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ORIGINAL RESEARCH

Coexistence of European hares and Alpine mountain hares in the Alps: what drives the occurrence and frequency of their hybrids?

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Abstract

As a glacial relict species, mountain hares are adapted to cold and snowy conditions. Conversely, European hares originate from the grasslands of the Middle East and spread from there throughout low-lying agricultural areas of Europe. Mountain hares and European hares generally occur allopatrically, however, sympatry occurs in some areas. In sympatric areas, introgressive hybridisation poses a threat to the Alpine mountain hare by reducing its genetic integrity. Introgressed individuals can be found in both species but are far more frequent in European hares than in mountain hares. The ecology of hybrids is poorly known in these species. To examine the Alpine mountain hare and European hare populations in the Alps with a particular focus on the occurrence and ecology of their hybrids, we performed molecular genetic analysis of hare faecal samples collected in four study areas in the Alps in South Tyrol for three winters and compared habitat associations of the genotyped samples. We recorded 150 individuals (i.e. 14 hybrids, 25 European hares and 111 Alpine mountain hares). Four introgressed individuals were at levels consistent with F2 hybrids, whereas the others showed an older interspecific gene flow. We found that hybrid faeces tended to be at lower elevations compared to those of Alpine mountain hare but at higher elevations than those of the European hare. The frequency of Alpine mountain hares decreased as the proportion of Alpine grassland increased but was positively correlated with the proportion of dwarf shrub heaths. No effect of vegetation type was found for the frequency of European hares and hybrids. Our results support the widely raised concerns that the European hare, as a generalist, is a strong competitor with the Alpine mountain hare in the Alpine ecosystem in the time of global climate change.

Introduction

The mountain hare (*Lepus timidus*) is well adapted to cold and snowy conditions. Hence, the distribution of this leporid is continuous from Scandinavia eastwards (Schai-Braun & Hackländer, 2016). On the contrary, European hares originate from the grasslands of the Middle East and from there, spread over the agricultural areas of Europe (Hackländer, 2023). Its geographical range today includes European lowland areas and extends eastwards to Lake Baikal (Schai-Braun & Hackländer, 2016). Mountain hares and European hares in general occur allopatrically. Sympatry only occurs under particular climatic conditions (see Thulin, 2003). In sympatry, European hares and mountain hares hybridise with each other. In most hybridization events, a male European hare mates with a female mountain hare due to the typical mating behaviour of both hare species (Flux, 1970; Hewson, 1990; Holley, 1986; Wiley & Poston, 1996). Areas of overlap between hare species in northern Europe are not stable (Jansson & Pehrson, 2007; Thulin, 2003). It is unclear whether the recent signs of a restriction in the range of the mountain hare in Scandinavia (Pohjoismäki et al., 2021) were caused by global warming, change of land use (from pastural to arable in the south, Angerbjörn & Schai-Braun, 2023), by direct competition with the European hare (see Schai-Braun & Hackländer, 2016), or a combination of all three factors. As a generalist, the European hare inhabits diverse habitat types (see Hackländer, 2023). Consistently reported habitat preferences of this species are fallow land (Bertolino, Cordero di Montezemolo, & Perrone, 2011; Bertolino, Perrone, et al., 2011; Schai-Braun et al., 2013; Smith et al., 2004) due to its characteristic open vegetation. Habitat preferences of the European hares inhabiting the Alps are as yet unknown. Alpine mountain hares select dwarf mountain pine as a habitat throughout the year (Bisi et al., 2013). Preferences for habitat types and vegetation characteristics of these two lagomorphs are vital to understand the mechanisms of competition between the two hare species and the occurrence of hybrids.

The Alpine mountain hare subspecies *Lepus timidus varronis*, which is restricted to the Alps, inhabits elevations between 800 and 4000 m above sea level (a.s.l., Angerbjörn & Schai-Braun, 2023), whereas the European hare has been observed up to 2800 m a.s.l. (Hackländer, 2023). In the Alps, climatic variation along the elevation gradient provides suitable conditions for the coexistence of the two congeneric hare species. An evaluation of hunting bag statistics collected over 30 years in Grisons (Switzerland) showed that both hare species shifted their minimum elevations towards higher elevations (Schai-Braun et al., 2021). Moreover, European hares shifted their ranges faster to higher elevations than Alpine mountain hares. The data suggest that European hares living in the Alps may benefit from global warming as they shift their range uphill. As well as spatial displacement, introgressive hybridisation poses a threat to the Alpine mountain hare by reducing its genetic integrity. Hybridisation has been confirmed in hares from Grisons (Marques et al., 2017; Zachos et al., 2010). In the Alps, it is expected that the distribution of the two lagomorph species and their hybrids proceeds along the elevation gradient with European hares inhabiting lower elevations than hybrids, and hybrids inhabiting lower elevations than Alpine mountain hares.

