Received: 28 September 2022 | Revised: 13 May 2023 | Accepted: 16 May 2023

DOI: 10.1002/jwmg.22454

RESEARCH ARTICLE

THE JOURNAL OF WILDLIFE MANAGEMENT

THE WILDLIFE SOCIETY

Red deer in the Pyrenees: a risky secondary contact zone for conservation genetics

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Abstract

Natural events over time, and human interventions, influence the genetic structure of species. The red deer (*Cervus elaphus*) is widely distributed in Europe, with a large-scale genetic structure largely determined by Pleistocene climatic oscillations. The Iberian Peninsula acted as one of the main glacial refuges for many species; a particular red deer lineage remains on the peninsula and is subjected to special conservation policies. The mountain range of the Pyrenees might be a contact zone where Iberian red deer (*Cervus elaphus hispanicus*) could hybridize with other central European genetic lineages. In the late twentieth century, the natural distribution areas of red deer on both sides of the Pyrenees became closer because of restocking from central Iberia to areas south of the Pyrenees and from French populations to areas north of the Pyrenees. We analyzed the genetic structure of red deer populations in the Pyrenees to investigate the underlying processes of population contact and hybridization. The analysis of microsatellite genotypes showed 2 genetic clusters. One of these clusters associated with Iberian red deer, whereas the other presented European non-Iberian genetic composition. Migration and hybridization events occurred between both genetic

Funding information
Generalitat de Catalunya, Grant/Award Number: AG-2021-1064; Government of Andalusia and ERDF of European Union, Grant/Award Number: P18-RT-4642

clusters, mostly in the eastern part of the sampling area. The Pyrenees is currently a secondary contact zone caused by anthropogenic translocations, and a risky hybrid belt for red deer genetic conservation.

KEYWORDS

Cervus elaphus, genetic structure, hybridization, Iberian Peninsula, microsatellite markers, sex-specific dispersal

Intraspecific genetic structure is a fundamental element of biodiversity that yields valuable information on species' conservation and is applicable to wildlife management (Frankham 2005, Hughes et al. 2008, Laikre et al. 2020, White et al. 2020). The genetic structure of species was affected by Pleistocene climatic oscillations that caused contractions and expansions of species' distribution ranges and the existence of southern refugia in which populations remained relict during glacial periods (Hewitt 1996, 2000; Taberlet et al. 1998; Schmitt 2007). In Europe, the 3 large Mediterranean peninsulas (Iberian, Italian, Balkan) acted as glacial refuges where different genetic lineages evolved (Hewitt 1996, 1999, 2000; Taberlet et al. 1998; Schmitt 2007). These differentiated genetic lineages increase the genetic diversity of species and can constitute infraspecific taxa that in certain circumstances deserve conservation efforts (Crandall et al. 2000, Coates et al. 2018).

The Pyrenees mountain range is located between the Iberian Peninsula and the rest of Europe (Figure 1). The range's west-east orientation and the cover of ice sheets during glaciations acted as a barrier for the expansion and contraction of species during the Pleistocene climatic oscillations (Taberlet et al. 1998). Therefore, this geographical barrier had a relevant role in the demography, distribution, and genetic structure of species (Hewitt 1996, 1999, 2000; Taberlet et al. 1998; Schmitt 2007). Furthermore, after the glacial periods, the Pyrenees had the potential to become a secondary contact zone where different evolutionary lineages might hybridize (Taberlet et al. 1998, Asztalos et al. 2020). Hybrid zones are of interest to scientists and managers because of the evolutionary and conservation issues (Barton and Hewitt 1985, Allendorf et al. 2001).

Anthropocene-related changes and anthropogenic manipulations of wildlife have relevant effects on the density, distribution, and, hence, the genetic structure of wild populations (Weeks et al. 2011, Dirzo et al. 2014, Newbold et al. 2016, Lamb et al. 2019). For instance, translocations (movement of individuals from one locality to another) and augmentations (release of individuals into an existing population) have been used to combat declining populations or to intentionally modify features of local populations (Singer et al. 2000, Carranza et al. 2003, Barbanera et al. 2010, Weeks et al. 2011). These human activities can alter the genetic structure of species and cause conservation issues (Allendorf et al. 2001, Carranza et al. 2003, Barbanera et al. 2010).

The current genetic structure of red deer (*Cervus elaphus*) has been greatly influenced by Quaternary glacial pulses that restricted populations to Mediterranean refugia, mainly in the Iberian, Italian, and Balkan peninsulas (Taberlet et al. 1998; Hewitt 1999, 2000; Meiri et al. 2013). These glacial refugia determined the evolution of ≥3 differentiated genetic lineages that shape the current genetic structure of red deer in Europe: western European lineage, eastern European lineage, and Mediterranean lineage in North Africa and the Tyrrhenian islands (Skog et al. 2009). Moreover, genetically distinctive groups have been detected in some areas that acted as glacial refugia (Zachos and Hartl 2011, Zachos et al. 2014, Carranza et al. 2016). For instance, Skog et al. (2009) reported that red deer from Mesola, Italy present a D-loop ancient haplotype with uncertain placement between the western and eastern European lineages.

The Iberian Peninsula has red deer populations with differentiated genetic composition of the genetic pool of the western European lineage (Fernández-García et al. 2014; Carranza et al. 2016; Queirós et al. 2019, 2020). The genetic identity of red deer from the Iberian Peninsula corresponds to morphological particularities that made researchers propose the existence of a subspecies: Iberian red deer (*Cervus elaphus hispanicus*; Geist 1998). Some of

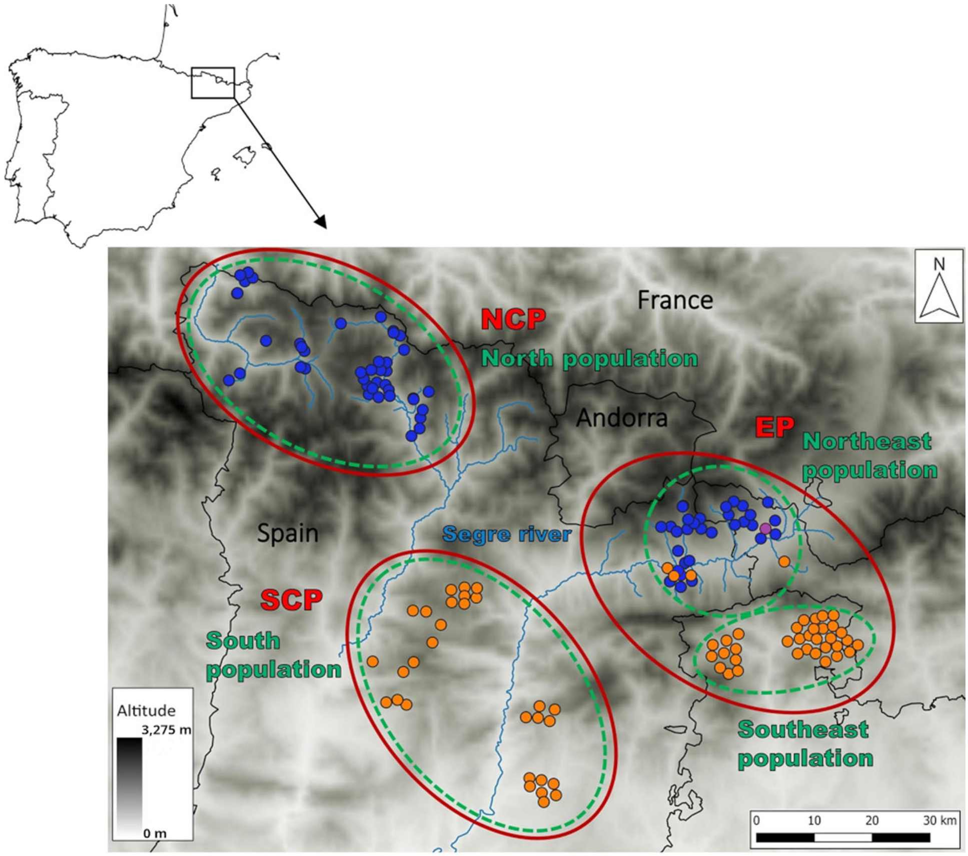

FIGURE 1 Study areas and location of red deer that were hunted in the Pyrenees, Spain, 2020–2021. We present the 3 study areas (red ellipses): north-central Pyrenees (NCP), south-central Pyrenees (SCP), and eastern Pyrenees (EP). Green ellipses indicate the populations established after considering the distribution of geographic proximity and genetic structure. Points indicate the locations of red deer individuals assigned to cluster 1 (blue; *n* = 72; qi [membership coefficient] >0.7 to cluster 1) or cluster 2 (orange; *n* = 68; qi > 0.7 to cluster 2), and 1 individual with an intermediate probability of assignation to each cluster (purple; qi = 0.56 to cluster 2). We used jitter points for those individuals with the same location. Blue lines indicate main rivers and streams and black lines in Spain divide provincial boundaries.

these morphological particularities are smaller size, smaller antlers, the lack of mane in the neck of males, or the protrusion of the tongue when males roar (Geist 1998, Frey et al. 2012). Accordingly, conservation efforts have been proposed to preserve the Iberian red deer (Carranza et al. 2003, 2016).

