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Demography reveals populational expansion of a recently extinct Iberian ungulate

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Abstract

Reconstructing the demographic history of endangered taxa is paramount to predict future fluctuations and disentangle the contributing factors. Extinct taxa or populations might also provide key insights in this respect by means of the DNA extracted from museum specimens. Nevertheless, the degraded status of biological material and the limited number of records may pose some constraints. For this reason, identifying all available sources, including private and public biological collections, is a crucial step forward. In this study, we reconstructed the demographic history based on cytochrome-*b* sequence data of the Pyrenean ibex (*Capra pyrenaica pyrenaica*), a charismatic taxon of the European wildlife that became extinct in the year 2000. Moreover, we built a database of the museum specimens available in public biological collections worldwide and genotyped a privately owned 140-year-old trophy from the Spanish Pyrenees to confirm its origin. We found that the population of the Pyrenean ibex underwent a recent expansion approximately 20,000 years ago, after which trophy hunting and epizootics triggered a relentless population decline. Our interpretations, based on the genetic information currently available in public repositories, provide a solid basis for more exhaustive analyses relying on all the new sources identified. In particular, the adoption of a genome-wide approach appears a fundamental prerequisite to disentangle the multiple contributing factors associated with low genetic diversity, including inbreeding depression, acting as extinction drivers.

Key Words

ancient DNA, anthropocene biodiversity crisis, biotic impoverishment, *Capra pyrenaica pyrenaica*, Cytochrome-*b*, epizootics, extinction, museum specimens, public repositories, trophy hunting

Introduction

The Anthropocene biodiversity crisis is causing a drastic biotic impoverishment worldwide (Leakey and Lewin 1996; Barnosky et al. 2011; Pievani 2014). Characterising genetic variation of endangered species is key to reconstruct and predict past and future demographic fluctuations (Collen et al. 2011). Such knowledge may shed light on the proximate and ultimate causes underlying the collapse of extinct taxa and of their populations, elucidating pivotal information for highly endangered species that may undergo a similar fate (Farrington et al. 2019).

The Iberian ibex (*Capra pyrenaica*) includes two extant (*C. p. hispanica* and *C. p. victoriae*) and two extinct subspecies (*C. p. lusitanica* and *C. p. pyrenaica*) (Herrero et al. 2020) distinguished on the basis of pelage characteristics and horn development (Cabrera 1911). Among the latter, *C. p. pyrenaica* (Fig. 1a), commonly known as the Pyrenean ibex or the bucardo, inhabited the Pyrenees until the year 2000 (Pérez et al. 2002). Morphological studies indicate this taxon as being the largest and stoutest of its group (with special reference to females), with cranial measurements placing it in an intermediate position between the Alpine ibex (*C. ibex*) and the other *C. pyrenaica* subspecies (García-González 1991, Granados et al. 1997). The distinctiveness of *C. p. pyrenaica* was later confirmed by genetic studies employing mitochondrial (Villalta et al. 1997, Manceau 1999) and microsatellite (Jiménez et al. 1999) markers pointing to a divergence between this subspecies and the others which is comparable to that between the latter and the Alpine ibex.

Now extinct due to human activities, *C. p. pyrenaica* was, and still is, one of Europe's most charismatic animals. Recently, interest in this subspecies has surged enough to motivate researchers to a controversial cloning attempt (Folch et al. 2009; Kupferschmidt 2014). Initiatives to re-establish populations of the Iberian ibex have been taken across the Iberian Peninsula, where a population of the subspecies *C. p. victoriae* now occurs in the former range of *C. p. lusitanica* (Moço et al. 2006, 2015). Plans to relocate *C. p. victoriae* also into the historical range of *C. p. pyrenaica* (Crampe 1991) have been enacted by releasing and monitoring of animals since 2014 onwards (Crampe et al. 2015). This population now consists of 250 individuals in the Pyrénées National Park and over 500 in the entire mountain chain (http://www.bouquetin-pyrenees.fr/).

Medieval sources such as Gaston Phébus' hunting chronicles (1387–1389) revealed that the Pyrenean ibex was abundant (Astre 1952), but this iconic animal became a common target for trophy hunters during the 19th and 20th century, which triggered a relentless decline reported in detail by several authors (e.g. Gourdon 1908, Cabrera 1914, Labarère 1985, Crampe et Crégut-Bonnoure 1994, García-González and Herrero 1999, Jiménez 2016). Moreover, infectious diseases like the sarcoptic mange have also been listed among the causes of its disappearance (Pèrez et al. 2002, Acevedo and Cassinello 2009), while pathogens like the bluetongue virus (BTV), which has recently caused the sharp demographic collapse of other ungulates (e.g. the pronghorn antelope *Antilocapra americana*: Thorne et al. 1988, the goitred gazelle *Gazella subgutturosa subgutturosa*: Gür 2008), have been recurrently detected in Iberian ibex populations across Spain (García et al. 2009; Lorca-Oró et al. 2014; Gómez-Guillamón et al. 2020).