After hybridisation, mitochondrial DNA (mtDNA) of mountain hares can be detected in European hares (Levänen, Kunnasranta, & Pohjoismäki, 2018; Thulin et al., 1997) if the hybrids backcrossed with European hares. This is called introgression. Introgressed individuals can be found in both species, however, far more often in European hares than in mountain hares (Levänen, Kunnasranta, & Pohjoismäki, 2018; Levänen, Thulin, et al., 2018), which is due to predominantly unidirectional hybridisation from the expansion front of the invading species (Thulin, 2003). 15% of European hares in areas of current sympatry in Sweden were hybrids (Thulin & Tegelström, 2002). In the Alps, 4% of analysed Alpine mountain hares were hybrids (Zachos et al., 2010). In areas of current sympatry, the proportion of European hares with mountain hare mtDNA is higher than in areas where both species lived in sympatry in the past (Thulin & Tegelström, 2002). It seems that European hares with mountain hare mtDNA have lower fitness than European hares with species-specific mtDNA. In contrast, mountain hare mtDNA has successfully remained in all three hare species (i.e. Iberian hare *Lepus granatensis*, European hare and broom hare *Lepus castroviejoi*) present in the colder and mountainous parts of Portugal and Spain, although mountain hares disappeared at the end of the last ice age from the Iberian Peninsula (Melo-Ferreira et al., 2005). A contribution of mountain hare mtDNA towards adaptation to colder climates and higher elevation areas might explain this evolutionary success.

Areas of sympatry are probably more localised and narrower in the Alps than in Sweden, as changes in habitat characteristics are more pronounced along the elevation gradient than along increasing latitude. Hence, the proportion of hybrids in the Alps is expected to be less than in Sweden. The proportions of first (F1) and second-generation (F2) or older hybrids in both hare species' populations are unknown. As F1 and F2 hybrids are only separated by one generation, proportions of F1 and F2 hybrids are expected to be similar. Despite the lack of empirical information about hybrids, backcrossed individuals will likely show similar habitat associations (e.g. elevation range) as the genetically closer hare species.

The occurrence of hybrids in Sweden increased with latitude, altitude, hilliness and where the proportion of arable land was low (Jansson et al., 2007). These abiotic factors complemented the main aspect influencing hybridisation frequency, namely the relative frequency of the two species (Jansson et al., 2007). In the Alps, the frequency of hybrids is anticipated to reflect both the prevailing vegetation types and the relative frequency of the two hare species.

The goal of this study was to investigate Alpine mountain hare and European hare populations in the Alps with a particular focus on the occurrence and habitat selection of hybrids. Our hypotheses were: (1) the two hare species have contiguous distributions in the Alps, with Alpine mountain hares inhabiting higher elevations than hybrids and hybrids inhabiting higher elevations than European hares; (2) the frequency of hybrids is dependent on the ratio of the two lagomorph species in an area but is <10%; (3) the frequency of hybrids, Alpine mountain hares and European hares differ between vegetation types; (4) in an area where hybrids occur, F1 and F2 hybrids are recorded in equal proportions; (5) backcrossed individuals inhabit elevation ranges similar to the genetically closer hare species. To test these, we performed molecular genetic analysis of hare faecal samples collected in four study areas in the Alps in South Tyrol (Italy) for three winters. We then investigated habitat associations (e.g. elevation and vegetation type) along with relative frequency of the genotypes.