Human-mediated factors such as introductions from non-Iberian populations threaten the genetic conservation of Iberian red deer (Carranza et al. 2016, Queirós et al. 2020). Hunting biases towards males also alter population structures that affect behavioral processes and, hence, the genetic structure of populations (Torres-Porras et al. 2014). For instance, Pérez-González and Carranza (2009) reported that Iberian red deer populations with altered population structures presented a female-biased dispersal pattern rather than the typical male-biased dispersal (Clutton-Brock et al. 1982) based on the sex-specific genetic structure (Chesser 1991, Aars and Ims 2000, Prugnolle and de Meeus 2002). Genetic cohesion among populations is maintained by the dispersing sex, and the genetic structure tends to be established by the philopatric sex (Mossman and Waser 1999). Consequently, at a local scale, the pairwise genetic distance between individuals of the philopatric sex should be higher than between individuals of the dispersing sex. As the geographical distance between individual pairs increases, the genetic distance between individuals of the philopatric sex should increase. In the case of the dispersing sex, the positive relationship between the geographical and genetic distance should decrease (Nussey et al. 2005, Coulon et al. 2006).

Pyrenean populations of red deer disappeared during the last centuries (Gortázar et al. 2000, Carranza 2007). Starting in the 1970s, Iberian red deer were translocated to the southern part of the mountain range from populations of central Spain (Marco et al. 1995; Figure S1, available in Supporting Information). These individuals present morphological features typical of the nominal Iberian subspecies. Since the 1980s in the Spanish northern part of the Pyrenees, individuals have been observed with morphological features not typically associated with Iberian red deer (mainly big body sizes, neck mane, and lower number of antler tines). These individuals in the northern Pyrenees were migrants from populations located in southern France that, in turn, were translocated from other French northern areas. Although they continued to expand, the deer from France and those of Mediterranean origin released farther south remained geographically separated for ≥2 decades. During the last 5–10 years, the distribution centers of both populations have been getting closer, which has meant that the distribution is already more or less continuous in some areas (Figure S1).

The current situation of red deer in the Pyrenees deserves attention because this mountain range can act as a contact and hybrid zone of genetically different lineages of red deer. In such a case, the hybridization would be the consequence of artificial translocations of individuals with potential risk to the conservation of the genetic identity of Iberian red deer.

We hypothesized that the Pyrenees range is an anthropogenic secondary contact zone for red deer. We predicted that in the study area there are 2 set of individuals with different morphologies, genetic compositions, and anthropic origins; genetically different individuals are in contact; and there are hybrid individuals in the contact zone. Thus, the aims of this study were to 1) confirm the presence of morphometric differences between males from the northern and southern Pyrenees, 2) find the number of genetic clusters and the genetic structure of red deer in the Pyrenees, 3) determine the level of hybridization between the genetic clusters, 4) establish the origin of the different genetic clusters, 5) characterize the sex-specific genetic structure, and 6) propose actions to further research and to manage red deer in the Pyrenees.

STUDY AREA

We carried out the present study in Spain from 2015–2021 in 3 geographic areas with ecological differences: north-central (NCP; 101,720 ha), south-central (SCP; 114,854 ha), and eastern Pyrenees (EP; 127,202 ha; Figure 1). The NCP had an Atlantic, montane and subalpine climate, with temperate wet summers and autumns, and very cold days and a large snow cover in winter and spring. Averaged precipitation per month during 2020 was 66.5 mm in the area (https://www.idescat.cat; accessed 1 Feb 2023). Mean temperature in 2020 was 12°C. The maximum altitude is >3300 m. This area is made up of a group of mountains that are subdivided geographically into a series of valleys that coincide with the headwaters of the main river basins of the western Pyrenees. For that reason, the landscape was composed of several altitudinal strata that give rise to varied ecosystems from alpine, very similar to taiga or tundra, subalpine and middle mountain ecosystems with extensive forests (deciduous and coniferous) between, and a mostly anthropized ecosystem in parts of the valley bottoms. The dominant fauna in this area was boreoalpine, such as white partridge (*Lagopus muta*), grouse (*Tetrao urogallus*), and chamois (*Rupicapra rupicapra*). Other ungulates including red deer, fallow deer (*Dama dama*), and wild boar (*Sus scrofa*) were also present. In the NCP, the red deer was present until recent times and came from spontaneous natural recolonization from deer translocated from other parts of France. Here, males had morphological features not typically associated with Iberian red deer: they appeared to have larger bodies and antler sizes.

The SCP had a continental Mediterranean high mountain climate, with hot and dry summers (often resulting in periods of drought), cold winters with snow accumulation (but clearly less than in the NCP), and most rainfall concentrated in spring and autumn. Averaged precipitation per month during 2020 was 50.8 mm. Mean temperature in 2020 was 13.2°C. The maximum altitude is around 2100 m. This area is located between 2 large valleys, and includes a series of mountain ranges arranged transversely, among which 3 of the largest ones stand out. The dominant ecosystem was Mediterranean and Sub Mediterranean with pine forests (black pine [*Pinus nigra*], Scotch pine [*P. sylvestris*], bog pine [*P. uncinata*]) with some subalpine meadows in the culminating levels, and holm oaks (*Quercus ilex*), accompanied by large areas of thermophilic bushes at low mountain ecosystems. The most characteristic species of this area was red deer, although necrophagous birds such as the griffon vulture (*Gyps fulvus*), black vulture (*Aegypius monachus*), and bearded vulture (*Gypaetus barbatus*) were also prominent. In SCP, red deer were introduced from diverse areas of the Iberian Peninsula, and males had morphologies typically associated with Iberian red deer.

The EP was an area similar to SCP with some peculiarities. The maximum altitude is 2915 m. This area is made up of a main range with several other peaks >2000 m and a set of smaller companion ranges. Averaged precipitation per month during 2020 was 55.4 mm. Mean temperature in 2020 was 10°C. The climate was also continental Mediterranean high mountain; however, it was very humid, which gave rise to ecosystems such as fir (silver fir [*Abies alba*], Scotch pine, black pine) and deciduous forests (beech [*Fagus sylvatica*], oaks, birches [*Betula* spp.], common hazel [*Corylus avellana*]). The most representative ungulate was the chamois, although deer species and wild boar were also present. In the southern part of EP, red deer were introduced from an Iberian population (Quintos de Mora, central Spain; Figure S2, available in Supporting Information), and males presented Iberian morphologies. In the northern part, close to the French border and around the Segre River (Figure 1), deer also came spontaneously and naturally from the French side. Here, non-Iberian morphologies, characterized by smaller bodies and antler sizes, were abundant. The main land uses in the 3 areas were for livestock, forestry, agriculture in the valley bottoms, and tourism.

METHODS

Morphometric measures and age

To confirm the existence of 2 morphologies in the study area, we collected morphometric measures of males from the areas where all individuals looked morphologically different. Because we observed individuals in EP with both morphologies, we collected morphometric measures of 41 males from NCP ($n = 27$) and SCP ($n = 14$). The measurements were body length (from the lip to the tail, in cm), body height (at the withers, in cm), antler thickness (diameter of the antler between candles and central; average of both antlers, in cm), antler length (length between the base of the antler and the tip of the larger tine of the crown; average of both antlers, in cm), and the number of antler tines (sum of both antlers). Forest rangers and hunters obtained these data during regular hunting and management culling by local regulated hunting plans at each area from 2015 to 2019.

Forest rangers and hunters collected the lower first molar of each male. We determined the age of individuals using skeletochronological techniques and a standardized method of counting growth marks in the cementum of the lower first molar (Azorit et al. 2004, Azorit 2011).

We assessed morphometric differences between males from NCP and SCP by linear regression models (Chambers 1992). We performed 5 linear models using the morphometric measures (body length, body height, antler length, antler thickness, and the number of tines) as dependent variables, and both area (northern vs. southern) and age as explanatory variables. Initially, we included the interaction term in the models, but we removed it from all models because of the lack of significance. We used adjusted *P*-values for multiple comparisons (Holm 1979).

Collection of samples, DNA isolation, and microsatellite genotyping

Forest rangers and hunters collected a piece of ear cartilage from 141 red deer from the 3 geographic areas: NCP ($n = 43$), SCP ($n = 30$), and EP ($n = 68$). Out of the 141 samples, 75 were females, 42 were males, and 24 samples did not have a recorded sex. Forest rangers and hunters provided exact harvest locations for 56 individuals. For the remaining 85 individuals, we considered the centroid of hunting areas as location sites. Specimens were hunted between 2020 and 2021 during regular sport hunting and management culling by local regulated hunting plans at each area.