Considered a serious threat to wildlife at continental level (Rossi et al. 2019), it is likely that this virus (most often transmitted from livestock) also contributed to the vertiginous decline of the Pyrenean ibex. Despite the fact that hunting and epizootics from sympatric livestock are deemed to have played a major role (along with habitat fragmentation) in drastically reducing its populations over the last two centuries, the relative contribution of multiple threatening factors remains largely unknown (Hidalgo and García-González 1995; García-González et al. 1996). Nonetheless, there is strong evidence that the severe bottleneck caused by hunting amplified high levels of inbreeding and homozygosis (Jiménez et al. 1999) which likely reduced fertility of the fast-shrinking population brought beyond the minimum viable population size (Acevedo and Cassinello 2009) into an extinction vortex (Soulé 1987).

In line with these considerations, a recent genome-wide study of all European ibex species evidenced a pattern of low genetic diversity and high inbreeding (Grossen et al. 2018), consistent with an overall well-defined genetic structure following genetic drift after isolation (Angelone-Alasaad et al. 2017). The scenario portrayed for the Pyrenean ibex would be similar to that unveiled for the cheetah (*Acinonyx jubatus*), in which minimal genetic diversity was correlated with major bottlenecks occurred ca. 100,000 (Dobrynin et al. 2015) and then again 12,000 years ago (O'Brien et al. 1985, Driscoll et al. 2002), and for both the Eurasian lynx (*Lynx lynx*) and Iberian lynx (*Lynx pardinus*), which experienced a drastic decline 700,000–100,000 thousand years ago (Abascal et al. 2016). Among ungulates, recurrent and non-anthropogenic bottlenecks have been indicated as the cause lessening genetic diversity in the muskox (*Ovibos moschatus*) (Prewer et al. 2020).

In order to shed some light on the demographic history of the Pyrenean ibex and possibly get insights into the drivers underlying its extinction, we reconstructed its demographic history relying on mitochondrial DNA sequences (cytochrome-*b*: cyt-*b*) currently accessible in public repositories. We also built a database of extant Pyrenean ibex museum specimens, most of which have not been used for genetic analyses, and we genetically confirmed the identity of an additional record from a private collection. Our results suggest that the population of the Pyrenean ibex underwent a recent expansion approximately 20,000 years ago, while trophy hunting and epizootics triggered a relentless decline over the last two centuries. These inferences provide a solid basis for more exhaustive analyses relying on all the new sources identified and a genome-wide approach bearing potential to

Figure 1. *C. p. pyrenaica*: **a.** Female individual (visitor centre of Ordesa y Monte Perdido National Park, Torla-Ordesa, Huesca, Spain). Photo: courtesy of Jose Miguel Pintor Ortego. **b.** Picture of the *C. p. pyrenaica* trophy of the 3-year-old male genotyped in this study and preserved at the English Circle of Pau (France). Photo: Jean P. Crampe.

assess the role of multiple contributing factors underlying the extinction of this animal. We finally discuss our results and compare them to those of other ungulates which recently experienced sharp population declines or even species extinction, and we address the key points for further studies to consider on the Pyrenean ibex.

Materials and methods

Demographic inferences

We used GenBank *C. p. pyrenaica* cyt-*b* sequences (alignment length 1,140 bps) and run preliminary phylogenetic trees to test for monophyly. This locus was chosen as the one with the highest number of *C. p. pyrenaica* records available in public repositories. We used the samples obtained by Ureña et al. (2018), which show clear monophyly and two additional samples from the Pyrenees (Manceau et al. 1999). The final data set consisted of 17 records (Fig. 2; Suppl. material 1: Table S1). Sequences were translated to amino acids to check against stop codons and aligned in SEAVIEW v4.2.11 (Gouy et al. 2010) under CLUSTALW2 default settings (Larkin et al. 2007).

