Biodiversity and Conservation (2023) 32:3875–3891
https://doi.org/10.1007/s10531-023-02664-1

ORIGINAL RESEARCH

Global warming is promoting the rapid invasion of the mountain hare range by the european hare in the Alps

Valentina La Morgia[1,2] · **Igor Martini**[2] · **Elena Tosatto**[2] · **Eleonora Mazza**[2] · **Sandro Bertolino**[2] · **Alfredo Santovito**[2]

Received: 10 December 2022 / Revised: 5 April 2023 / Accepted: 23 June 2023 / Published online: 5 July 2023

Abstract

In the European Alps, where the mountain hare *Lepus timidus* partly overlaps with the European hare (*L. europaeus*), climate change, hybridization and gene introgression are thought to interact, with synergic adverse effects on the mountain hare. However, to depict future scenarios for conserving the mountain hare is not feasible without knowledge of the magnitude and geographical pattern of hare distribution.

As a first attempt to fill this gap and assess the impacts of climate change, we investigated the distributional pattern of hare species in a valley in the Western Italian Alps using non-invasive genetic techniques based on mtDNA, and resampling the same locations after 12 years (2009–2021).

The mountain hare was more common as altitude increases, in pioneer vegetation and rocky areas. However, we also detected the European hare at high altitudes, in atypical landscapes for this lowland species, and the overlap area between hares was quite broad. The proportion of European hare samples increased in time, and the co-occurrence pattern changed, with an increase of the European hare at intermediate-high altitudes.

Our data allowed us to assess directly, and for the first time, the impact of climate change on hare species in the Italian Alps. The mountain hare-only areas seem restricted to a very narrow, high-altitude strip. The considerable spatial overlap suggests that the European-mountain hare system could evolve towards a complete admixture situation. Conservation measures should aim to mitigate climate change effects and appropriately manage European hare populations.

Keywords Climate change · Conservation · Contact zones · *Lepus timidus* · *Lepus europaeus* · Wildlife management

---

Communicated by Adeline Loyau.

✉ Valentina La Morgia
valentina.lamorgia@isprambiente.it

[1] ISPRA, Istituto Superiore per la Protezione e la Ricerca Ambientale, sede di via Ca' Fornacetta 9, Ozzano Emilia (BO) 40064, Italia

[2] Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino, via dell'Accademia Albertina 13, Torino 10123, Italia

Introduction

One of the main mechanisms used by species to cope with human-driven global warming is dispersing to track the original climatic conditions over space. At temperate regions, global warming is driving species ranges toward higher latitudes and elevations (Lenoir and Svenning 2015; Lenoir et al. 2020). Adaptation can also include a range contraction at the warmer lower latitudes and elevations, contractions that occur through local extinctions (Wiens 2016).

In mountainous areas, steep slopes result in neighbouring isotherms, and the distance species must cross to track suitable temperature is short compared to flatter regions (Brito-Morales et al. 2018). However, species living close to mountain summits will progressively be limited in migrating because moving upward generally means less available land surface. Moreover, dispersal to higher elevations may not be possible at a certain point, eventually leading to local extinction (Wiens 2016; Giezendanner et al. 2019). In the Yosemite National Park, Moritz et al. (2008) observed substantial upward changes in elevation limits for half of 28 small mammals monitored across a century, with high-elevation species contracting their range. Freeman et al. (2018) showed that recent warming led to the decline in range and abundance of high-elevation birds, with eight species being extinct. At the same time, these climate changes might allow low-elevation species to expand their ranges (Moritz et al. 2008). Lowland species can benefit from temperature increases, expanding their altitudinal distribution. The ascent in elevation of species adapted to warmer conditions can lead them to compete against alpine species, which are better adapted to colder conditions and are less competitive in a new climate. In the European Alps, climate warming occurs faster than in other parts of the northern hemisphere (Gobiet et al. 2014). As a result, numerous elevational range shifts have already been observed for plants and animals over recent decades (Vitasse et al. 2021).

Among other Alpine species, the mountain hare (*Lepus timidus*) is likely to be affected by climate change. The mountain hare is a boreo-alpine species with a wide distribution area (Angerbjörn and Flux 1995). It is widespread between Scandinavia and Poland to the Pacific coast of Russia (Flux and Angermann 1990; Angerbjör 2018). Isolated populations are present in Ireland, Scotland, the Alps and Japan (Flux and Angermann 1990; Mitchell-Jones et al. 1999). Even though populations appear stable across much of their range, possible declines seem to occur in the Alps, Sweden, and Russia (Thulin 2003). In Europe, isolated alpine populations of the sub-species *L. t. varronis* occur in the Alps above 1,300 m (Mitchell-Jones et al. 1999). According to the reporting for the Habitats Directive (2013–2018), the conservation status of these populations is considered unfavourable/inadequate in Italy and France. The causes of regional declines are not clear. However, climate change seems to impact the species through two interconnected, additive mechanisms. Firstly, being adapted to the cold climate (Angerbjörn and Flux 1995), the species probably suffered from the temperature increase over the last decades. In the Alps, the mountain hare is expected to thrive at the highest altitudes, where the typical alpine vegetation is dominant, and at the montane level (Amori et al. 2008; Bisi et al. 2013), however, as a mountaintop species, its possibility to shift towards higher altitudes is minimal. Secondly, warmer temperatures promote a rise in altitude of the European hare's (*Lepus europeaus*) range. In the Alps, the optimal habitat for the European hare is mainly located in lowlands and valley bottoms, and only occasionally the species occurs up to 2000 m (Amori et al. 2008). Global warming, however, could lead to a loss and fragmentation of the mountain hare habitat (Bisi et al. 2015; Rehnus et al. 2018) and it could determine the ascent in altitude of the European hare, which will then penetrate the range of the mountain hare (Schai-Braun et al. 2021). The reduction in snow persistence caused by climate change affects both species and it can play an important role in this respect. It leads to camouflage mismatch for mountain hare, potentially increasing its mortality, and at the same time it can facilitate the movement and search for food for the European hare (Levänen et al. 2017; Zimova et al. 2020). An increase of an at least temporary overlap between the two species at high elevations (Bisi et al. 2015) could also indirectly threaten the mountain hare populations exacerbating hybridization with the European hare (La Morgia and Venturino 2017; Thulin 2003; Thulin et al. 2006; Thulin and Tegelström 2002) and possibly competition (Thulin 2003). In Fennoscandia, the two hare species are highly sympatric (e.g., Levänen et al. 2017, 2018; Pohjoismäki et al. 2021; Levänen et al. 2019). However, hybrid zones between them are well documented (Thulin 2003; Thulin et al. 2006; Thulin and Tegelström 2002). In Scandinavia, Thulin and Tegelström (2002) and Thulin et al. (1997) documented the widespread introgression of mountain hare mitochondrial DNA (mtDNA) in European hares. In Russia, Thulin et al. (2006) also found specimens with European hare mitochondrial genome and apparent mountain hare nuclear genome, suggesting a reciprocal transfer of mtDNA between the two species. Hybridization between the two species is also reported in the Italian Alps (Perlini 1923; Scandura et al. 2008). The competition between the two species was inferred because a comparison of their distributions shows that in most places where they are in contact, the mountain hare seems restricted to high altitudes and deep forests (Thulin 2003). Lind (1963) found that in sympatry with the European hare, mountain hares live in denser forests and further away from open fields in respect to where they are allopatric. In Sweden, the reductions in the mountain hare range coincided with the establishment of European hares (Thulin 2003). This research indicates a potential for European hares to expand into the mountain hare range to the detriment of the latter.