Materials and methods

Study area

We studied hare populations in South Tyrol (Italy) in four study areas near Ulten (46°33′N, 10°52′ E), Durnholz (46°44′N, 11°26′E), Reinswald (46°41′N, 11°25′E) and Mühlwald (46°53′N, 11°52′E, see Fig. S1) during the three winters 2016/17, 2017/18 and 2018/19. South Tyrol is a central Alpine region and the elevations within our four study areas ranged from 1600 to 2500 m a.s.l. Each study area covers an area of 225–343 ha (see Table S1). The study areas consisted of the typical vegetation types for the subalpine altitudinal zones of South Tyrol, comprising forest areas, dwarf shrub heaths and alpine grasslands (see Table S2). Proportions of vegetation types in each study area were ascertained using orthophotos (Geokatalog, 2017, Autonome Provinz Bozen) and, in case of ambiguity, by verifications on site. Because steep slopes (>30°) with fresh snow could not be walked for safety reasons, only southwest to southeast exposed sites were sampled.

Data collection

We collected faecal pellets for genetic analysis to determine the hybridisation levels and distribution ranges of both leporids. Line transects were surveyed using touring skis or snowshoes. The exact course of the transects was recorded using a GPS device. As the walks took place 3 days after snowfall, the freshness of the faeces was guaranteed. Fieldwork in the winter of 2016/17 could not begin until 10 February 2017 and ended on April 30 due to reduced snowfall. Sampling was extended from 30 December 2017 to 20 February 2018 in winter 2017/18. Fieldwork was delayed again until February 7, 2019 and ended on April 9 in winter 2018/19. The Mühlwald area was not surveyed in the first winter, whereas the Durnholz area was not surveyed in the third winter.

A grid of points (200 m × 200 m) was laid out over the study areas in advance to ensure a complete survey. The size of the grid was considered sufficient, as radio-collared female Alpine mountain hares in Reinswald covered an average daily distance of 800 m (Amt für Jagd und Fischerei Südtirol, unpublished). The observer visited each of these points along the line transect. The survey team consisted of two to seven people per study area. The survey personnel were trained in advance. Up to two consecutive days were necessary to survey the study area by the survey team.

Hare faeces were picked up with tweezers and preserved in tubes filled with ethanol (90%). The exact location of the faeces was located using a GPS device. If there were multiple groups of pellets of the same colour, size or shape at one location, only one sample (consisting of several pellets) was collected. When different colouration, size or shape suggested several individuals, one sample of faeces per colour, size or shape was taken. A total transect length of 320 km was covered in the three survey winters. Transects ranged in horizontal length from 2.710 to 9.260 m, with a mean length of 5.343 m. Twenty-eight transects were sampled in winter 2016/17, 27 transects were sampled in winter 2017/18 and 12 transects were sampled in winter 2018/19.

Data analysis

Tubes with hare faeces were preserved at room temperature. Drying in silica gel and further processing was performed at the laboratory according to Roeder et al. (2004). The storage time in ethanol was reduced after winter 2017/18 to achieve better results. DNA was extracted from faecal pellets using the GenElute™ Mammalian Genomic DNA Miniprep kit (Sigma Aldrich, Darmstadt, Germany). Ten microsatellite loci with different levels of polymorphism were selected for this study: Sat02, Sat12, Sol30, Sol08, Sol28, Sol33, Lsa1, Lsa2, Lsa3 and Lsa8 and have been repeatedly tested on hares (Ben Slimen et al., 2008). Consensus genotypes were used to initially confirm species identity and subsequently duplicate genotypes. We used two factors as criteria for accepting multilocus genotypes as duplicates of the same individuals. First, allelic mismatches were allowed at just a single locus to account for potential genotype scoring errors. Second, the genotyping success rate was set to seven overlapping loci between samples as the minimum number accepted for sample comparison in winter 2018/2019. The sample quality was lower for the other two sample periods, and we relaxed the threshold for overlapping loci to a minimum of five for comparison, but again only allowed one allelic mismatch in the winters 2016/2017 and 2017/2018. Genotyping was supported by GENEMARKER 2.6.7 software (SoftGenetics LLC). Identical genotypes were identified using the Excel add-in GenAlEx 6.5 (Peakall & Smouse, 2012).