We used JMODELTEST (Posada 2008) to choose the optimal model. We specified a relaxed lognormal clock for the cyt-*b* data in BEAST v1.8.2 (Drummond et al. 2012, http://beast.bio.ed.ac.uk) and the nucleotide substitution model HKY as suggested by JMODELTEST. To time-calibrate the population tree, we used fossil dates as tip calibrations as reported in *C. p. pyrenaica* (Ureña et al. 2018). These dates were between GModerrand and Keraval 1 at 16,294 years ago, between Valdegoba 1 and all other *C. p. pyrenaica* at 40,093 years ago, and the clade composed by Chaves 56, 42, 61, 64, 63, Bolinkoba 3 and Chorrugues 1 at 29,386 years ago. We used a 10% standard deviation for all calibrations. Changes in effective population size through time were inferred using a Bayesian skyline plot (BSP) model (Drummond et al. 2005). We conducted two independent Markov Chain Monte Carlo (MCMC) runs, each with 30 million states and sampling every 3,000th state. Independent runs were evaluated for convergence and mixing by observing and comparing traces of each statistic and parameters in TRACER v1.6 (Rambaut et al. 2007, http://beast.bio.ed.ac.uk/tracer). We considered effective sampling size (ESS) values > 200 to be good indicators of parameter mixing. The first 10% of each run were discarded as burn-in, and samples were merged using LogCombiner v1.8.2 (Drummond et al. 2012). All BEAST analyses were performed through the CIPRES platform (Miller et al. 2010).

In order to test for past population demographics, we used Tajima's D (Tajima 1989) and Fu's FS (Fu 1997) statistics. Demographic changes were also examined by calculating Harpending's raggedness index (Harpending et al. 1993) and the sum of squared deviations (SSD) (Schneider and Excoffier 1999). All neutrality tests were calculated in ARLEQUIN v3.5 (Excoffier and Lischer 2010) under a demographic expansion model and 1,000 simulations. The results of mismatch distribution analyses were plotted in DNAsp v5.10 (Rozas 2009).