Despite the potential pressure from climate change to the mountain hare and the possible increasing spatial overlap with the European hare, no detailed data are available to describe the distributional pattern and the potential for interaction between the two species at the regional scale and to infer the impacts of climate change on the mountain hare. So far, no study has investigated the distribution of mountain and European hare in the Alps over time. Our research aims to fill this gap by resampling broad elevational transects after 12 years, repeating the same survey in 2009 and 2021. Given the different ecology of the mountain and the European hares (Amori et al. 2008), in our study area in the Western Italian Alps, within an altitudinal range of 1500–3000 m, we expected an expansion over time in the European hare presence due to warmer temperature, and therefore, an increased overlap between the two species. During the first survey, in 2009, we expected (1) a low proportion of samples belonging to the European hare, (2) a difference in the distribution of the two species across habitats and altitudes, and (3) the occurrence of an ‘overlapping zone’, where both species could be found, at low altitudes. In 2021, we expected (4) an increase in the proportion of European hare samples with respect to 2009, and (5) changes in the distribution of the two species along the altitudinal gradient and in different habitat use. The data presented here allows us to test these hypotheses and quantify the impact of more than a decade of climate change on these two species.

Materials and methods

Study area

We investigated the spatial pattern of *Lepus* species in the Orco Valley, Gran Paradiso National Park, Western Italian Alps (45°26’N, 7°08’E). We collected data in a study area of about 180 km^2, ranging in altitude from 1500 to about 3000 m a.s.l. The mean temperature fluctuates between −7 °C in winter (February) and 10/11°C in summer (August), with precipitations showing a peak in spring (April to June, depending on the year) (climate data provided by the Regional Environmental Agency, ARPA Piemonte). Like for other Alpine valleys, climate change is expected to have a high impact in this area and on alpine species in general (IPCC 2022). Since the late 19th century, the Alps have warmed twice the global average, and larger changes in the Alpine zone and summer season are expected in the scenarios with more intense climate change, for which temperature rise may reach 5 °C by the end of the century. Recent studies indicate for Italy, for the period 2021–2050, a temperature increase of up to 2 °C on a seasonal scale, with more pronounced increases in the Alpine zone and summer season (Spano et al. 2020).

The vegetation at lower altitudes is composed mainly of larch (*Larix decidua*) woods, sometimes mixed with spruce (*Picea excelsa*), with rhododendron (*Rhododendron ferrugineum*) and juniper (*Juniperus communis*) as the main shrubs. As altitude increases, the percentage of shrubs becomes more relevant. Above 1800 m, the vegetation is represented by alpine meadows, with extensive *Festuca varia* grasslands, which gradually gives way to the nival zone vegetation, with pioneer species, mosses, cluster-forming plants such as prostrated willows (*Salix* spp.) and lichens (Fig. 1).

Field data collection

In summer 2009, considering a spatially homogeneous survey of *Lepus* presence in the study area performed by the park rangers, we searched for faecal pellets in 47 locations where at least one hare species was previously detected (Fig. 1). Within each location, we collected pellets along transects interspersed with six circular plots. We randomly selected the direction and starting point for a 250 m line transect and then searched for faecal pellets along the line. Moreover, we established six plots (10 m diameter) along the line (50 m inter-plot distance), and within each of them, we counted and collected all faecal pellets. The sampling design was chosen to gather spatially replicated data (for occupancy based on spatial replicates, see also Srivathsa et al. 2018, and an example in Bisi et al. 2019) and allow for subsequent occupancy analyses (La Morgia et al. *in prep*). Samples were stored in polyethene bags and frozen at -20 °C within two days. Transects and plots were resampled in 2021.

Genetic analyses

Reference DNA material for genetic analyses was obtained from tissue samples from European and mountain hares hunted in areas neighbouring the study site. We adopted the protocol described in Laird et al. (1991) for DNA extraction from tissue samples.

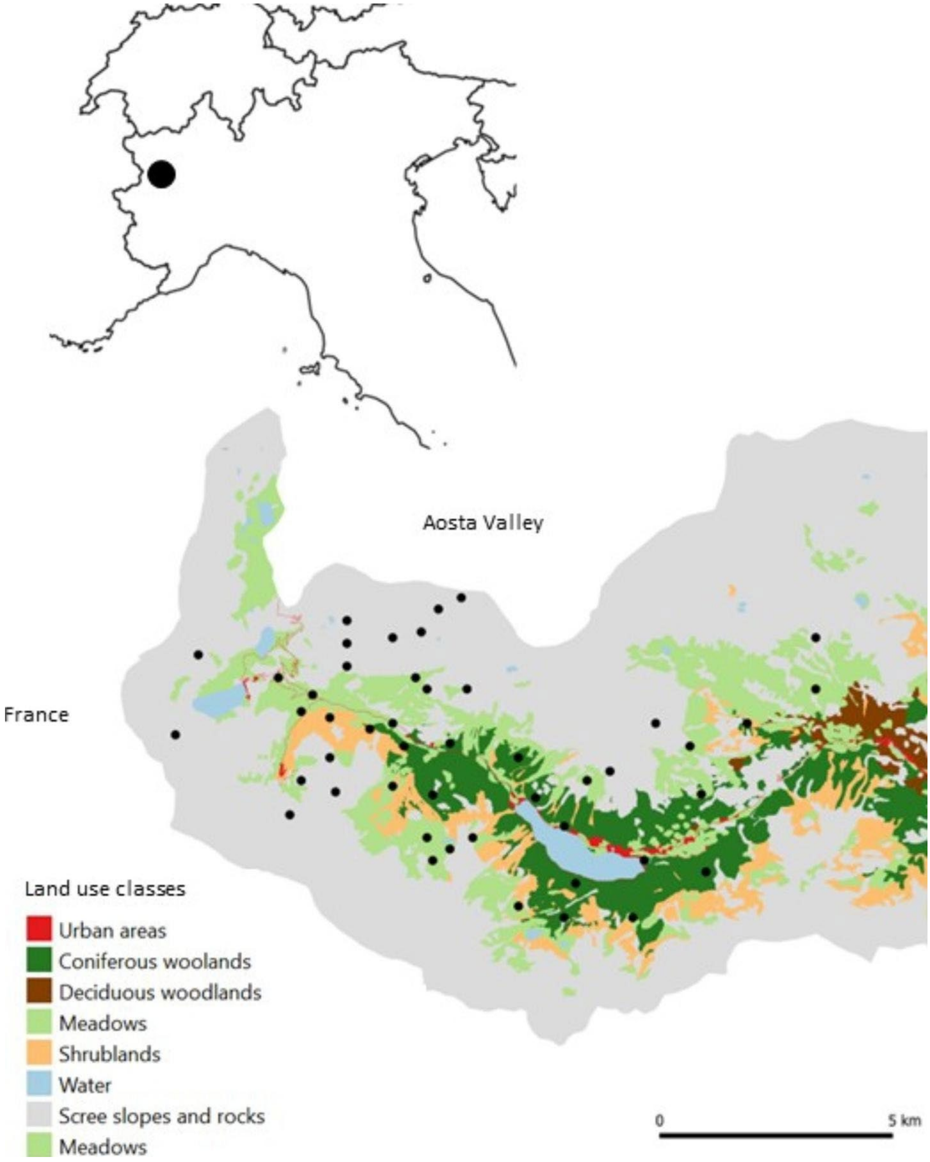

Fig. 1 The study area is located in the Turin district (black dot), NUTS-3 (Piedmont Region, Northwest Italy); below the simplified vegetation map of the high Orco Valley, Gran Paradiso National Park, with the 47 investigated locations (black dots on the vegetation map)

