-C. 2016. Genetic structure and effective population sizes in European Red Deer (*Cervus elaphus*) at a continental scale: Insights from microsatellite DNA. The Journal of Heredity 107:318–326. https://doi.org/10.1093/jhered/esw011.

Zachos FE, Hartl GB. 2011. Phylogeography, population genetics and conservation of the European Red Deer *Cervus elaphus*. Mammal Review 41:138–150. https://doi.org/10.1111/j.1365-2907.2010.00177.x.