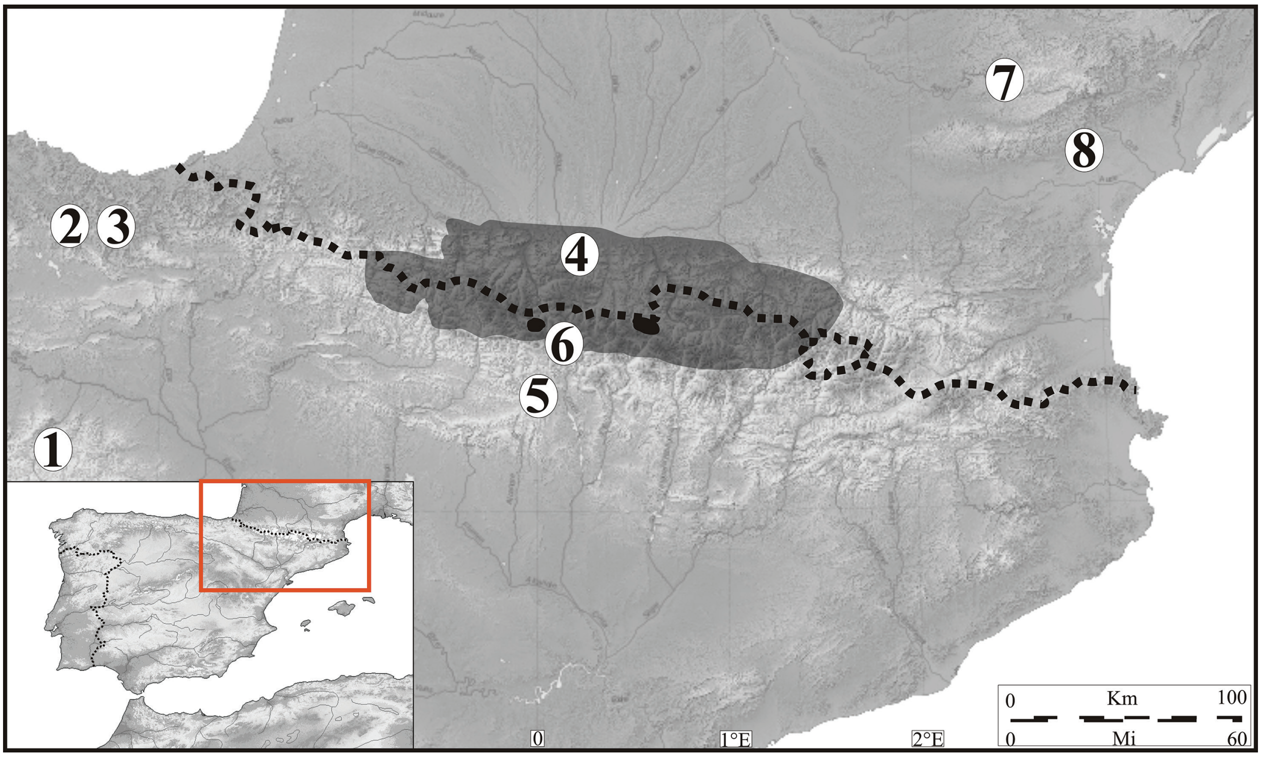

Figure 2. *C. p. pyrenaica* hypothetical distribution during the 19th (dark grey) and at the beginning of the 20th (black) century, when this taxon occurred only in Ordesa Valley and La Maladeta Massif (east to west: Woutersen 2019). Numbers refer to sampling localities in Suppl. material 1: Table S1.

Identification, origin and provision of museum specimens

We made a thorough bibliographic search to retrieve information on *C. p. pyrenaica* and built a comprehensive database of historical specimens preserved in museums and smaller collections, both public and private, thus localising new valuable source of information to elucidate the natural history of this iconic animal. For this purpose, we first relied on literary sources (including letters exchanged by trophy hunters and museum curators) presented in a previous review on the Pyrenean ibex (Woutersen 2012, 2019) and then expanded our search by using the platform VertNet (http://vertnet.org/). Hence, we examined the sex ratio and age of all known specimens to assess whether the occurrence of adult males, persistently persecuted for hunting, decreased across time. For this purpose, we plotted ages of male and female specimens versus collection date to detect the occurrence of possible sex-biased discrepancies. Only a subset (n = 32) of the entire sample was used as some records were data deficient and were therefore excluded from analyses. For three specimens we lacked ages, but they were catalogued as 'young' and hence incorporated in the graph as juveniles (ca. 3 years of age). Specimens with dates '1835–1836' were included in the analyses as 1836, those with '1837 or later' as 1837 and those with '1878 or later' as 1878 (Suppl. material 1: Table S2). In addition, we examined the sex ratio and age of all specimens in the 1800s and 1900s to assess for possible differences in collection records, and particularly whether the occurrence of adult males decreased across time.

Genetic analyses

We obtained a cranium from a *C. p. pyrenaica* trophy of a 3-year-old male preserved in a private collection (Fig. 1b) and hunted by British hunter Arthur Post on 10th April 1881 (Leslie 1894) in the Ordesa Valley (Spanish Pyrenees), now part of the Ordesa and Monte Perdido National Park, a protected area instituted in 1918 to rescue the Pyrenean ibex from extinction. DNA extraction was performed using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) as in Forcina et al. (2015) at the National Institute of Ecology (NIE: Gunsan, South Korea) by strictly adhering to the protocol for nucleic acid isolation from archival samples. A 310 bp-long Control Region (CR) fragment of the mitochondrial DNA (mtDNA) was amplified in a single PCR with thermal conditions detailed in Forcina et al. (2015) using the primers D-loop-FW (GATCCCTCTTCTCGCTCCG) and D-loop-cabra (CCATGCCTACCATTATGGGGA) (Amills et al. 2004a). Amplicon purification was performed with the Genelute PCR Clean-up Kit (final volume 40 µL; Sigma Aldrich) and directly sequenced on both DNA strands (BigDye Terminator v3.1 Cycle Sequencing Kit, ABI 3730 DNA automated sequencer, Applied Biosystems). We then used the Basic Local