We randomly selected pellets from each pellet group for each location and we used them for DNA extractions. Extraction from randomly selected faecal pellets was performed using the "QIAamp Stool" kit (Qiagen, Helden, Germany), following the manufacturer's instructions. Mitochondrial DNA fragments were amplified through a nested-PCR, using cytochrome *b* primers described in Scandura et al. (2008). In the first amplification, we used primers Le-cyt-F1 (5'-GAGGACAAATATCATTCTGAGG-3') and Le-cyt-R1 (5'-GTCT-

GATGGGATACCTGATGG-3’), suitable for both hare species. The amplified products (239 bp fragments) were used as a template for a second multiplex PCR, where the primer Le-cyt-R1was used in association with a European hare (Le-cyt-F3, 5’-CTATCAGCCATTCCTTATATCG-3’) and a mountain hare (Le-cyt-F4, 5’-TCATTCTCCCATTCATCATCG-3) specific primers. Amplification products were 195 bp and 95 bp, for the European and the mountain hare, respectively. In order to double check the PCR results, about 10% of the samples (N = 10 and N = 20, for 2009 and 2021 samplings, respectively) were re-typed by single PCR. The results obtained were completely concordant with those of multiplex-PCR.

Nuclear DNA extraction and PCR amplification were also performed for sex identification. Again, we followed a protocol defined by Scandura et al. (2008). First, to determine sex, we amplified the sex-determining region Y (Sry) using primers Le-sry-F (5’- TCCCAAAATGCGAAACTCAG-3) and Le-sry-R (5’-GGCGAGGTCTGTACTTGTAGTC-3’). The amplification product was 152 bp. Negative samples were amplified again, using the above-mentioned Sry primers and the generic primers used for the first mtDNA amplification as an internal control. Individuals were classified as (i) males if both 152 and 239 bp amplification products were detected, and as (ii) females, when only the 239 bp amplification product was detected. For all other samples, we considered the sex determination unsuccessful.

PCR reactions were performed in a 25 µL volume containing 10 µl template DNA, with a final concentration of 1X Reaction Buffer, 1.5 mM of $MgCl_2$, 5% of DMSO, 250 µM of dNTPs, 0.5 µM of each primer, and 1 U/sample of Taq DNA polymerase (Fischer, U.S.). Ultra-pure water was added to reach the final volume of 25 µL. For DNA amplification, we used a Bio-Rad Thermocycler. Cycles were set as follows: a first initialization step of 5 min at 94 °C was followed by 35 cycles including 1 min at 94 °C, 1 min at 60 °C, 1 min at 72 °C, and a final extension step of 10 min at 72 °C. Amplification products were detected by ethidium bromide staining after 3% agarose gel electrophoresis.

Statistical analyses

Proportion of samples with european hare mtDNA

We estimated the proportion of pellets with the European hare mtDNA collected in 2009 with standard methods, as the fraction of samples determined as European hare, out of the total number of successfully amplified hare samples, describing the sampling uncertainty with the Wald interval.

Since data on the presence of European hare mtDNA in alpine environments were also available from the nearby Aosta Valley (Scandura et al. 2008), we adopted a Bayesian approach to incorporate this knowledge into our estimate for 2009. We represented the prior information about the proportion of European hare mtDNA, θ , with a beta probability distribution $beta\,(a, b)$, with expected value 0.14, mode at 0.13, and $Pr(0.07 < \theta < 0.26)$. Given the prior and a sampling model described by a binomial (57, θ) probability distribution, the posterior distribution for 2009, $p\,(\theta|\,y)$, is again described by a beta distribution, $beta\,(a + y, b + n - y)$, where *y* is the observed number of samples determined as European hare, and *n* the total number of hare samples. We then adopted the same approach to incorporate the 2009 results in the estimate for 2021.

Spatial distribution of Lepus mtDNAs

To describe the spatial distribution of *Lepus* mtDNAs across the study area, we first calculated the proportion of cells with European or/and mountain hare samples and mapped their occurrences. We obtained data on altitude and the habitat types for our sampling locations in a GIS environment (QGIS Development Team 2016) using a DEM with a 10 m resolution (Regione Piemonte) and a forest map of the Piedmont Region (1:25000), as revised by the park for high altitude habitats. For altitude, we considered that our locations ranged from 1544 to 3010 m (median = 2269 m), and we divided our study area into four intervals, corresponding to the quantiles of the altitudinal distribution. For habitat types, we drew a buffer of 250 m diameters (cf. length of the transects) around each location, and we identified the dominant habitat type as the one with the largest extent within the buffer. For this analysis, we considered three main habitat types (woodlands and shrublands, alpine meadows, pioneer vegetation and rocky areas).

We then assessed whether each species occurred in different proportions in the quantiles of the altitudinal distribution and in the main habitat types, within and between years and species, through Bayesian tests of proportions (Bååth 2014) using the number of locations. We used a 'non-informative' beta prior $\theta \sim Beta(1,1)$, and estimated the relative frequencies of European and mountain hares assuming $x \sim Binom\left(\theta, n\right)$ for each altitudinal interval/habitat type. For altitudinal data, we also tested differences in the mean altitude between species or, for the same species, between years, through Bayesian *t*-tests.

We performed statistical analyses in R, version 3.5.0 (R Core Team 2015), using the RStudio IDE (RStudio Team 2016).

Results

We found hare faecal pellets in 27 (2009) and 17 (2021) out of the 47 investigated locations.

In 2009, one location was excluded from subsequent analyses for problems in faecal sample storage. Out of the 66 attempts, we successfully amplified mtDNA and discriminated the two species in 57 cases, determining the sex of the animals in 34 cases. In 2021, we extracted DNA for all 17 locations. For a total of 176 DNA extractions, we successfully amplified mtDNA and determined the sex of the animals.

The proportion of samples with European hare mtDNA increased in 2021, as reported in Table 1 and shown in Fig. 2.

In 2009, we found mountain hare samples in 25 out of the 26 locations for which faecal samples were available (0.53 out of the 47 surveyed locations; Fig. 3a). We classified 17 samples as males and only 7 as females. Males occurred throughout the altitudinal range of the study area. Females were detected at altitudes ranging from 1887 to 2674 m. European hare samples were found in 5 out of the 26 locations (0.11 out of the 47 surveyed locations), at different altitudes (range 1745–2674 m). The sex determination was not successful for one individual, and most European hares (4 out of 6) were classified as females. We classified as male only one individual at 2287 m, where a mountain hare male was also detected. Indeed, as shown in Fig. 3a, we found both mountain and European hares in 4 locations.