We isolated DNA from ear-tags using the BioSprint® 96 DNA Blood kit (Qiagen, Carlsbad, CA, USA) according to the manufacturer protocol. To the nuclear DNA amplification, we genotyped the samples for a set of 12 microsatellite markers according to the methodology described by Carranza et al. (2016). For each polymerase chain reaction (PCR), we used the same positive control with a known genotype and a negative control without a sample. We used the positive control to ensure that the peaks were correctly identified and the negative control to determine the existence of unspecific amplifications. We have used the same positive control in hundreds of PCRs and the identified genotype was always the same. The Central Research Support Service of the University of Córdoba, Spain, resolved the PCR products by capillary electrophoresis using a DNA sequencer (3130 xl; Applied Biosystem, Waltham, MA, USA). We determined the allele sizes after processing the raw data with GeneMapper® software 5 using a LIZ 500 bp as the internal size standard (Applied Biosystems). We used Microchecker software (Van Oosterhout et al. 2004) to determine the existence of genotyping errors due to null alleles, large allele dropout, and the scoring of stutter peaks. We did not detect genotyping errors. We also used Genepop on the Web (Raymond and Rousset 1995) to detect linkage disequilibrium between loci. After adjustment for multiple comparisons, 2 pairs of loci had linkage disequilibrium in the Pyrenean red deer populations (BM1818-CSSM19, $P < 0.001$; BM1818-CSSM43, $P = 0.006$). We repeated the downstream analyses after removing the BM1818 locus and results did not change (Table S3, available in Supporting Information).

Genetic structure, diversity, and differentiation

We used the Bayesian methodology implemented by Structure version 2.3.4 (Pritchard et al. 2000) to infer genetic structure in the Pyrenees independently of the sampling location of individuals. We ran 10 independent runs from $K = 1$ to $K = 4$, with 500,000 iterations, following a burn-in period of 100,000 iterations. Even though our hypothesis predicted that the red deer in the Pyrenees might exhibit 2 divergent sets of genetic compositions, we chose $K = 4$ as the upper limit in the Structure analysis because we studied red deer in 3 geographic areas (the ΔK method [see below] requires ≥4 K values to assess ΔK in $K = 3$). We tested higher upper limits in the analyses, but the results remained consistent. We used an admixture model, correlated allele frequencies and the ancestdist output option as run parameters. We analyzed the model output using Structure Harvester (Earl and VonHoldt 2012) according to the ΔK method described in Evanno et al. (2005). We averaged the 10 runs of the K value with the highest ΔK value using CLUMPP version 1.1.2 (Jakobsson and Rosenberg 2007) and displayed by Distruct version 1.1 (Rosenberg 2003).

We considered geographical proximity (individuals sampled in NCP, SCP, or EP) and the distribution of genetic clusters to obtain the number and distribution of populations in the study area. We quantified genetic diversity measures (number of alleles [A], observed heterozygosity [Ho], and expected heterozygosity [He]) and departures from Hardy-Weinberg equilibrium (HWE) by exact test using Markov chain methods across loci and populations with adegenet package version 2.1.5 (Jombart 2008) in R software (R Core Team 2019). We quantified the number and proportion of private alleles per locus and cluster or population in R software. We also used adegenet to perform a principal component analysis (PCA) summarizing the genetic diversity among sampled individuals. For this PCA, we replaced missing data by the mean allele frequency and retained the 2 principal components (PCs) that explained the highest amount of variance in the dataset. Because the plot of eigenvalues sharply decreased beyond the third PC (see Results), we also show the results of the PCA analysis after retaining 3 PCs. Finally, we quantified the overall and pairwise fixation index (Fst) values with Genetix 4.05 (Belkhir et al. 2004). We also used Genetix to assess whether Fst values were significantly different from 0 with 10,000 permutations.

Hybridization analyses, origin of the genetic clusters, and sex-specific genetic structure

We used NewHybrids (Anderson and Thompson 2002) to perform a hybrid detection analysis between the genetic clusters of Pyrenees. We performed 10 replicate runs with 100,000 burn-in repetitions and 500,000 sweeps and averaged posterior probabilities for each individual. We considered 6 genotype frequency classes: pure non-Iberian (non-Iberian cluster in Structure analysis), pure Iberian (Iberian cluster in Structure analysis), first generation hybrid (F1), second generation hybrid (F2), backcross non-Iberian (the product of the mating between a non-Iberian individual and an F1), and backcross Iberian (the product of the mating between an Iberian individual and an F1).

The red deer population in our study area south of the Pyrenees originated from restocked individuals from central Spanish populations, whereas red deer north of the Pyrenees presumably come from French populations. We are not completely sure about the provenance of all individuals that might have been translocated from other European areas. We used additional genotypes from a reference panel of 228 red deer samples from diverse areas (Figure S2). These samples were genotyped with the same set of microsatellite markers as the Pyrenees samples between 2010 and 2021. Those additional red deer samples included 136 individuals from 8 Iberian populations, 35 individuals from the French population of Petite Pierre, 17 individuals from Norway, 19 individuals from Scotland, and 21 individuals from Germany. We chose a population from mainland Europe (Germany) and 2 peripheral European populations (Scotland, Norway) to comprise the highest amount of genetic diversity of red deer in western Europe (Carranza et al. 2016).

We pooled the Pyrenean and the additional red deer samples and used the Bayesian methodology described above to infer the genetic structure in these 369 microsatellite genotypes. We used the same run parameters, although here we ran 10 independent runs from $K = 1$ to $K = 15$. We chose $K = 15$ as the maximum value considered because we analyzed 2 hypothetical Pyrenean populations and 12 additional populations (the ΔK method requires ≥ 15 K values to assess ΔK in $K = 14$). We retained the K value with the highest ΔK value. As above, we averaged the results of the independent runs using CLUMPP and displayed by Distruct. Additionally, we quantified pairwise Fst values among Pyrenean populations, the red deer population in France, and significant differences from 0, with Genetix. We used R to construct a cladogram with these pairwise Fst values.

For each obtained population, we conducted genetic structure analyses separately for males and females. In this case, we performed isolation by distance (IBD) analyses with the adegenet package. We tested IBD using Mantel test between the matrix of genetic distance (Nei's distance) and the matrix of geographical distances (Euclidean distances between sampling points). We also performed graphical representation of the relationship between genetic and geographical distances and kernel density estimations with the adegenet package.

RESULTS

Morphometric differences

The males sampled in NCP were taller and presented thicker antlers than the males sampled in SCP (body height: NCP, mean ± SD = 120.074 ± 7.195 cm; SCP = 110.000 ± 7.059 cm; antler thickness: NCP = 11.071 ± 1.912 cm; SCP = 9.500 ± 1.920 cm). Males from NCP were 9.16% taller and had antlers 16.54% thicker than those from

SCP. Males that were 4.5 years old from NCP ($n = 9$) were 11.16 cm taller and had antlers 1.62 cm thicker than those from SCP ($n = 5$).

The data indicated body height ($P < 0.001$) and antler thickness ($P = 0.009$) differed by geographic region after correcting by the age of individuals (Table S1, Figure S3, available in Supporting Information). Differences in body height tended to be higher for younger males, whereas differences in antler thickness tended to be higher for older males. The interaction between age and geographical area (northern vs. southern) did not reach significance for any morphological variable (not shown).

Genetic structure, diversity, and differentiation

The ΔK value for the Structure analysis was higher for K = 2 (ΔK = 894.406) than for K = 3 (ΔK = 0.365). Moreover, after the increase of the upper limit, the Structure analysis supported 2 genetic clusters in the Pyrenees. Individuals from NCP were mostly assigned to cluster 1 (97.67% of the individuals with probability intervals including 1 for cluster 1); individuals from SCP were assigned to cluster 2 (100% of the individuals with probability intervals including 1 for cluster 2); and individuals from EP assigned to both clusters 1 and 2 (Figure 2). The spatial distribution of individuals was related to the cluster for which they had the highest membership probability (Figure 1). Geographic distribution of individuals and clusters support the existence of 4 populations: the north population that coincides with NCP, the south population that coincides with SCP, the northeast population around the Segre riverbed, and the southeast population several kilometers south of the Segre River (northeast and southeast populations in EP; Figure 1). The Structure analysis assigned 4 individuals from the northeast population to the cluster that was mainly formed by south and southeast populations (these individuals had probability intervals including 1 for cluster 2). Furthermore, there was 1 individual from the northeast population that had an intermediate probability for each cluster (Figures 1 and 2). The number of private alleles in cluster 1 (north and northeast populations) was 6.68% higher than in cluster 2 (south and southeast populations; Table 1). The proportion of private alleles in cluster 1 was 24.11% higher than in cluster 2 (Table 1).

The south population had the highest mean number of alleles (9.19% higher than in the northeast population; Table 2). The southeast population had the highest values of Ho (3.58% higher than in the south population; Table 2). The northeast population had the highest value of He (4.49% higher than in the southeast population; Table 2). Three out of the 4 populations presented ≥1 locus with significant departures from HWE. The south population had 6 loci with significant departures from HWE. Two loci (FCB193, JP38) were not in equilibrium in 2 populations. We repeated the Structure analysis without these 2 loci and results did not change.

Overall Fst considering the 4 populations was 0.104 (significantly different from 0, 10,000 permutations, $P < 0.001$). All pairwise Fst values were also significantly different from 0 (10,000 permutations $P < 0.001$). The north-southeast pair of populations had the highest genetic differentiation (Fst = 0.155) and south-southeast pair of populations had the lowest genetic differentiation (Fst = 0.050; Table S2, available in Supporting Information).