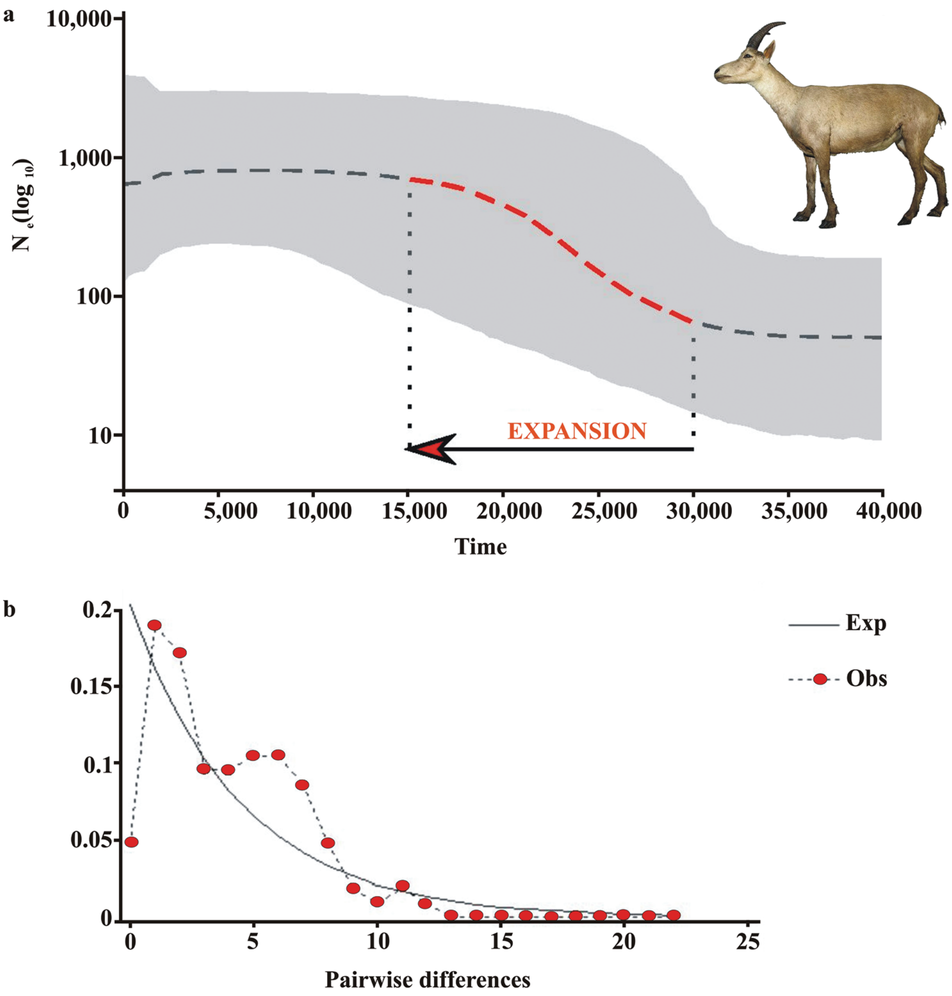

Figure 3. Demographic analyses: **a.** Bayesian skyline plot (Ne values were rescaled based on a generation time of 10 years and a 1/4 conversion factor for mtDNA data); **b.** Mismatch distributions. Exp: expected; Obs: observed.

Alignment Search Tool (BLAST) as implemented in the National Center of Bioinformatic Information (NCBI) website to confirm species identity. The newly produced CR sequence was deposited in GenBank (accession number: MW659860).

Results

Demographic inferences

No stop codons were found. The runs showed high Effective Sample Size (>1,500), corresponding to an adequate sampling of the posterior distribution. BSPs indicated a stable population within the last 15,000 years and a population expansion between 15,000 and 30,000 years ago (Fig. 2a). The mismatch distribution analysis shows a bell shape distribution suggesting population expansions (Fig. 2b).

Negative neutrality tests, Fu's Fs (-4.38, $p = 0.01$) and Tajima's D (-1.94, $p = 0.012$) statistical values observed in the population supports for evidence of past population expansions. Non-significant values for SSD (0.008, $p = 0.71$) indicate that the data do not deviate from those expected under the model of expansion. Similarly, non-significant raggedness values (0.0296, $p = 0.71$) indicate population expansion. Non-significant value in goodness-of-fit distribution for all populations suggest that this phenomenon is likely recent (Rogers 1995). The gene diversity (0.95 ± 0.043) and the mean number of pairwise differences (3.92 ± 2.08) point to high population differentiation.

Identification, origin and provision of museum specimens

We located 45 specimens of *C. p. pyrenaica* collected between 1818 and 2000. Of these, 43 came from two massifs in the Spanish Pyrenees, La Maladeta and Monte Perdido (which includes the Ordesa Valley), while two more records from nearby sites in France were found through VertNet at the LACM Vertebrate Collection Natural (History Museum of Los Angeles County) and the Cowan Tetrapod Collection (University of British Columbia Beaty Biodiversity Museum) (Suppl. material 1: Table S2). Archival sources revealed that the specimens preserved in the biological collections (which in the 19th century were still mostly private) of Toulouse, Zurich, Mainz, St. Petersburg, Strasbourg and Vienna had been specifically bought for the purpose of exhibiting animals killed on specific request (Woutersen 2019). For instance, around 1835, several collectors of zoological specimens in Germany and Switzerland asked the naturalist Alfred Moquin-Tandon from Toulouse (France) for *C. p. pyrenaica* records. The latter commissioned professional hunters in Benasque, a village at the feet of Maladeta Massif, to kill some individuals for the provision of museum specimens. Significantly, the documents also reveal that more was paid for males than for females. In 1841, the existence of the population of the Ordesa Valley (then called Val d'Arras) attracted trophy hunters who ended up enlarging incidentally the list of *C. p. pyrenaica* specimens preserved in natural history museums worldwide until 1913, when the Pyrenean ibex was lawfully protected.