In 2021, we found mountain hare samples in 14 out of the 17 locations for which faecal samples were available (0.30 out of the 47 surveyed locations; Fig. 3b). Males (69 samples)

Table 1 Summary of the sampling effort and main results obtained in 2009 and 2021

		2009	2021
Effort	No. locations surveyed	47	47
	No. amplified samples – total	66	172
	No. amplified samples – median per location (range in brackets)	3 (1–6)	8 (2–43)
Proportion of European hare mtDNA in the faecal samples	Standard point estimate with Wald intervals	0.11 (0.03–0.18)	0.24 (0.18–0.30)
	$\widehat{\theta}$ (expected mean of the posterior distribution)	0.124 ($0.072<\Pr<0.187$)	0.187 ($0.145<\Pr<0.232$)
Lepus spp. distribution	No. of locations with pellets	27	17
Mountain hare spatial distribution	No. of locations with mountain hare mtDNA	25	14
	Mountain hare altitudinal range	1595–3010	1763–3010
European hare spatial distribution	No. of locations with European hare mtDNA	5	9
	European hare altitudinal range	1745–2674	1763–2432
Co-occurrence of hare species	No. of locations with mtDNA of both species	4	6
	Co-occurrence of different sexes of the two species (e.g., male European hare with female mountain hare, etc.)	0	6

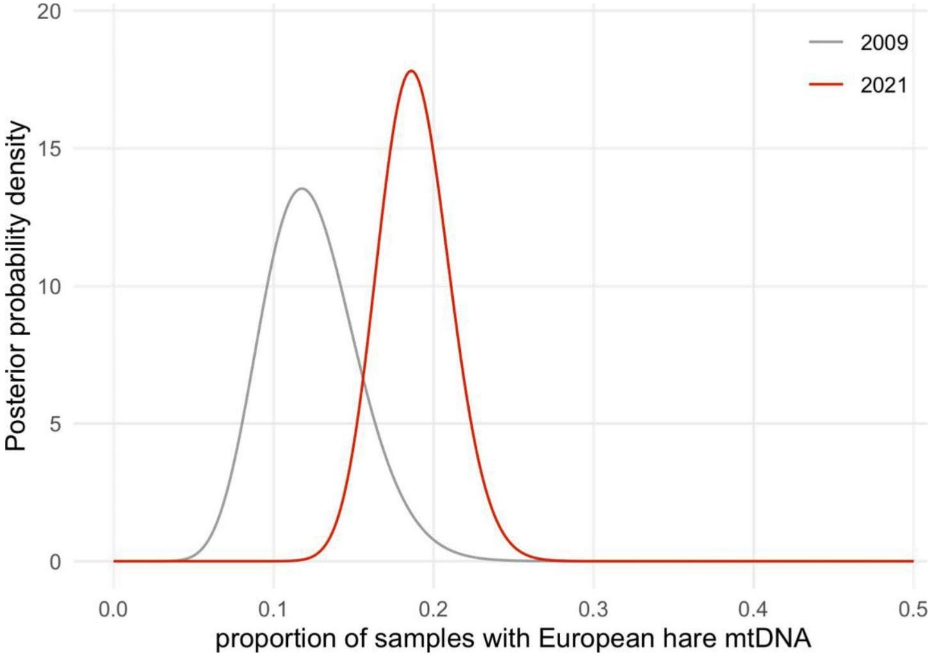

Fig. 2 Posterior probability densities of samples with European hare mtDNA (θ). For the 2009 posterior, the peak is at 0.118 and the expectation of θ is 0.124. For 2021, the peak is at 0.185 and the expectation of θ is 0.187 (see also Table 1 for credibility intervals)

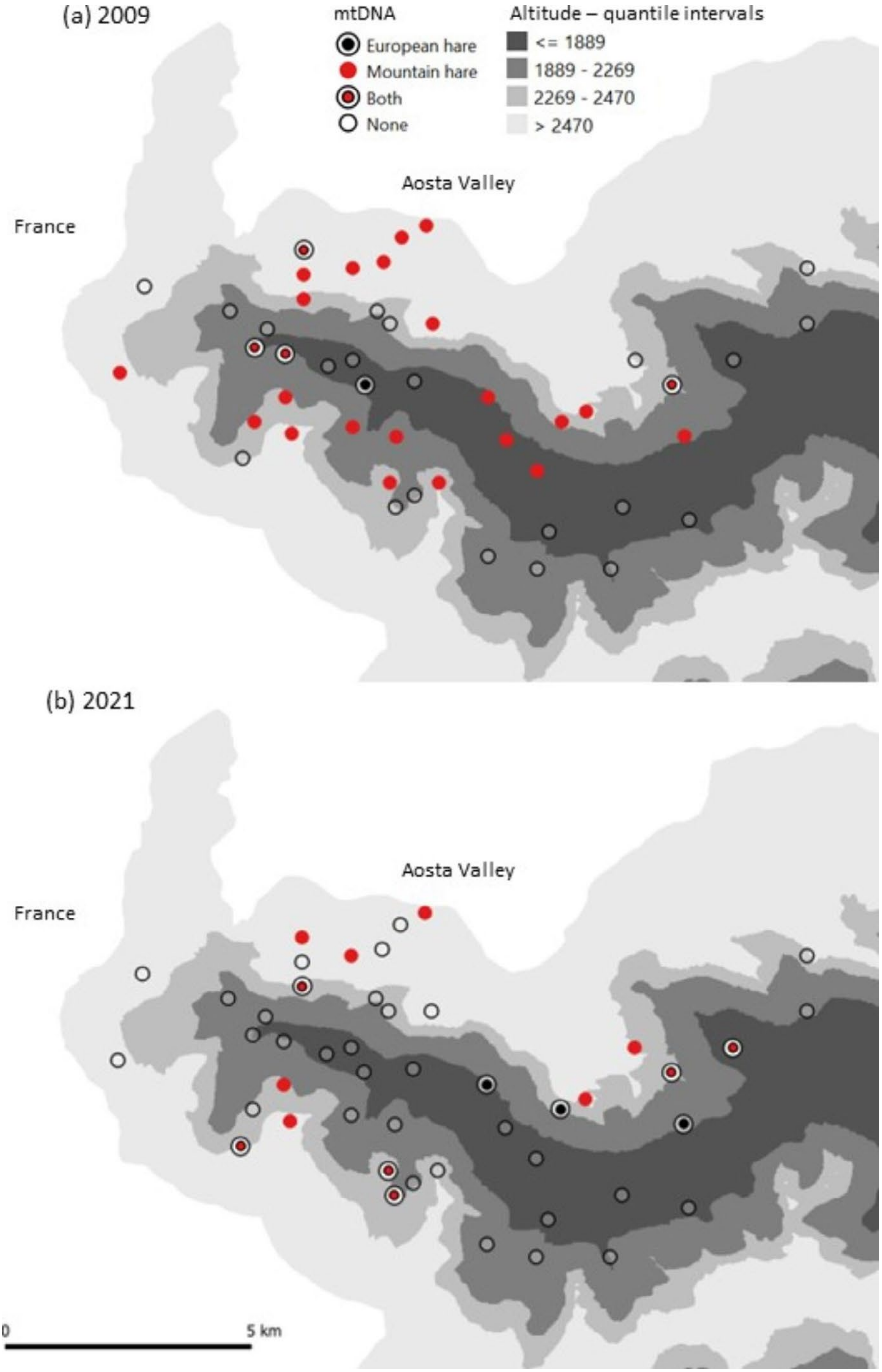

Fig. 3 Distribution of the mtDNA of European and mountain hares in the study area in 2009 (**a**) and 2021 (**b**). The map also shows the altitudinal intervals (quantiles of the altitudinal distribution)

and females (64 samples) occurred in the altitudinal range 1763–3010 m and 2270–3010 m respectively, and they co-occurred in 10 locations. European hare samples were found in 9 locations (0.20 out of the 47 surveyed locations), within an altitudinal range (1763–2432 m) similar to 2009. 18 European hare samples were classified as females, and 24 as males. The two sexes co-occurred in 7 locations. As shown in Fig. 3b, we found the mountain and European hare in 6 locations. In these locations, data indicated that individuals of mountain hare coexist with European hare individuals of different sex, thus suggesting a potential for hybridisation.