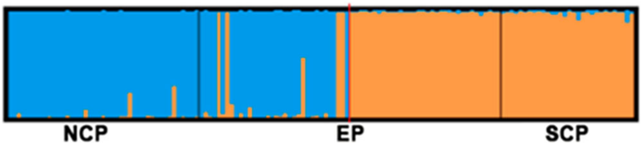

FIGURE 2 Structure results for red deer thar were hunted in the Pyrenees, Spain, 2020–2021, in 3 regions: north-central Pyrenees (NCP; $n = 43$), eastern Pyrenees (EP; $n = 68$), and south-central Pyrenees (SCP; $n = 30$). We present the probability of membership for each cluster (K = 2): cluster 1 (blue) or cluster 2 (orange). The vertical red line divides individuals from EP into those individuals sampled around the Segre River (left of the red line) and those several kilometers south of the Segre River (right of the red line).

TABLE 1 Number and proportion of private alleles (PA) per locus for the 2 genetic clusters for red deer that were hunted in the Pyrenees, Spain, 2021–2021.

	Number of PA		Proportion of PA	
Locus	Cluster 1	Cluster 2	Cluster 1	Cluster 2
BM1818	0	0	0.000	0.000
CP26	2	4	0.125	0.250
CSSM19	4	0	0.286	0.000
CSSM43	10	11	0.345	0.379
ETH225	2	4	0.143	0.286
FCB193	3	5	0.150	0.250
FCB304	3	2	0.231	0.154
FCB5	2	1	0.154	0.077
JP38	2	0	0.200	0.000
MM12	1	0	0.167	0.000
RME25	1	1	0.167	0.167
TGLA53	2	2	0.133	0.133
Mean	2.667	2.500	0.175	0.141

TABLE 2 Genetic diversity measures and significant departures from HWE (*) across loci and populations for red deer that were hunted in the Pyrenees, Spain, 2020–2021. We present the number of alleles (A), the raw number of private alleles (PA), observed heterozygosity (Ho), and expected heterozygosity (He) for the 4 populations: north (*n* = 43), south (*n* = 30), northeast (*n* = 34), and southeast (*n* = 34).

	North			South			Northeast			Southeast		
Locus	A/PA	Ho	He	A/PA	Ho	He	A/PA	Ho	He	A/PA	Ho	He
BM1818	5/0	0.58	0.62	8/0	0.53	0.81*	8/0	0.68	0.80	5/0	0.65	0.64
CP26	9/1	0.71	0.76	10/1	0.78	0.78	10/0	0.64	0.68	12/1	0.79	0.85
CSSM19	8/1	0.65	0.70	9/0	0.79	0.84*	12/3	0.76	0.81	7/0	0.85	0.80
CSSM43	11/2	0.82	0.81	16/7	0.75	0.89*	14/2	0.80	0.87	9/2	0.83	0.79
ETH225	7/2	0.63	0.64	12/1	0.93	0.85	5/0	0.56	0.65	9/0	0.74	0.82
FCB193	13/1	0.67	0.78	14/2	0.70	0.90*	10/1	0.39	0.84*	8/3	0.38	0.75*
FCB304	7/0	0.74	0.72	8/0	0.63	0.75	10/3	0.91	0.82	9/1	0.76	0.78
FCB5	10/0	0.74	0.73	10/0	0.77	0.86*	10/1	0.76	0.82	9/1	0.85	0.84
JP38	7/1	0.72	0.69	7/0	0.53	0.61*	8/1	0.65	0.77*	8/0	0.76	0.78
MM12	6/1	0.72	0.74	5/0	0.37	0.39	4/0	0.79	0.69	4/0	0.53	0.52
RME25	4/0	0.35	0.40	4/0	0.62	0.62	5/1	0.50	0.50	5/1	0.71	0.68
TGLA53	10/1	0.86	0.85	11/2	0.97	0.86	9/0	0.74	0.83*	8/0	0.82	0.80
Mean	8.1/0.8	0.683	0.703	9.5/1.1	0.698	0.708	8.7/1.0	0.682	0.757	7.7/0.7	0.723	0.754

The south population had the highest mean number of private alleles (Table 2). The northeast population presented the highest mean proportion of private alleles (mean proportion of private alleles: north = 0.061, south = 0.051, northeast = 0.076, southeast = 0.051; Figure S4, available in Supporting Information). Therefore, mean number of private alleles in the northeast population was 24.59% higher than in the north population, and 49.02% higher than in the south and southeast populations.

For the PCA analysis, we retained the 2 PCs that explained the highest amount of variance (Figure S5, available in Supporting Information). Individuals from the north population were grouped in the PCA plot (Figure 3; Figure S6, available in Supporting Information), whereas the scores for the south population were scattered along axis 2 (PC2; Figure 3). Individuals from the northeast and southeast had scores that overlapped with those from the north and south populations, respectively (Figure 3). Some individuals from the northeast population had scores that overlapped with those from the south and southeast populations (Figure 3).

Hybridization analyses, origin of the genetic clusters, and sex-specific genetic structure

Most individuals (84.09%) from the north population had a high probability (>0.9) of being pure non-Iberian (Figure 4). There were 2 individuals in the north population (4.7%) with a high probability (>0.9) of being hybrids (F2 or backcross non-Iberian). For the south population, the percentage of individuals with a high probability (>0.9) of being pure Iberian was 71.43% (Figure 4). There were 5 individuals in the south population (16.7%) with intermediate probabilities of being hybrids (F2 or backcross Iberian). For the northeast population, the percentage of individuals with a high probability (>0.9) of being pure non-Iberian was 61.76% (Figure 4). There were 4 individuals (males) with a high probability of being pure Iberian that can be considered as migrant individuals and 1 individual with a high probability (>0.9) of being hybrid. Most individuals (94.12%) from the southeast population had a high probability (>0.9) of being pure Iberian (Figure 4).

The Structure analysis with the Pyrenean and additional red deer samples supported 3 genetic clusters (K = 3, Figure 5A). The French individuals assigned to 1 genetic cluster, the non-Iberian populations mostly assigned to a second cluster, and the Iberian samples had high assignment values to a third cluster

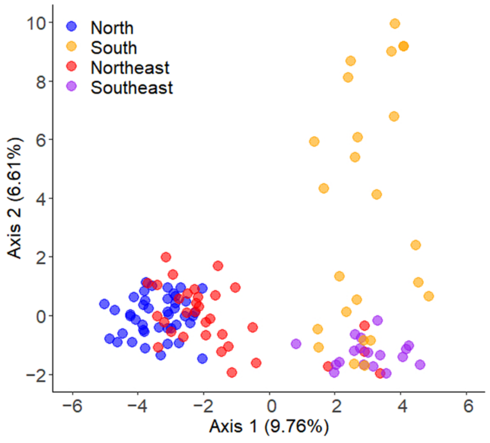

FIGURE 3 Representation of the principal component analysis (PCA) with adegenet for red deer that were hunted in the Pyrenees, Spain, 2020–2021: north population (n = 43), south population (n = 30), northeast population (n = 34), and southeast population (n = 34). Two principal components were retained in the PCA. The first principal component (axis 1) explained 9.761% of the total variance in the sample and the second principal component (axis 2) explained 6.107% of the variance.

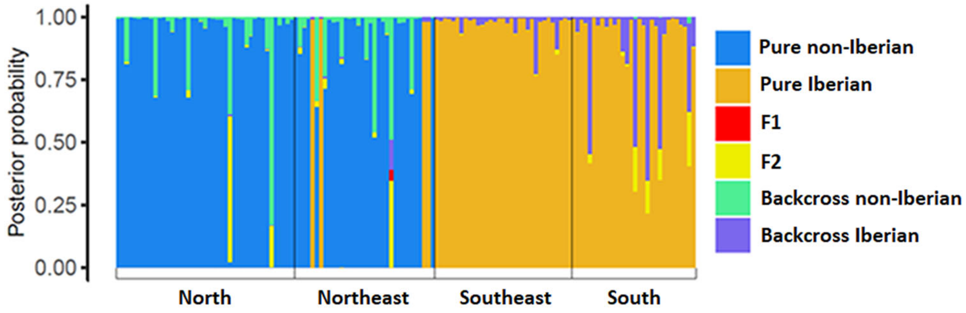

FIGURE 4 Posterior probabilities of the genotype frequency classes for the red deer that were hunted in the Pyrenees, Spain, 2020–2021. Individuals from each population are represented on the *x*-axis ($n = 141$). Different colors indicate the probability an individual is pure non-Iberian, pure Iberian, first generation hybrid (F1), second generation hybrid (F2), backcross non-Iberian (the product of the mating between a non-Iberian individual and an F1 hybrid) and backcross Iberian (the product of the mating between an Iberian individual and an F1 hybrid).