Attributes of museum specimens

The origin, collection date, sex and other features of *C. p. pyrenaica* specimens are listed in Suppl. material 1: Table S2. Two thirds of the animals currently available in museums come from La Maladeta massif and were killed between 1818 and 1892. Those hunted on Monte Perdido were killed between 1852 and 1910. The first of the eight specimens collected by the staff of Ordesa and Monte Perdido National Park is from 1958. Males collected both only prior to (71.4%, n = 25) and also after (73.3%, n = 33) the 1913 hunting ban were more numerous than females (Fig. 4). We can hence conclude that males were probably hunted more than females for their trophies before 1913, and that museum curators were perhaps more willing to secure male rather than female specimens for their collection. The average age of individuals collected from 1800–1900 (n = 25) was 7.9 years, while that for those collected from 1900–2000 (n = 7) reached 12.1 years.

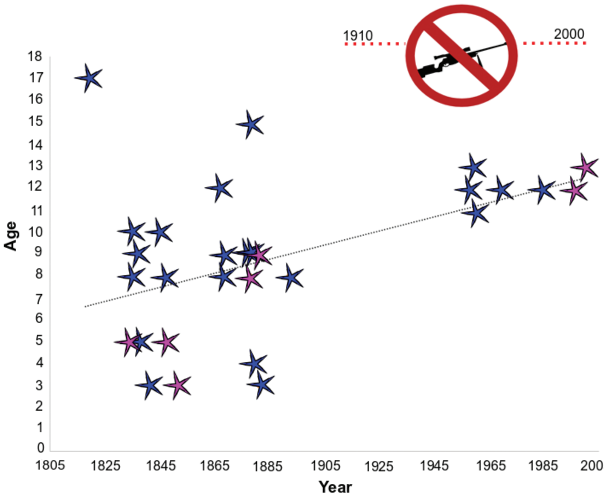

Figure 4. Scatter plot representation of museum specimen ages and year of collection over time with an emphasis on before and after the 1913 hunting ban (as indicated by the gun icon). The trend line is $r^2 = 0.21$. Blue and purple stars are used for males and females, respectively.

Genetic analyses

The NCBI-blast revealed that the *C. p. pyrenaica* CR fragment was 100% identical to the CR isolate CPP1 obtained from the last living individual of *C. p pyrenaica*, which died in Ordesa y Monte Perdido National Park (Central Pyrenees, Huesca, Spain) in the year 2000 (Folch et al. 2009), only two kilometres away from where the 3-year old male had been hunted 140 years before. Moreover, the CR fragment differed by two to six nucleotides when compared to the homologous regions of GenBank records AJ233741 and AJ233739–AJ233740 (Manceau et al. 1999), respectively, which were also retrieved from animals belonging to the same Spanish population.

Discussion

The Pyrenean ibex is an emblematic example of wildlife that went extinct in recent times in spite of its popularity among the broader public and evidently belated conservation efforts that started only a decade before the last individual died (e.g., García-González and Herrero 1999). Among European ungulates, the last two centuries have witnessed the extinction of other taxa such as the Caucasian moose (*Alces alces caucasicus*), the Caucasian wisent (*Bison bonasus caucasicus*), the tarpan (*Equus ferus ferus*) and the Portuguese ibex (*C. p. lusitanica*). However, in more recent times captive breeding programs have prevented the extinction of other taxa as with the Corsican red deer (*Cervus elaphus corsicanus*, Hmwe et al. 2006, Kidjo et al. 2007) and, outside Europe, with the scimitar-horned oryx (*Oryx dammah*; Little et al. 2016) and Père David's deer (*Elaphurus davidianus*, Zeng et al. 2007). Admittedly, several studies have been carried out to deepen our knowledge of the Pyrenean ibex over the last two decades of the 20th century, including two to assess the minimum viable population size (García-González et al. 1991, 1996), which nonetheless were futile in rescuing the subspecies from the brink of extinction. However, no demographic reconstructions had been performed to understand historical population sizes of the Pyrenean ibex thousands of years ago. In order to fill this knowledge gap, we infer temporal changes in its effective population size by means of the BSP, mismatch distributions, and neutrality tests.