Figure 4 shows our sample's altitudinal distribution of the mtDNA of the hare species. In 2009, the 'overlap area', where we found both species, ranged in altitude from 1595 (min altitude of the mountain hare) to 2674 (max altitude of the European hare). In 2021, it

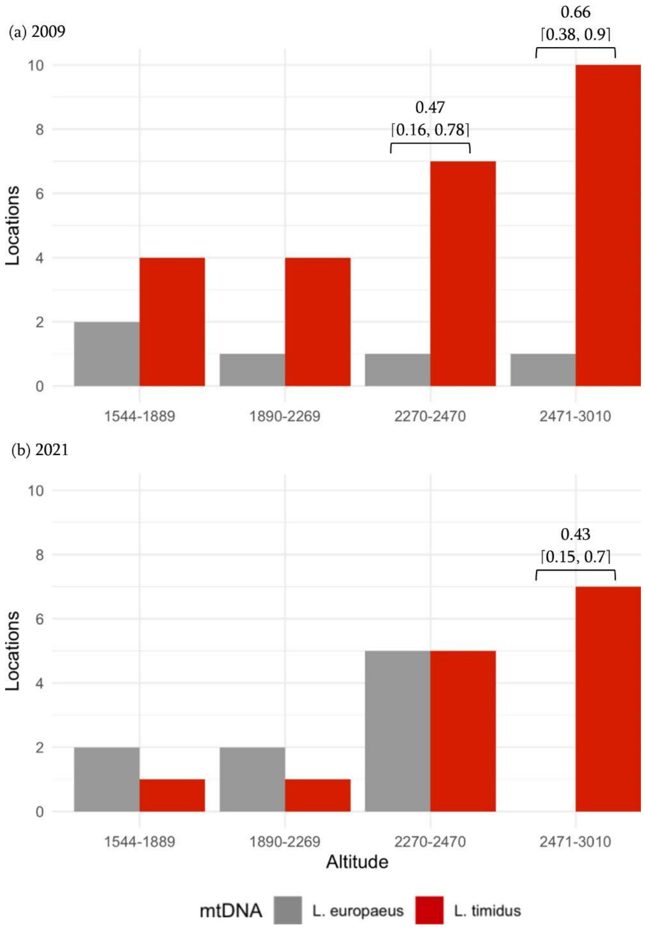

Fig. 4 Frequency of locations with mountain (red) and European (grey) hare mtDNA in the altitudinal quantiles −2009 in panel (**a**) and 2021 in panel (**b**). Estimated pairwise differences with 95% credible intervals are reported only when there is evidence of an actual difference between the two species. Details on the differences between altitudinal intervals within each species per year are provided in the Supplementary Materials

ranged in altitude from 1763 (min altitude of the mountain hare) to 2432 (max altitude of the European hare). We found the same samples of the two species in the range 2270–2470 m.

The analysis of the posterior distributions of the samples of the two species in the altitudinal intervals revealed that in 2009 the frequency of occurrence of the European hare was low but even (Fig. S1). However, in 2021 the frequency of occurrence in the interval (2270–2470) was larger and there were no detections in the last interval (2471–3010) (see Fig. S2 for details). On the contrary, in both 2009 and 2021, the frequency of the mountain hare mtDNA increased with altitude. Indeed, in 2009 the ratio of the two species was in favour of the mountain hare as altitude increased (Fig. 4a) and the frequency was larger in the last altitudinal interval (2471–3010) than in the first two intervals (<2270) (Fig. S4). In 2021 (Fig. S5), the mountain hare frequency was larger in the intervals (2471–3010) and (2270–2470) than in the first two intervals (<2270). The estimated mean altitude of the mountain hare was larger than that of the European hare, especially in 2021 (pairwise difference with 95% credible interval = 274 [16, 543]; Table T1).

For the habitat types, we verified that in both 2009 and 2021 the estimated relative frequency of occurrence of the European hare was relatively even (Fig. S7 and S8), with a slight decrease in pioneer vegetation and rocky areas, where the mountain hare was dominant (Fig. 5). Indeed, for the mountain hare, in 2009 the estimated relative frequency of occurrence was large in pioneer and rocky areas and limited in alpine meadows (Fig. S10). In 2021, the large relative frequency of occurrence of the mountain hare in pioneer and rocky areas was confirmed, especially with respect to woodlands and shrublands (Fig. S11).

Discussion

The data gathered in this study allows us to assess directly, and for the first time, the impact of climate change on hare species in the Italian Alps. In our study area, we confirmed the spatial overlap between the two species and the potential for hybridization. Indeed, the mountain hare was always more common at the highest altitudes, both in 2009 and 2021. Still, the co-occurrence pattern changed over time: in 2021, at intermediate-high altitudes (2270–2470 m), we could no longer detect a difference in the occurrence of the two species. Moreover, in the last decade, the European hare increased and expanded at the expenses of the mountain hare, which seems now relegated mainly to the highest altitudes and present in a limited number of locations with respect to 2009.

In 2009 the mountain hare was relatively common in the upper part of the Orco valley, being detected in 53% of investigated transects. As expected (hypothesis 1), during the first survey we found a low proportion of samples belonging to the European hare. The species was present but rare, being found only in 10% of localities. The proportion of European hare samples observed in our study was similar to what Scandura et al. (2008) obtained by their investigations in neighbouring valleys, where they also confirmed the ongoing hybridization between the two species through nuclear DNA analyses.

Consistently with our second hypothesis, we also observed that the mean altitude of the two species was different, with the frequency of the mountain hare increasing with altitude. Moreover, the estimated relative frequency of occurrence of the mountain hare was larger than for the European hare in the areas with pioneer vegetation and rocks.