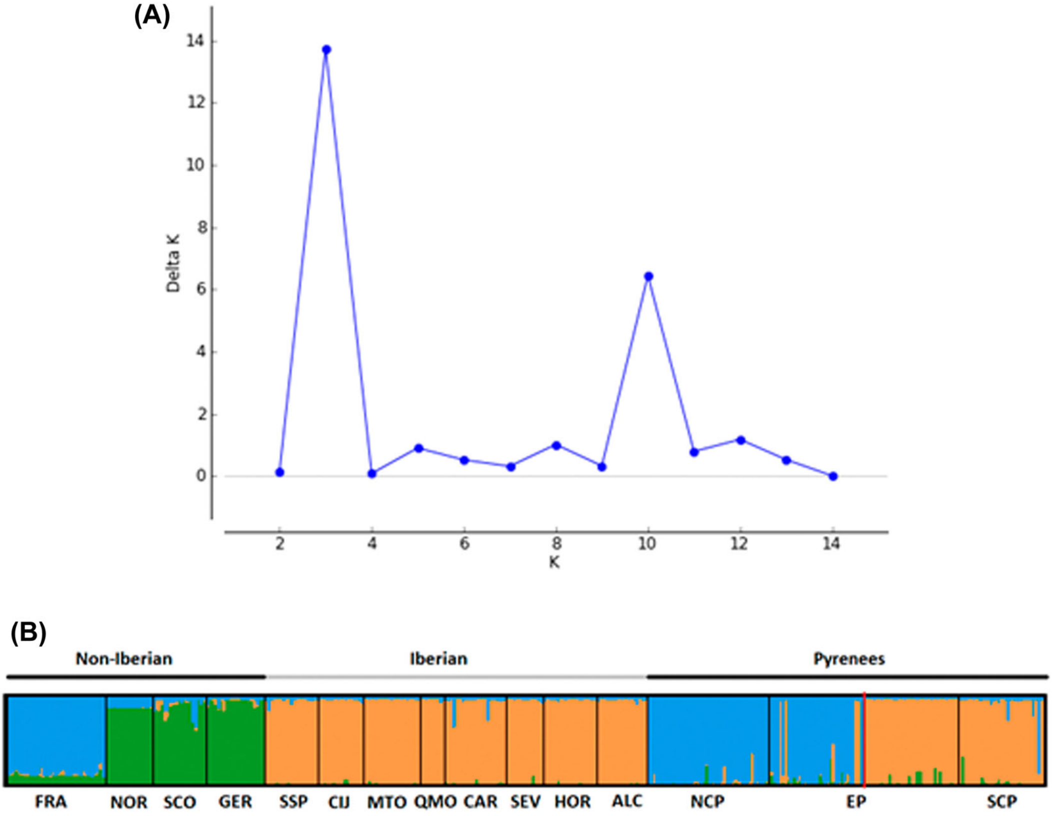

FIGURE 5 Structure results for red deer from samples that were collected in the Pyrenees, Spain, and other Spanish areas (SSP through ALC), France (FRA), Norway (NOR), Scotland (SCO), and Germany (GER), 2010–2021. A) Delta K (ΔK) results obtained with Structure Harvester. B) Membership coefficient of probability for each cluster ($K = 3$). Pyrenees samples were collected in the north-central Pyrenees (NCP), eastern Pyrenees (EP), and south-central Pyrenees (SCP). Pyrenean individuals ($n = 141$) and red deer from the other areas ($n = 228$) constituted the database.

(Figure 5B). Red deer from the Pyrenees were assigned to the French or the Iberian cluster (Figure 5B). Individuals from NCP were assigned to the French cluster, most individuals from SCP had high assignment values to the Iberian cluster, and individuals from EP assigned to the French cluster or the Iberian cluster (Figure 5B). The individuals assigned to cluster 1 of Pyrenees (Figure 2) tended to be assigned to the French cluster (Figure 5B). Individuals assigned to cluster 2 of Pyrenees (Figure 2) tended to have high assignment values to the Iberian cluster (Figure 5B).

Even though the French population had lower genetic differentiation with the northeast population than with the other Pyrenean populations (France-northeast: Fst = 0.102; France-southeast: Fst = 0.140; France-north: Fst = 0.133; France-south: Fst = 0.127), all pairwise Fst values were significantly higher than 0 (10,000

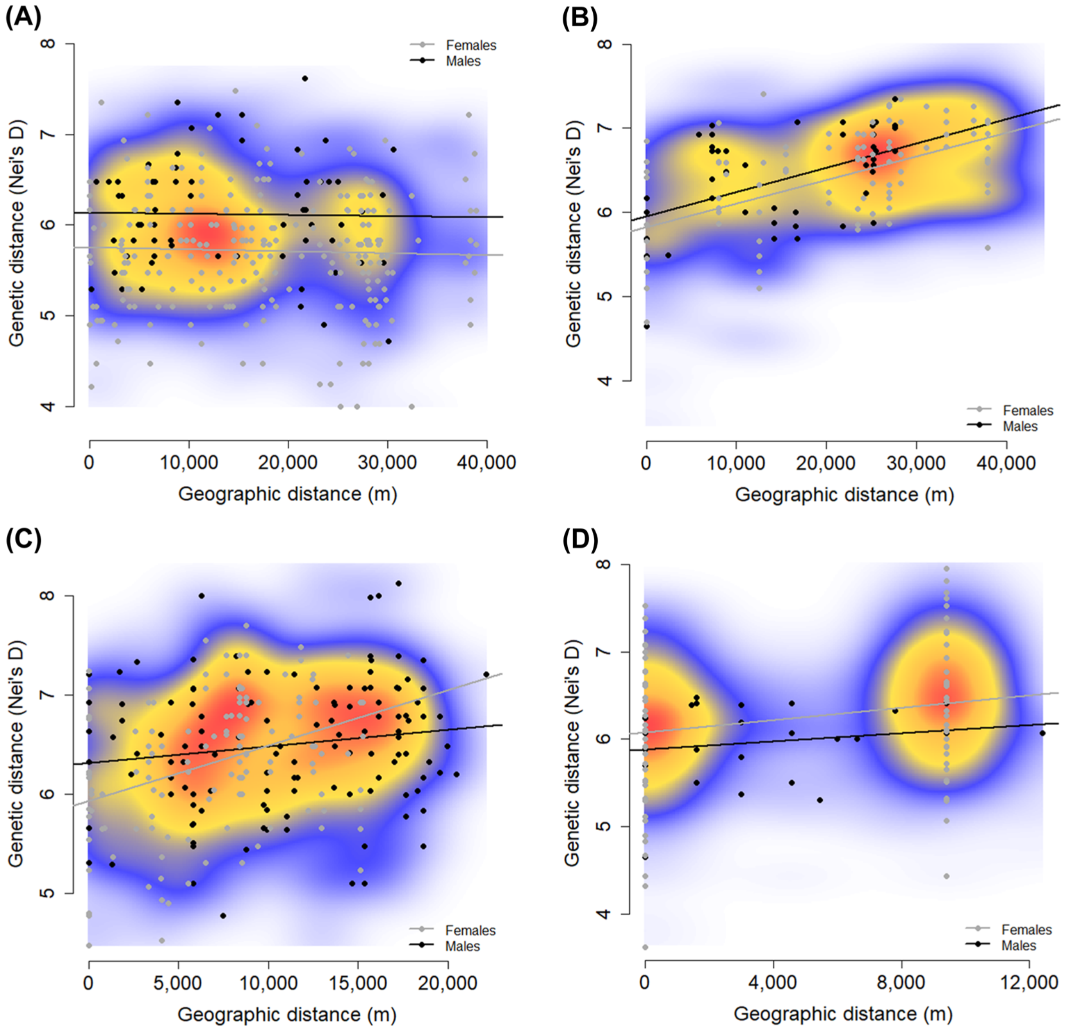

FIGURE 6 Isolation by distance analyses across populations and sexes for red deer that were hunted in the Pyrenees, Spain, 2020–2021, in 4 populations: the north population (A; *n* = 43). the south population (B; *n* = 30), the northeast population (C; *n* = 34), and the southeast population (D; *n* = 34). Gray points and line represent the observed values and fit for females. Black points and line represent the observed values and fit for males. We used Nei's genetic distance and Euclidean geographical distances between sampling points. Two-dimensional kernel density estimations (high density [red], low density [blue]) were obtained for all individuals within each population.

permutations, $P < 0.001$). Moreover, the cladogram with pairwise Fst shows that the French population was genetically different from all the Pyrenean populations (Figure S7, available in Supporting Information).

For the north population, neither males nor females presented an IBD pattern (males, correlation coefficient = −0.021, 10,000 replicates, $P = 0.535$, $R^2 = 0.0004$; females, correlation coefficient = −0.035, 10,000 replicates, $P = 0.669$, $R^2 = 0.0001$; Figure 6A). Here, genetic distances among males were higher than among females (Nei's distance, mean ± SD = 6.122 ± 0.598 for males and 5.719 ± 0.646 for females, $t_{108.5} = 4.799$, $P < 0.001$; Figure 6A). Therefore, genetic distance among males from the north population was 7.05% higher than for females. For the south population, both males and females presented a very similar IBD pattern (males, correlation coefficient = 0.473, 10,000 replicates, $P = 0.006$, $R^2 = 0.224$; females, correlation coefficient = 0.557, 10,000 replicates, $P < 0.001$, $R^2 = 0.310$; Figure 6B). For the northeast population, males and females had a similar IBD pattern, females presenting a higher slope (males, correlation coefficient = 0.157, 10,000 replicates, $P = 0.047$, $R^2 = 0.025$; females, correlation coefficient = 0.354, 10,000 replicates, $P < 0.001$, $R^2 = 0.125$; Figure 6C). Similarly, females, but not males, showed an IBD pattern for the southeast population (males, correlation coefficient = 0.194, 10,000 replicates, $P = 0.121$, $R^2 = 0.038$, females: correlation coefficient = 0.245, 10,000 replicates, $P < 0.001$, $R^2 = 0.060$; Figure 6D).