Our analyses evidenced a population expansion during the Marine Isotope Stage 2 (MIS 2) period (14,000–29,000 years ago), a time marked by abrupt climatic oscillations in Southern Europe (Last Glacial Maximum: 20,000 years ago). The BSP shows a contrasting trend to other critically endangered ungulates (which, albeit, live in different ecological contexts and latitudes) such as the hirola (*Beatragus hunteri*, Jowers et al. 2020) and the saiga antelope (*Saiga tatarica*, Campos et al. 2010). Both species underwent a genetic bottleneck as expected after a severe demographic contraction.

DNA data from ancient and modern samples of the saiga antelope have shown increased haplotype diversity (with similar values to *C. p. pyrenaica*) in ancient times and a reduction of diversity in modern times. Concordantly, the BSP analyses of combined samples reveals a Pleistocene/Holocene bottleneck (Campos et al. 2010), a pattern not observed in *C. p. pyrenaica*. On the other hand, Ureña et al (2011) detected a significant loss of genetic diversity in samples of this taxon from Cueva de Chaves. This trend started between the Paleolithic and Neolithic (14,932 and 7,504 yrs BP: Ureña et al. 2018) and persisted to the present day (Manceau et al. 1999, Amills et al. 2004b). In accordance to the observed BSP expansion, the genetic differentiation as inferred from haplotypic divergence and genetic distance was still high in comparison to other ungulate species with larger distribution ranges, such as the dorcas gazelle (*Gazella dorcas*, Godinho et al. 2012), the scimitar-horned oryx (Iyengar et al. 2007), the roan antelope (*Hippotragus equinus*, Alpers et al. 2004) or the recently extinct in the wild Saudi gazelle (*Gazella saudiya*, Hammond et al. 2001).

Although we cannot rule out that the comparatively higher genetic diversity is at least partly due to the heterogeneity of the samples used, this outcome certainly deserves interest and should be readdressed in further studies relying on a larger dataset of samples from the last two centuries. By pointing to a demographic expansion of a still genetically diverse population over the last 15,000–30,0000 years, these results do not allow us to draw conclusions about the primary cause underlying the decline of the Pyrenean ibex nor which anthropogenic impact (including hunting but also diseases from livestock) played a major role for its extinction.

Indeed, previous studies acknowledge that hunting, either legal or illegal, was extremely detrimental not only in terms of the reduction in population size, but also in terms of the disruption of the social structure of the population (García-González et al. 1996; García-González and Herrero 1999; Acevedo and Cassinello 2009; Herrero et al. 2020). However, multiple contributing factors, such as epizootics, are typically invoked. In this respect, it is worth mentioning that epidemics have been known to wipe out populations of this species over extremely short periods. For example, the sarcoptic mange affecting the *C. p. hispanica* population inhabiting the Sierra de Cazorla (Andalucía, Southern Spain) during the 1980s caused the disappearance of 90% of the population (10,000 individuals) in just a few years (Granados et al. 2001). According to Amills et al. (2004b), the bottleneck experienced by the Pyrenean ibex and the consequent loss of genetic diversity turned into depleted variation at genes of the Major Histocompatibility Complex (MHC), thus enhancing susceptibility to epidemics.

On the other hand, the extinction of several other ungulates since the 1800s has ultimately been linked to indiscriminate poaching. This is surely the case of the Saudi gazelle, the South African bluebuck (*Hippotragus leucophaeus*), the Queen of Sheba's gazelle or Yemen gazelle (*Gazella bilkis*), and the Schomburgk's deer (*Rucervus schomburgki*), while other heavily hunted taxa such as the Barbary deer (*Cervus elaphus barbarous*: Kingdon et al. 2013), the Alpine ibex (Grossen et al. 2017) and the Apennine chamois (*Rupicapra pyrenaica ornata*: Pérez et al. 2014) were rescued from the brink of extinction thanks to last resort conservation initiatives.

Although the ultimate culprit factor responsible for a species extinction remains challenging to ascertain, it is reasonable to argue that multiple confounding anthropogenic causes likely contributed to the demise of these populations. In other cases, the occurrence of such detrimental factors is widely acknowledged. For instance, the ure (*Bos primigenius*) was believed to have become extinct in the 16th century by unrestricted hunting, habitat reduction due to expanding farmlands, and diseases transmitted by domesticated cattle (Rokosz 1995; Van Vuure 2005). Likewise, the drastic decline in bluebuck populations just before the 16th century has been largely attributed to competition with livestock for forage and habitat deterioration (Klein 1974), as was the fate of the Quagga's (*Equus quagga quagga*, Weddell 2002). Illnesses (e.g. rinderpest virus, sarcopses) are known to have decimated almost entire ungulate populations in a relatively short period of time; however, this scenario alone does not explain why other subspecies of ungulates in the Iberian Peninsula were not affected by similar pathogens. Furthermore, species like the hirola and the coastal topi (*Damaliscus lunatus topi*), whose populations crashed by illnesses, were able to recover fully (Jowers et al. 2020).