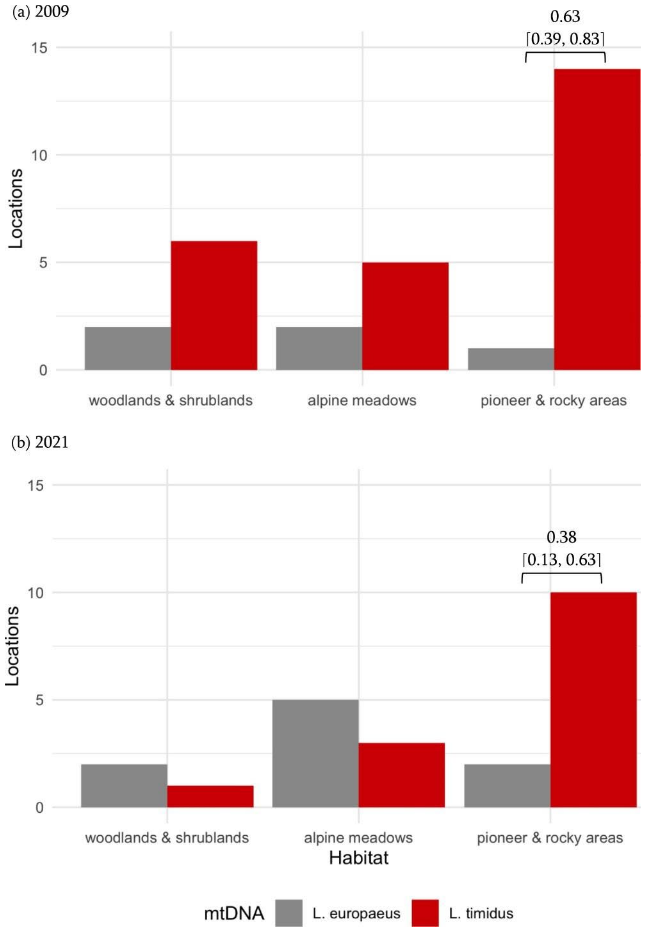

Fig. 5 Frequency of locations with mountain (red) and European (grey) hare mtDNA in the dominant habitat types – 2009 in panel (**a**) and 2021 in panel (**b**). Estimated pairwise differences with 95% credible intervals are reported only when there is evidence of an actual difference between the two species. Details on the differences between habitat types within each species per year are provided in the Supplementary Materials

The observed distributional patterns are consistent with the ecology of the two species. The mountain hare has a broad altitudinal range (400–4000 m, Trocchi and Riga 2005) and in forested landscapes, it usually prefers dense woodlands or shrublands, i.e., habitat providing shelter and concealment from predators (Bisi et al. 2013). However, it also shows a preference for the highest altitudes (1500–2800 m) and the habitats therein, which appears more marked in the Western than in the Central and Eastern Alps (Artuso and Paganin 1993). Our data refer to the summer season, and we do not know the altitudinal range and the habitats used in winter. The species shows high site fidelity, with seasonal home ranges overlapping considerably (Bisi et al. 2011); therefore, we do not expect a completely different distribution in winter.

On the contrary, in our study area, the optimal habitat for the European hare should correspond to valley bottoms. Nevertheless, we recorded the species up to 2674 m. Interestingly, the highest locality with European hare was characterized by a landscape atypical for the species, dominated by rocky debris, and where herbaceous coverage was almost completely absent. In the Alps, the European hare could occur up to 2000 m, although in the past it rarely reached this altitude (Amori et al. 2008; Trocchi and Riga 2005). The species has high ecological plasticity; it can thrive in a variety of habitats, including agricultural areas, moorlands, and sparse woods, but it usually prefers flat and hilly landscapes, avoiding dense woods, overgrown forests, shady slopes and cold and damp soils (Amori et al. 2008).

The data also confirmed the presence of an ‘overlap area’ between the two species (hypothesis 3). Based on our data we could not estimate the species densities in this range. However, in these atypical European hare habitats, where one species dominates in numbers, or when both species display low densities, increased frequency of hybridization is expected due to low availability of conspecific partners, a phenomenon referred to as Hubbs’ principle, as also observed by Jansson et al. (2007). Data on hare mating behaviour are scarce, and even if our sex determination approach could certainly be improved in future studies, our results already allowed us to verify the overlap of different sexes of the two species, thus confirming the potential for ongoing hybridization.

In 2021 the largest frequency of occurrence of the mountain hare at the highest altitudes was confirmed. In general, the mountain hare seemed more and more linked to habitats at high elevations, and it was always more frequent than the European hare in pioneer vegetation and rocky areas. The latter slightly increased in the alpine meadows and at intermediate-high altitudes. These findings support our hypothesis (5), on changes in the distribution of the two species along the altitudinal gradient and in different habitat types. Schenker et al. (2020) documented the occurrence of a European hare at high elevation in the Swiss Alps and changes in the distributional patterns of the two species have recently been observed also in other Italian Alpine areas (Hackländer et al. 2022).

Finally, our Bayesian analyses to estimate the proportion of the European hare mtDNA in the samples supported hypothesis (4). The analyses used an informative prior based on the data gathered in 2009. It was thus very conservative in the estimate of the proportion for 2021. Nevertheless, it revealed an increase over time in the proportion of the European hare.

Conclusions on the trends of hare populations need support from long-term studies, also because hares are certainly susceptible to population fluctuations. Nevertheless, our two surveys represent a first attempt in this direction and they can be regarded as a reference points for future studies. Our results confirm with field data the predictions of modelling attempts conducted to assess the future evolution of the mountain and European hares in the Italian (Bisi et al. 2015; La Morgia and Venturino 2017) and Swiss (Rehnus et al. 2018) Alps. The evidence that rapid climatic changes will facilitate the expansion of European hare populations at the expense of mountain hares has also been recently supported by the models developed by Caravaggi et al. (2017). According to these authors, niche overlap metrics between the two species describe an increasing probability of geographic overlap and, hence, interspecific interaction between European hares and neighbouring mountain hare subspecies in the coming decades.

Global warming can affect the survival and distribution of the two hare species, e.g., because of the shortening of the snow-cover duration (Levänen et al. 2017; Zimova et al. 2020), and under climate change scenarios, Rehnus et al. (2018) predicted a loss of habitat area and an increased fragmentation for the mountain hare. Although their models did not include interspecific interactions, these authors recognized that increasing temperatures could favour the European hare and increase the relevance of processes such as competition and introgression. In England, Bedson et al. (2021) also predicted a range contraction, on hilltops, for the mountain hare, and a contrasting expansion of the European hare. In this context, some alleles transferred from the mountain to the European hare might also offer adaptive advantage for the latter (Pohjoismäki et al. 2021), offering an additional advantage to the expanding species. In some areas of the Italian Alps, the proportion of introgression in areas of sympatry as recently been estimated around 5–14% (Hackländer et al. 2022).

Conclusions

Overall, our survey supports the hypothesis of a retreat of the mountain hare to high elevations, where the species is now restricted to a very narrow, high-altitude strip, and an expansion of the European hare, probably favoured by climate change.