DISCUSSION

In the Pyrenees, red deer populations are constituted by individuals that present heterogeneities in morphometric and genetic features. Males from NCP were bigger and had thicker antlers than males from SCP. We cannot rule out that morphological differences could be due to spatial or temporal variations in environmental conditions (Azorit et al. 2002, Torres-Porras et al. 2009). These morphometric differences, however, are supported by the existence of 2 genetic clusters associated with NCP and SCP, respectively. Both genetic clusters occurred in EP. One was close to the French border and around the Segre riverbed, and the other was several kilometers south of the Segre River. There was evidence of migration and hybridization between both genetic clusters, mainly in EP where heterozygosity levels reached greater values. The genetic composition of the northern cluster was associated with that of European non-Iberian red deer populations, while the genetic composition of the southern cluster was related to that of Iberian red deer populations (Carranza et al. 2016, Queirós et al. 2020).

Genetic structure analyses with additional red deer samples from several Iberian areas and populations from other countries resulted in 3 genetic clusters. A similar arrangement of samples was also used in previous studies (Carranza et al. 2016, Queirós et al. 2020) and the results were also similar: a pool of Iberian samples assigning to a genetic clusters and a pool of non-Iberian samples assigning to another cluster. Our data identified a new genetic cluster shaped by the French population. The relatively high number of French samples used in our analysis might explain the appearance of the new cluster. The northern Pyrenean population had high probabilities of belonging to the same Structure cluster as individuals from France, but pairwise Fst values indicated relatively high levels of genetic differentiation. The French population of Petite Pierre might not be the direct source of individuals for the northern Pyrenees, or they might have suffered processes of genetic drift or hybridization with other populations.

All 3 sampling areas show contrasting situations regarding the genetic structure of red deer. The NCP had non-Iberian individuals that came from the south of France and bear genetic compositions similar to those of the French red deer population of Petite Pierre. Most individuals in this area showed high assignment probability to the northern cluster (cluster 1), and 2 of them might be hybrids. This northern area coincided with the location of the north population, where we did not identify an IBD pattern for females or males. Therefore, this population might be interpreted as consisting of non-philopatric individuals whose movements remain mainly within the NCP. The SCP contains Iberian red deer. Most individuals in this area showed high assignment values to the southern cluster (cluster 2), but some of them might also be hybrids. Individuals from SCP showed relatively high genetic variation in the PCA analysis. This can be due to the diverse origin of the Iberian individuals that were reintroduced in this area. The SCP coincided with the location of the south population, where both sexes showed IBD, therefore consisting of individuals with philopatric tendencies. Finally, EP presented non-Iberian individuals with French origin around the Segre riverbed (northeast population), and Iberian individuals mainly several kilometers south of the Segre River (southeast population). Consequently, we observed the highest level of genetic variation in this area. Migrant males (probably from the southeast population) were in the northeast population and hybrids were detected in both eastern populations (northeast and southeast). In both eastern populations, we mainly detected IBD for females; this result is typical of male-biased dispersal pattern (Prugnolle and de Meeus 2002, Nussey et al. 2005, Coulon et al. 2006). Despite the expected male-biased dispersal pattern in red deer, the IBD result in the southeast population should be taken with caution because of the low sample size and few locations for females.

The current presence of red deer in the Pyrenees is the product of the spreading and movements from areas where individuals were reintroduced. Therefore, the Pyrenees can be considered as a secondary contact zone (Mayr 1942, 1963; Thorpe 1984) for red deer, where 2 genetically different clusters are hybridizing. Currently, red deer populations in the Pyrenees do not seem to have contact with other Iberian populations. Pyrenean populations, however, have increased their size and range during the last decades (Figure S1) and movement of individuals to Iberian populations in northern Spain can be expected.

The role of the Pyrenees as a secondary contact zone and a hybrid belt (Hewitt 1988, Schmitt and Zimmermann 2012, suture zone in Remington 1968) has been previously described in species such as the European grasshopper (*Chorthippus parallelus*), common lizard (*Zootoca vivipara*), tawny owl (*Strix aluco*), capercaillie (*Tetrao urogallus*), and grass snakes (*Natrix astreptophora* and *N. helvetica*; Cooper et al. 1995, Brito 2007, Rodríguez-Muñoz et al. 2007, Milá et al. 2013, Asztalos et al. 2020). These examples are the products of natural recolonization before the complete deglaciation of the Pyrenees (Hewitt 1999, Schmitt and Zimmermann 2012). We detected a hybrid belt that is a consequence of human intervention. Therefore, the case of red deer in the Pyrenees can pose conservation concerns (Bohling 2016) rather than an opportunity to study natural evolutionary processes (Harrison 1986, Hewitt 1988, Durrett et al. 2000).

In hybrid belts a balance exists between dispersal favoring hybridization and selection against hybrids (Barton and Hewitt 1985, 1989). Therefore, the evolution of the hybrid belts depends on the rate of gene flow and the relative fitness of hybrids. We detected migrant individuals and inferred hybrids mainly in the northeast population. On the other hand, hybridization between different subspecies and lineages of red deer has been confirmed across Europe (Haanes et al. 2010, 2013; Fernández-García et al. 2014; Krojerová-Prokešová et al. 2015; Frantz et al. 2017). Consequently, the dispersal and selection balance for red deer in the Pyrenees should be biased to the hybridization process and an increase of the width of the hybrid zone can be expected (Barton and Hewitt 1985, 1989). Despite migration and hybridization appearing to be higher in the northern genetic cluster (associated with non-Iberian red deer), hybridization might also occur in southern areas where males are smaller and probably less competitive in mating encounters. Thus, the hybrid belt in the Pyrenees must be considered as a potential risk for the genetic conservation of red deer in the Iberian Peninsula and in western Europe (Carranza et al. 2003, 2016).

Despite the importance of hybridization for evolutionary studies (Harrison 1986, Hewitt 1988, Durrett et al. 2000) and the value of hybrids for conservation and ethical considerations (Allendorf et al. 2001, Ellstrand et al. 2010, Jackiw et al. 2015, Wayne and Shaffer 2016), hybridization and introgression can be considered as a potential threat to biodiversity conservation (Rhymer and Simberloff 1996, Bohling 2016). In the case of red deer in the Pyrenees, the observed hybridization process could result in the introgression of genetic material from non-Iberian red deer into the Iberian lineages (Carranza et al. 2003, 2016; Queirós et al. 2020), with important consequences on the genetic conservation of red deer in western Europe. Therefore, management actions that are practical, ethical, and economical should be designed to handle this hybrid system.

The management of hybrids depends on the distinction between natural and anthropogenic hybridization (Allendorf et al. 2001). Allendorf et al. (2001) categorized hybridization into 6 types, 3 for natural hybridization and 3 for anthropogenic hybridization. The situation we described for red deer in the Pyrenees might be included in type 5: anthropogenic hybridization where there was a high probability of backcrosses, but high differentiation remained between clusters. In this circumstance, Allendorf et al. (2001) confer low conservation value for hybrids and propose efforts to maintain and expand the remaining pure populations. Because red deer are a game species, the persecution and hunting of hybrids might be proposed. The culling of animals in an active hybrid system like red deer in the Pyrenees is problematic (Bohling 2016), however. Identifying the pure and hybrid individuals can be complicated in the field, and impractical by using genetic tools for decision making. Therefore, the removal of hybrids cannot be recommended.

Under this scenario, practical management actions with low ecological and ethical concerns might be proposed. The existence and evolution of the active hybridization system of deer in the Pyrenees could be accepted. But it is feasible to establish a safety belt in north-western Spain, stretching from the Cantabrian to the Mediterranean coasts (see below).

Despite the current genetic structure of Pyrenean red deer described in this study, there is no genetic information about the red deer living in the Pyrenees and the closer territories south until 1 or 2 centuries ago. We cannot rule out the dominant or partial presence of cluster 1 in this area in historical times, the presence of a form of the Iberian lineage different from the one that currently exists (and has been studied here), or a natural presence of hybrid individuals between both lineages in the past. Perhaps there was already a cline and progressive variation between the 2 lineages that we find today. This is the case, for example, of the Spanish ibex (*Capra pyrenaica*) in the Iberian Peninsula according to recently published results (Barros et al. 2022). Here, we discuss alternatives to manage the threat for the conservation genetics of Iberian red deer due the existence of the anthropogenic hybrid belt in the Pyrenees. The study of the past genetic structure red deer in the Pyrenees could make the approach and the management actions to be focused differently. In this case, to confirm or rule out this possible situation, we consider it necessary and urgent to study the genetics of the deer that lived until 1 or 2 centuries ago in the Pyrenees and its surroundings.

MANAGEMENT IMPLICATIONS

Because of the anthropogenic origin of red deer populations in the area, we recommend the establishment of a safety belt in northwestern Spain to prevent further admixture resulting from southward deer movements from the Pyrenees to the Iberian Peninsula. The safety belt should take advantage of areas without red deer because of strong human presence and large infrastructures such as cities and rural areas without current red deer populations. In areas with low human presence that might act as natural corridors, local authorities and hunting societies can be advised on increasing hunting pressure on red deer appearing in the area.