The new database of *C. p. pyrenaica* museum specimens allowed us to make some inferences about the extinction pattern followed by these animals across the last 200 years. Since male trophies are more attractive for collection than female ones, a male-bias is expected, with a progressive decrease of adult males and a disproportionately high numbers of females and young individuals over time (Loveridge et al. 2006). Likewise, older animals are favoured since they correspond to bigger trophies (Geist 1966). We can cautiously state that this overall scenario is mirrored by our data (73.3% males vs 26.7% females). However, we did not observe an increase of female individuals in the biological collections over time, while we observed an increase in the average age of the collected individuals after the 1913 hunting ban. This age-related pattern could be explained by admitting that collectors were mostly interested in securing a male specimen for their museum and that after 1913 the majority of specimens were obtained from aged individuals *post mortem* by natural causes.

The information retrieved to build our database also allowed us to flag one specimen of potentially high historical value from a private collection. Indeed, the trophy we genotyped in this study turned out to hold the same *C. p. pyrenaica* CR haplotype to the attempted cloned individual in the year 2000 (Folch et al. 2009; Kupferschmidt 2014), which also originated from the last population of this taxon located on the Spanish side of the Pyrenees, and precisely from the female whose skin is exposed at the visitor centre of Ordesa y Monte Perdido National Park (Huesca, Spain). This finding conforms to the believed pattern of low levels of both neutral and adaptive genetic diversity of the Pyrenean ibex (Amills et al. 2004b) and confirms the geographic origin of trophy. Significantly, the Pyrenean ibex was referred to as extremely rare since its first mention in an official document dating back to 1767. Low levels of genetic diversity are expected in ungulate populations experiencing strong bottlenecks, which may even turn into fixation of unique mtDNA haplotypes, as in the case of the Cypriot mouflon *Ovis orientalis ophion* (Guerrini et al. 2015), and inbreeding, thus triggering an extinction vortex. Experimental data from another related taxon, the bighorn sheep (*Ovis canadensis*) (Berger 1990) suggest that the small population size and the associated low genetic variability might have well been the main extinction driver for the Pyrenean ibex. Concordantly, we found that the young male killed during a hunting trip organised by British villagers based in Pau (France) beyond the Spanish border (since probably the French populations had gone already extinct or were already extremely scarce) held the same mtDNA found in a member of the same population which survived until two decades ago in the last stronghold of this animal: Ordesa y Monte Perdido National Park.

This study further evidences the importance of biological collections as an invaluable repository of historical material in studies on extinct taxa. However, achieving spatial other than temporal representativeness of past populations is challenging. Private collections and small exhibits are an invaluable yet often overlooked repository of missing information (Casas-Marce et al. 2012) and this could well be the case with the Pyrenean ibex. In this context, citizen science may represent a useful tool to locate such specimens (European Commission 2013). Mitochondrial DNA data have recently pointed to *C. p. pyrenaica* as a distinct group from living heterospecifics other than the Alpine ibex (Ureña et al. 2018), thus making it a candidate Evolutionary Significant Unit (ESU: Manceau et al. 1999). We call for the need of genome-wide sequencing on already sampled and non-sampled specimens from private collections and small exhibits to clarify the still debated systematic relationships between the Iberian ibex and other European relatives, as well as with the potentially closely related *C. caucasica* (Ureña et al. 2018). Such studies would also allow to characterise the unique traits of *C. p. pyrenaica* underlying adaptation to extreme environments and a likely higher vulnerability to infectious diseases, as well as to quantify the amount of genetic diversity that became lost during its decline.

Conclusions

This case study shows the importance of identifying historical biological material in venues other than large and well-known public biological collections and corroborating the taxonomic identity and origin by means of preliminary genetic analyses. We call for the creation of an online public database of private collections hosting biological material to the benefit of biodiversity studies.

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Supplementary material 1

Tables S1, S2

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Data type: Table

Explanation note: **Table S1.** Details of *C. p. pyrenaica* samples used in this study. FR.: France; SP.: Spain. **Table S2.** Database of the 45 *C. p. pyrenaica* specimens identified in this study as a reference for further research. IPE: Pyrenean Institute of Ecology; PNOMP: Ordesa and Monte Perdido National Park.

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