For these reasons, there is now an urgent need for conservation measures to mitigate the adverse effects of global warming and avoid the evolution of the European-mountain hare alpine system towards a complete admixture situation (Allendorf et al. 2001). In the Alps, nowadays, climate change is undoubtedly a main driver of change. However, wildlife management interacts with global warming and it could potentially exacerbate its effects on hares. Despite a clear difference in the spatial and temporal scales, the two drivers affect the local communities. Climate change must be contrasted at broad scales, but locally, wildlife managers should at least try to mitigate any adverse effect due to improper management practices. Rehnus et al. (2018) suggested that activities for improving habitat quality should focus on patches with high importance for connectivity, and that habitat improvements should be put beside by other measures such as limiting the impact of winter recreational activities in critical habitat patches and applying schemes for sustainable hunting. In this respect, strategies for conserving the Alpine mountain hare should also aim to manage the European hare appropriately. As already proposed by La Morgia and Venturino (2017), management practices such as the deliberate release of European hares for hunting purposes in the Alps should be avoided, as they may lead to local ”founder effects”, with an artificial increase in the contact zones between European and mountain hares that would ultimately promote hybridization and biotic homogenization (Allendorf et al. 2001). Finally, more research is needed to clarify the extent, magnitude, and direction of the European-mountain hare hybridization in the Alps. Data from other geographical regions published so far mainly supported the hypothesis that the introgression is highly asymmetrical in the direction of gene flow from mountain hare to brown hare (e.g., Levänen et al. 2017; Melo-Ferreira et al. 2005), but a reciprocal transfer of mtDNA has been observed in Russian populations (Thulin et al. 2006) and in Finland (Levänen et al. 2017). In this respect, the study carried out by Scandura et al. (2008) represented a first, valuable attempt for the Alpine region. Nevertheless, an increase in the sampling effort and the spatial extent of investigated areas seems mandatory to adequately describe the current scenario of hybridization and introgression in the Alps and to produce better estimates of its future evolution.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10531-023-02664-1.

Acknowledgements This work was supported by Fondazione CRT and Gran Paradiso National Park.

Author contributions VLM, SB and AS conceived the ideas and defined the general methodology; VLM conceived the sampling design; EM collected the data; IM and ET collected the data and performed the laboratory analyses, under the supervision of AS; AS defined and coordinated the genetic analyses; VLM performed the statistical analyses; VLM, SB and AS wrote the paper. All authors reviewed and accepted the manuscript.

Data availability The datasets analysed during the current study will be made available in a github repository, upon acceptance of the paper.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

References

Allendorf FW, Leary RF, Spruell P (2001) The problems with hybrids: setting conservation guidelines. Trends Ecol Evol 16:613–622. https://doi.org/10.1016/s0169-5347(01)02290-x

Amori G, Contoli L, Nappi A (2008) Fauna d'Italia. *Mammalia* II: *Erinaceomorpha, Soricomorpha, Lagomorpha, Rodentia*. Vol. XLIV. Edizioni Calderini de Il Sole-24 Ore. ISBN: 978-88-506-5302-7

Angerbjörn A, Flux JEC (1995) Lepus timidus. Mammal Spec 495:1–11

Artuso I, Paganin M (1993) Alcuni risultati del "Progetto Alpe" (U.N.C.Z.A. - F.I.d.C.). Suppl Ric Biol Sel XXI 463–466

Bååth R (2014) Bayesian First Aid: A Package that Implements Bayesian Alternatives to the Classical *.test Functions in R, Presented at the UseR! 2014 - the International R User Conference

Bedson CPE, Devenish C, Symeonakis E, Mallon D, Reid N, Harris WE, Preziosi R (2021) Splitting hares: current and future ecological niches predicted as distinctly different for two congeneric lagomorphs. Acta Oecol 111:103742. https://doi.org/10.1016/j.actao.2021.103742

Bisi F, Nodari M, Dos Santos Oliveira NM, Masseroni E, Preatoni DG, Wauters LA, Tosi G, Martinoli A (2011) Space use patterns of mountain hare (*Lepus timidus*) on the Alps. Eur J Wildl Res 57:305–312. https://doi.org/10.1007/s10344-010-0429-2

Bisi F, Nodari M, Dos Santos Oliveira NM, Ossi F, Masseroni E, Preatoni DG, Wauters LA, Martinoli A (2013) Habitat selection and activity patterns in Alpine mountain hare (*Lepus timidus varronis*). Mammalian Biology 78:28–33. https://doi.org/10.1016/j.mambio.2012.05.004

Bisi F, Wauters LA, Preatoni DG, Martinoli A (2015) Interspecific competition mediated by climate change: which interaction between brown and mountain hare in the Alps? Mammal Biol 80:424–430. https://doi.org/10.1016/j.mambio.2015.06.002

Bisi F, Cremonesi G, Gaffi L, Zibordi F, Gagliardi A, Gueli L, Martinoli A, Preatoni DG (2019) Watching a movie or going for a walk? Testing different Sun bear (*Helarctos malayanus*) occupancy monitoring schemes. Hystrix. Italian J Mammalogy (n s. https://doi.org/10.4404/hystrix-00246-2019

Brito-Morales I, Molinos JG, Schoeman DS, Burrows MT, Poloczanska ES, Brown CJ, Ferrier S, Harwood TD, Klein CJ, McDonald-Madden E, Moore P, Pandolfi JM, Watson JEM, Wenger AS, Richardson AJ (2018) Climate velocity can inform conservation in a warming world. Trends Ecol Evol 33(6):441–457. https://doi.org/10.1016/j.tree.2018.03.009

Caravaggi A, Leach K, Santilli F, Rintala J, Helle P, Tiainen J, Bisi F, Martinoli A, Montgomery WI, Reid N (2017) Niche overlap of mountain hare subspecies and the vulnerability of their ranges to invasion by the european hare; the (bad) luck of the irish. Biol Invasions 19:655–674. https://doi.org/10.1007/s10530-016-1330-z

Freeman BG, Scholer MN, Ruiz-Gutierrez V, Fitzpatrick JW (2018) Climate change causes upslope shifts and mountaintop extirpations in a tropical bird community. Proc. Natl. Acad. Sci. U.S.A., 115(47):11982–11987. https://doi.org/10.1073/pnas.1804224115

Giezendanner J, Bertuzzo E, Pasetto D, Guisan A, Rinaldo A (2019) A minimalist model of extinction and range dynamics of virtual mountain species driven by warming temperatures. PLoS ONE 14(3):e0213775. https://doi.org/10.1371/journal.pone.0213775

Gobiet A, Kotlarski S, Beniston M, Heinrich G, Rajczak J, Stoffel M (2014) 21st century climate change in the european Alps—A review. Sci Total Environ 493:1138–1151. https://doi.org/10.1016/j.scitotenv.2013.07.050

Hackländer K, Schwienbacher S, Smith S (2022) New insights into interspecific competition within the genus *Lepus* in the Alps. 6th World Lagomorph Conference, Montpellier, France, July 4–8 2022. Abstract book, p. 118

IPCC, Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change (2022) Climate Change 2022: Impacts, Adaptation and Vulnerability. Pörtner, H.-O, Roberts, D.C, Tignor, M, Poloczanska, E.S, Mintenbeck, K, Alegría, A, Craig, M, Langsdorf, S, Löschke, S, Möller, V, Okem, A, Rama, B. (eds.). Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp, https://doi.org/10.1017/9781009325844

Jansson G, Thulin C-G, Pehrson Ã (2007) Factors related to the occurrence of hybrids between brown hares *Lepus europaeus* and mountain hares *L. timidus* in Sweden. Ecography 30:709–715. https://doi.org/10.1111/j.2007.0906-7590.05162.x

La Morgia V, Venturino E (2017) Understanding hybridization and competition processes between hare species: implications for conservation and management on the basis of a mathematical model. Ecol Modell 364:13–24. https://doi.org/10.1016/j.ecolmodel.2017.09.002

Laird PW, Zijderveld A, Linders K, Rudnicki MA, Jaenisch R, Berns A (1991) Simplified mammalian DNA isolation procedure. Nucleic Acids Res 19:4293. https://doi.org/10.1093/nar/19.15.4293