ACKNOWLEDGMENTS

We thank P. R. Krausman, Z. Olson, and 2 anonymous reviewers for their comments on the manuscript. Capillary electrophoresis was carried out by the Central Services for Research Support of the University of Córdoba. We also thank the national game reserves of Alt Pallars, Boumort, Cadí and Cerdanya-Alt Urgell, the controlled game area of Sort, Soriguera, Rialp and Tornafort, the General Council of Aran and the Catalan Hunting Federation. I. Afonso, J. Teixidó, J. Curià, J. Fernández Gil, M. Sala Jordana, A. Rosell, M. Llussà, R. Lladòs, J. Muntané, R. Garcia, S. España, S. Ferron, and J. Zarzoso coordinated the collection of samples. Financial support came from contract AG-2021-1064 (Government of Catalonia) and project P18-RT-4642 (Government of Andalusia and ERDF of European Union), to JC as PI.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ETHICS STATEMENT

No animal was harvested for the purpose of this study. All samples were obtained from red deer hunted during ordinary hunting activities, based on the hunting plans approved by the Director General of Forest Ecosystems and Environmental Management (Generalitat of Catalonia) for each of the hunting areas from which they came, both in public management (Alt Pallars National Hunting Reserve, Cerdanya-Alt Urgell National Hunting Reserve, Cadí National Hunting Reserve, Boumort National Hunting Reserve, Val d'Aran Controlled Hunting Area, Naut Aran Controlled Hunting Area and Sort, Soriguera, Rialp and Tornafort Controlled Hunting Area) and private management (B-10.331, B-10.336, G-10.158, L-10.141, L-10.232, L-10.293, L-10.543, L-10.635).

DATA AVAILABILITY STATEMENT

Data available in article supplementary material.

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REFERENCES

Aars, J., and R. A. Ims. 2000. Population dynamic and genetic consequences of spatial density-dependent dispersal in patchy populations. American Naturalist 155:252–265.

Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg. 2001. The problems with hybrids: setting conservation guidelines. Trends in Ecology & Evolution 16:613–622.

Anderson, E. C., and E. A. Thompson. 2002. A model-based method for identifying species hybrids using multilocus genetic data. Genetics 160:1217–1229.

Asztalos, M., N. Schultze, F. Ihlow, P. Geniez, M. Berroneau, C. Delmas, G. Guiller, J. Legentilhomme, C. Kindler, and U. Fritz. 2020. How often do they do it? An in-depth analysis of the hybrid zone of two grass snake species (*Natrix astreptophora* and *Natrix helvetica*). Biological Journal of the Linnean Society 131: 756–773.

Azorit, C. 2011. Guía para la determinación de la edad del ciervo ibérico (*Cervus elaphus hispanicus*) a través de su dentición: revisión metodológica y técnicas de elección. Anales 24:235–264.

Azorit, C., M. Analla, R. Carrasco, and J. Muñoz-Cobo. 2002. Influence of age and environment on antler traits in Spanish red deer (*Cervus elaphus hispanicus*). Zeitschrift für Jagdwissenschaft 48:137–144.

Azorit, C., J. Muñoz-Cobo, J. Hervás, and M. Analla. 2004. Aging through growth marks in teeth of Spanish red deer. Wildlife Society Bulletin 32:702–710.

Barbanera, F., O. R. W. Pergams, M. Guerrini, G. Forcina, P. Panayides, and F. Dini. 2010. Genetic consequences of intensive management in game birds. Biological Conservation 143:1259–1268.

Barros, T., J. Fernandes, E. Ferreira, J. Carvalho, M. Valldeperes, S. Lavín, J. Ruiz-Olmo, C. Fonseca, and E. Serrano. 2022. Genetic signature of blind reintroductions of Iberian ibex (*Capra pyrenaica*) in Catalonia, Northeast Spain. PloS ONE 17:e0269873.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. Annual Review of Ecology and Systematics 16:113–148.

Barton, N. H., and G. M. Hewitt. 1989. Adaptation, speciation and hybrid zones. Nature 341:497–503.

Belkhir, K., P. Borsa, L. Chikhi, N. Raufaste, and F. Bonhomme. 2004. Genetix 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier, France.

Bohling, J. H. 2016. Strategies to address the conservation threats posed by hybridization and genetic introgression. Biological Conservation 203:321–327.

Brito, P. H. 2007. Contrasting patterns of mitochondrial and microsatellite genetic structure among Western European populations of tawny owls (*Strix aluco*). Molecular Ecology 16:3423–3437.

Carranza, J. 2007. Ciervo—*Cervus elaphus*. Account *in* A. Salvador and I. Barja, editors. Enciclopedia Virtual de los Vertebrados Españoles. Museo Nacional de Ciencias Naturales, Madrid, Spain. http://www.vertebradosibericos.org/. Accessed 1 Feb 2022.

Carranza, J., J. G. Martínez, C. B. Sánchez-Prieto, J. L. Fernández-García, B. Sánchez-Fernández, R. Álvarez-Álvarez, J. Valencia, and S. Alarcos. 2003. Game species: extinction hidden by census numbers. Animal Biodiversity and Conservation 26:81–84.

Carranza, J., M. Salinas, D. Andrés, and J. Pérez-González. 2016. Iberian red deer: paraphyletic nature at mtDNA but nuclear markers support its genetic identity. Ecology and Evolution 6:905–922.

Chambers, J. M. 1992. Linear models. Chapter 4 *in* J. M. Chambers and T. J. Hastle, editors. Statistical models in S. Wadsworth & Brooks/Cole, Pacific Grove, California, USA.

Chesser, R. K. 1991. Gene diversity and female philopatry. Genetics 127:437–447.

Clutton-Brock, T., F. Guinness, and S. Albon. 1982. Red deer: behaviour and ecology of two sexes. University of Chicago Press, Chicago, Illinois, USA.

Coates, D. J., M. Byrne, and C. Moritz. 2018. Genetic diversity and conservation units: dealing with the species-population continuum in the age of genomics. Frontiers in Ecology and Evolution 6:165.

Cooper, S. J. B., K. M. Ibrahim, and G. M. Hewitt. 1995. Postglacial expansion and genome subdivision in the European grasshopper *Chorthippus parallelus*. Molecular Ecology 4:49–60.

Coulon, A., J.-F. Cosson, N. Morellet, J.-M. Angibault, B. Cargnelutti, M. Galan, S. Aulagnier, and A. J. Hewison. 2006. Dispersal is not female biased in a resource-defence mating ungulate, the European roe deer. Proceedings of the Royal Society B: Biological Sciences 273:341–348.

Crandall, K. A., O. R. P. Bininda-Emonds, G. M. Mace, and R. K. Wayne. 2000. Considering evolutionary processes in conservation biology. Trends in Ecology & Evolution 15:290–295.

Dirzo, R., H. S. Young, M. Galetti, G. Ceballos, N. J. B. Isaac, and B. Collen. 2014. Defaunation in the Anthropocene. Science 345:401–406.

Durrett, R., L. Buttel, and R. Harrison. 2000. Spatial models for hybrid zones. Heredity 84:9–19.

Earl, D. A., and B. M. VonHoldt. 2012. Structure Harvester: a website and program for visualizing Structure output and implementing the Evanno method. Conservation Genetics Resources 4:359–361.

Ellstrand, N. C., D. Biggs, A. Kaus, P. Lubinsky, L. A. McDade, K. Preston, L. M. Prince, H. M. Regan, V. Rorive, O. A. Ryder, et al. 2010. Got hybridization? A multidisciplinary approach for informing science policy. BioScience 60:384–388.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology 14:2611–2620.

Fernández-García, J. L., J. Carranza, J. G. Martínez, and E. Randi. 2014. Mitochondrial D-loop phylogeny signals two native Iberian red deer (*Cervus elaphus*) lineages genetically different to Western and Eastern European red deer and infers human-mediated translocations. Biodiversity and Conservation 23:537–554.

Frankham, R. 2005. Genetics and extinction. Biological Conservation 126:131–140.

Frantz, A. C., F. E. Zachos, S. Bertouille, M.-C. Eloy, M. Colyn, and M.-C. Flamand. 2017. Using genetic tools to estimate the prevalence of non-native red deer (*Cervus elaphus*) in a Western European population. Ecology and Evolution 7: 7650–7660.

Frey, R., I. Volodin, E. Volodina, J. Carranza, and J. Torres-Porras. 2012. Vocal anatomy, tongue protrusion behaviour and the acoustics of rutting roars in free-ranging Iberian red deer stags (*Cervus elaphus hispanicus*). Journal of Anatomy 220:271–292.

Geist, V. 1998. Deer of the world: their evolution, behavior, and ecology. Stackpole Books, Mechanicsburg, Pennsylvania, USA.

Gortázar, C., J. Herrero, R. Villafuerte, and J. Marco. 2000. Historical examination of the status of large mammals in Aragon, Spain. Mammalia 64:411–422.