Lenoir J, Svenning JC (2015) Climate-related range shifts–a global multidimensional synthesis and new research directions. Ecography 38(1):15–28. https://doi.org/10.1111/ecog.00967

Lenoir J, Bertrand R, Comte L, Bourgeaud L, Hattab T, Murienne J, Grenouillet G (2020) Species better track climate warming in the oceans than on land. Nat Ecol Evol 4(8):1044–1059. https://doi.org/10.1038/s41559-020-1198-2

Levänen R, Kunnasranta M, Pohjoismäki J (2017) Mitochondrial DNA introgression at the northern edge of the brown hare (Lepus europaeus) range. Ann Zool Fennici 55:15–24. https://doi.org/10.5735/086.055.0103

Levänen R, Thulin C-G, Spong G, Pohjoismäki JLO (2018) Widespread introgression of mountain hare genes into fennoscandian brown hare populations. PLoS ONE 13:e0191790. https://doi.org/10.1371/journal.pone.0191790

Levänen R, Pohjoismäki JLO, Kunnasranta M (2019) Home ranges of semi-urban brown hares (*Lepus europaeus*) and mountain hares (*Lepus timidus*) at northern latitudes. Ann Zool Fennici 56(1/6):107–120. https://www.jstor.org/stable/27100660

Lind EA (1963) Observations on the mutual relationship between the snow hare (*Lepus timidus*) and the field hare (*L. europaeus*). Suomen Riista 16:128–135

Melo-Ferreira J, Boursot P, Suchentrunk F, Ferrand N, Alves PC (2005) Invasion from the cold past: extensive introgression of mountain hare (*Lepus timidus*) mitochondrial DNA into three other hare species in northern Iberia. Mol Ecol 14:2459–2464. https://doi.org/10.1111/j.1365-294X.2005.02599.x

Mitchell-Jones AJ, Amori G, Bogdanowicz W, Kryštufek B, Reijnders PJH, Spitzenberger F, Stubbe M, Thissen JMB, Vohralik V, Zima J (1999) Atlas of European mammals. Academic Press, London, UK. ISBN: 978-0-85661-130-8

Moritz C, Patton JL, Conroy CJ, Parra JL, White GC, Beissinger SR (2008) Impact of a century of climate change on small-mammal communities in Yosemite National Park, USA. Science 322(5899):261–264. https://doi.org/10.1126/science.1163428

Perlini R (1923) Fauna alpina. Istituto Italiano di Arti Grafiche, Bergamo, Italia

Pohjoismäki JLO, Michell C, Levänen R, Smith S (2021) Hybridization with mountain hares increases the functional allelic reportoir in brown hares. Sci Rep 11:15771. https://doi.org/10.1038/s41598-021-95357-0

QGIS Development Team (2016) QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org Accessed 27 March 2023

R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing. https://www.r-project.org Accessed 27 March 2023

Rehnus M, Bollmann K, Schmatz DRK, Braunisch V (2018) Alpine glacial relict species losing out to climate change: the case of the fragmented mountain hare population (*Lepus timidus*) in the Alps. Glob Change Biol 24:3236–3253. https://doi.org/10.1111/gcb.14087

RStudio Team (2016) RStudio: Integrated Development Environment for R. RStudio, Inc, Boston, MA. https://posit.co/ Accessed 27 March 2023

Scandura M, Cossu A, Iacolina L, Apollonio M (2008) Indagine conoscitiva per il monitoraggio non-invasivo di una popolazione di lepre variabile (*Lepus timidus*). Dipartimento di Zoologia e Genetica Evoluzionistica, Università degli Studi di Sassari, Sassari, Italia

Schai-Braun SC, Jenny H, Ruf T, Hacklander K (2021) Temperature increase and frost decrease driving upslope elevatioanl range shifts in Alpine grouse and hares. Glob Change Biol 27:6602–6614. https://doi.org/10.1111/gcb.15909

Schenker L, Bollman K, Rehnus M, Brodbeck S, Gugerli F (2020) Hare's affair: lessons learnt from a noninvasive genetic monitoring for tracking mountain hare individuals. Ecol Evol 10:10150–10166. https://doi.org/10.1002/ece3.6676

Spano D, Mereu V, Bacciu V, Marras S, Trabucco A, Adinolfi M, Barbato G, Bosello F, Breil M, Chiriacò MV, Coppini G, Essenfelder A, Galluccio G, Lovato T, Marzi S, Masina S, Mercogliano P, Mysiak J, Noce S, Pal J, Reder A, Rianna G, Rizzo A, Santini M, Sini E, Staccione A, Villani V, Zavatarelli M (2020) Analisi del rischio. I cambiamenti climatici in Italia". Fondazione CMCC - Centro Euro-Mediterraneo sui Cambiamenti Climatici. https://doi.org/10.25424/cmcc/analisi_del_rischio

Srivathsa A, Puri M, Kumar NS, Jathanna D, Karanth KU (2018) Substituting space for time: empirical evaluation of spatial replication as a surrogate for temporal replication in occupancy modelling. J Appl Ecol 55(2):754–765. https://doi.org/10.1111/1365-2664.13005

Thulin CG (2003) The distribution of mountain hares *Lepus timidus* in Europe: a challenge from brown hares *L. europaeus*? Mammal Rev 33:29–42. https://doi.org/10.1046/j.1365-2907.2003.00008.x

Thulin CG, Tegelström H (2002) Biased geographical distribution of mitochondrial DNA that passed the species barrier from mountain hares to brown hares (genus *Lepus*): an effect of genetic incompatibility and mating behaviour? J Zool Lond 258:299–306. https://doi.org/10.1017/S0952836902001425

Thulin CG, Jaarola M, Tegelström H (1997) The occurrence of mountain hare mitochondrial DNA in wild brown hares. Mol Ecol 6:463–467. https://doi.org/10.1046/j.1365-294x.1997.t01-1-00199.x

Thulin CG, Fang M, Averianov AO (2006) Introgression from *Lepus europaeus* to *L. timidus* in Russia revealed by mitochondrial single nucleotide polymorphisms and nuclear microsatellites. Hereditas 143:68–76. https://doi.org/10.1111/j.2006.0018-0661.01952.x

Trocchi V, Riga F (2005) I Lagomorfi in Italia. Linee guida per la conservazione e la gestione. Min. Politiche Agricole e Forestali - Ist. Naz. Fauna Selvatica. Documenti Tecnici 25:1–128

Vitasse Y, Ursenbacher S, Klein G, Bohnenstengel T, Chittaro Y, Delestrade A, Monnerat C, Rebetez M, Rixen C, Strebel N, Schmidt BR, Wipf S, Wohlgemuth T, Yoccoz NG, Lenoir J (2021) Phenological and elevational shifts of plants, animals and fungi under climate change in the european Alps. Biol Rev 96:1816–1835. https://doi.org/10.1111/brv.12727

Wiens JJ (2016) Climate-related local extinctions are already widespread among plant and animal species. PLoS Biol 14(12):e2001104. https://doi.org/10.1371/journal.pbio.2001104

Zimova M, Giery Sean T, Newey S, Nowak JJ, Spencer M, Mills LS (2020) Lack of phenological shift leads to increased camouflage mismatch in mountain hares. Proc. R. Soc. B.2872020178620201786. https://doi.org/10.1098/rspb.2020.1786