Haanes, H., K. H. Røed, A. Mysterud, R. Langvatn, and O. Rosef. 2010. Consequences for genetic diversity and population performance of introducing continental red deer into the northern distribution range. Conservation Genetics 11: 1653–1665.

Haanes, H., J. Rosvold, and K. H. Røed. 2013. Non-indigenous introgression into the Norwegian red deer population. Conservation Genetics 14:237–242.

Harrison, R. G. 1986. Pattern and process in a narrow hybrid zone. Heredity 56:337–349.

Hewitt, G. 2000. The genetic legacy of the quaternary ice ages. Nature 405:907–913.

Hewitt, G. M. 1988. Hybrid zones-natural laboratories for evolutionary studies. Trends in Ecology & Evolution 3:158–167.

Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnean Society 58:247–276.

Hewitt, G. M. 1999. Post-glacial re-colonization of European biota. Biological Journal of the Linnean Society 68:87–112.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics 6:65–70.

Hughes, A. R., B. D. Inouye, M. T. J. Johnson, N. Underwood, and M. Vellend. 2008. Ecological consequences of genetic diversity. Ecology Letters 11:609–623.

Jackiw, R. N., G. Mandil, and H. A. Hager. 2015. A framework to guide the conservation of species hybrids based on ethical and ecological considerations. Conservation Biology 29:1040–1051.

Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806.

Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405.

Krojerová-Prokešová, J., M. Barančeková, and P. Koubek. 2015. Admixture of Eastern and Western European red deer lineages as a result of postglacial recolonization of the Czech Republic (Central Europe). Journal of Heredity 106: 375–385.

Laikre, L., S. Hoban, M. W. Bruford, G. Segelbacher, F. W. Allendorf, G. Gajardo, A. G. Rodríguez, P. W. Hedrick, M. Heuertz, P. A. Hohenlohe, et al. 2020. Post-2020 goals overlook genetic diversity. Science 367:1083–1085.

Lamb, C. T., A. T. Ford, M. F. Proctor, J. A. Royle, G. Mowat, and S. Boutin. 2019. Genetic tagging in the Anthropocene: scaling ecology from alleles to ecosystems. Ecological Applications 29:e01876.

Marco, X., D. García-Ferré, and J. Canut. 1995. Cérvol-*Cervus elaphus*. Pages 146–150 *in* J. Ruiz-Olmo and A. Aguilar, editors. Els grans mamífers de Catalunya i Andorra. Lynx Edicions, Barcelona, Spain.

Mayr, E. 1942. Systematics and the origin of species. Columbia University Press, New York, New York, USA.

Mayr, E. 1963. Animal species and evolution. Harvard University Press, Cambridge, Massachusetts, USA.

Meiri, M., A. M. Lister, T. F. G. Higham, J. R. Stewart, L. G. Straus, H. Obermaier, M. R. González Morales, A. B. Marín-Arroyo, and I. Barnes. 2013. Late-glacial recolonization and phylogeography of European red deer (*Cervus elaphus* L.). Molecular Ecology 22:4711–4722.

Milá, B., Y. Surget-Groba, B. Heulin, A. Gosá, and P. S. Fitze. 2013. Multilocus phylogeography of the common lizard *Zootoca vivipara* at the Ibero-Pyrenean suture zone reveals lowland barriers and high-elevation introgression. BMC Evolutionary Biology 13:192.

Mossman, C. A., and P. M. Waser. 1999. Genetic detection of sex-biased dispersal. Molecular Ecology 8:1063–1067.

Newbold, T., L. N. Hudson, A. P. Arnell, S. Contu, A. De Palma, S. Ferrier, S. L. L. Hill, A. J. Hoskins, I. Lysenko, H. R. P. Phillips, et al. 2016. Has land use pushed terrestrial biodiversity beyond the planetary boundary? A global assessment. Science 353:288–291.

Nussey, D. H., D. W. Coltman, T. Coulson, L. E. B. Kruuk, A. Donald, S. J. Morris, T. H. Clutton-Brock, and J. Pemberton. 2005. Rapidly declining fine-scale spatial genetic structure in female red deer. Molecular Ecology 14:3395–3405.

Pérez-González, J., and J. Carranza. 2009. Female-biased dispersal under conditions of low male mating competition in a polygynous mammal. Molecular Ecology 18:4617–4630.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Prugnolle, F., and T. de Meeus. 2002. Inferring sex-biased dispersal from population genetic tools: a review. Heredity 88: 161–165.

Queirós, J., P. Acevedo, J. P. V. Santos, J. Barasona, B. Beltran-Beck, D. González-Barrio, J. A. Armenteros, I. Diez-Delgado, M. Boadella, I. Fernandéz de Mera, et al. 2019. Red deer in Iberia: molecular ecological studies in a southern refugium and inferences on European postglacial colonization history. PLOS ONE 14:e0210282.

Queirós, J., C. Gortázar, and P. C. Alves. 2020. Deciphering anthropogenic effects on the genetic background of the red deer in the Iberian Peninsula. Frontiers in Ecology and Evolution 8:147.

R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.

Remington, C. L. 1968. Suture-zones of hybrid interaction between recently joined biotas. Pages 321–428 *in* T. Dobzhansky, M. K. Hetch, and W. C. Steere. Evolutionary biology. Springer US, Boston, Massachusetts, USA.

Rhymer, J. M., and D. Simberloff. 1996. Extinction by hybridization and introgression. Annual Review of Ecology and Systematics 27:83–109.

Rodríguez-Muñoz, R., P. M. Mirol, G. Segelbacher, A. Fernández, and T. Tregenza. 2007. Genetic differentiation of an endangered capercaillie (*Tetrao urogallus*) population at the Southern edge of the species range. Conservation Genetics 8:659–670.

Rosenberg, N. A. 2003. Distruct: a program for the graphical display of population structure. Molecular Ecology Notes 4: 137–138.

Schmitt, T. 2007. Molecular biogeography of Europe: pleistocene cycles and postglacial trends. Frontiers in Zoology 4:11.

Schmitt, T., and M. Zimmermann. 2012. To hybridize or not to hybridize: what separates two genetic lineages of the chalk-hill blue *Polyommatus coridon* (Lycaenidae, Lepidoptera) along their secondary contact zone throughout eastern Central Europe? Journal of Zoological Systematics and Evolutionary Research 50:106–115.

Singer, F. J., C. M. Papouchis, and K. K. Symonds. 2000. Translocations as a tool for restoring populations of bighorn sheep. Restoration Ecology 8:6–13.

Skog, A., F. E. Zachos, E. K. Rueness, P. G. D. Feulner, A. Mysterud, R. Langvatn, R. Lorenzini, S. S. Hmwe, I. Lehoczky, G. B. Hartl, et al. 2009. Phylogeography of red deer (*Cervus elaphus*) in Europe. Journal of Biogeography 36:66–77.

Taberlet, P., L. Fumagalli, A. G. Wust-Saucy, and J. F. Cosson. 1998. Comparative phylogeography and postglacial colonization routes in Europe. Molecular Ecology 7:453–464.

Thorpe, R. S. 1984. Primary and secondary transition zones in speciation and population differentiation: a phylogenetic analysis of range expansion. Evolution 38:233–243.

Torres-Porras, J., J. Carranza, and J. Pérez-González. 2009. Combined effects of drought and density on body and antler size of male Iberian red deer *Cervus elaphus hispanicus*: climate change implications. Wildlife Biology 15:213–221.

Torres-Porras, J., J. Carranza, J. Pérez-González, C. Mateos, and S. Alarcos. 2014. The tragedy of the commons: unsustainable population structure of Iberian red deer in hunting estates. European Journal of Wildlife Research 60: 351–357.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535–538.

Wayne, R. K., and H. B. Shaffer. 2016. Hybridization and endangered species protection in the molecular era. Molecular Ecology 25:2680–2689.

Weeks, A. R., C. M. Sgro, A. G. Young, R. Frankham, N. J. Mitchell, K. A. Miller, M. Byrne, D. J. Coates, M. D. B. Eldridge, P. Sunnucks, et al. 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. Evolutionary Applications 4:709–725.

White, P. S., A. Choi, R. Pandey, A. Menezes, M. Penley, A. K. Gibson, J. de Roode, and L. Morran. 2020. Host heterogeneity mitigates virulence evolution. Biology Letters 16:20190744.

Zachos, F. E., and G. B. Hartl. 2011. Phylogeography, population genetics and conservation of the European red deer *Cervus elaphus*. Mammal Review 41:138–150.

Zachos, F. E., S. Mattioli, F. Ferretti, and R. Lorenzini. 2014. The unique Mesola red deer of Italy: taxonomic recognition (*Cervus elaphus italicus* nova ssp., Cervidae) would endorse conservation #. Italian Journal of Zoology 81:136–143.

Associate Editor: Zach Olson.

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How to cite this article: Pérez-González, J., A. Gort-Esteve, J. Ruiz-Olmo, G. Anaya, C. Broggini, M. F. Millán, G. Vedel, E. de la Peña, A. Membrillo, J. M. Seoane, C. Azorit, and J. Carranza. 2023. Red deer in the Pyrenees: a risky secondary contact zone for conservation genetics. Journal of Wildlife Management 87:e22454. https://doi.org/10.1002/jwmg.22454