Species determination was analysed using the software STRUCTURE (Pritchard et al., 2000) with an assumed number of clusters (K = 2) for comparison with a reference dataset of both species from Alpine populations (Suchentrunk et al., 1999). Each sample was identified as either an Alpine mountain hare or a European hare based on its assignment probability. We collected 34 tissue samples from isolated European hare populations inhabiting South Tyrolean valleys as an additional localised reference for the European hare. An abundance ratio of both species was calculated for each study area. Each sample was further analysed with the software NEWHYBRIDS in order to obtain information on hybridisation and backcrossing events (Anderson & Thompson, 2002). NEWHYBRIDS quantifies the posterior probability of assignment for each sample to a hybrid class or the parent population, and probabilities are reported as percentages. A sample was defined as introgressed if the posterior probability of assignment was less than 90% in either of the parental classes. The software allowed the detection of hybridization events in the F1 or F2 generation along with F1 backcrosses to each parent species.

Statistical analysis

We examined the effect of multiple predictor variables (i.e. hare species, study area and study winter) on the elevation of the faecal pellets. As some faecal samples represented those from the same individuals in different years, we used generalised linear mixed models (GLMMs) to include hare identity as a random effect. GLMMs were computed using R (R Development Core Team, 2019) packages 'brms' (Bürkner, 2017) and 'rstan' (Carpenter et al., 2017). The Bayesian GLMM approach implemented in these libraries has the advantage that it can estimate random effects even when data are obtained partly as repeated samples of an individual among years and partly from a single reproductive bout in one hare. This data structure often causes singularities and prevents random effects from being estimated with other methods. Multicollinearity between the predictor variables was checked calculating the Variance Inflation Factor (VIF) with R package 'car' (Fox & Weisberg, 2019) for all independent variables in each model. All variables had a VIF < 1.4. All GLMM samples were drawn with the NUTS algorithm using four chains and 6000 iterations. We only report models that converged with all Rhat < 1.05. In addition to the above random effect, the two full models included the following three predictor variables: study area (Ulten, Durnholz, Reinswald and Mühlwald), study winter (2016/17, 2017/18 and 2018/19) and hare species (Model 1: European hare and Alpine mountain hare, data analysed by STRUCTURE and Model 2: European hare, Alpine mountain hare and hybrids, data analysed by NEWHYBRIDS). Model 1 also included the two-way interactions between these variables, whereas in Model 2 this was not possible due to smaller sample size (see Table S3).

We eliminated model terms to determine the model that minimised leave-one-out cross-validation Information Criterion (LOOIC; Vehtari et al., 2017) using the R package 'loo' (Vehtari et al., 2019). Leave-one-out cross-validation (LOO) is a method for estimating prediction accuracy from a fitted Bayesian model using the log-likelihood of the parameter values (Vehtari et al., 2017). We used the increment of the increase caused by its removal, Δ LOO, as a measure of support for the inclusion of a variable. We used the family function 'gaussian' for the response variable elevation.

The number of individuals pooled across genotypes (i.e. both hare species together) in each study area was estimated using capture-recapture estimation (Petersen, 1895). We used a model variant developed for population estimates based on genetic samples (Miller et al., 2005) implemented in the R package CAPWIRE (Miller et al., 2005; Pennell et al., 2013). The requirement for capture-recapture models that the capture probability is the same for all individuals in a population was fulfilled in our survey, as there was no change in the capture probability for an individual after each sampling event. CAPWIRE offers three models to estimate population size. The one with the most appropriate estimate was selected using a likelihood ratio test. The model calculated a maximum likelihood abundance with minimum and maximum values of the 95% confidence interval (henceforth, lower and upper CIs). The density of hares per 1 km^2 was calculated in relation to the area surveyed for each study area. Minimum population density was estimated as the lower CI. The surveyed area was calculated using the geographic information system QGIS (QGIS.org, 2021). The population density of the Alpine mountain hares was estimated separately from that of the total hare density after genetic determination of the two hare species. Population densities estimated for European hares were not possible due to low number of samples matching this species.

Two linear models were fitted to study the effect of the ratio of European hares to Alpine mountain hares on the relative frequency of hybrids and of F2 hybrids. We fitted models for the following response variables: relative frequency of hybrids ($n = 8$); and relative frequency of F2 hybrids ($n = 4$), including the fixed effect predictor ratio of European hares to Alpine mountain hares detected. The model for the response variable relative frequency of hybrids included a second fixed effect predictor study winter. This was not possible in the model for the response variable relative frequency of F2 hybrids due to small sample size (see Table S3).

Additionally, linear models were fitted to study the effect of the vegetation type on the relative frequency of the two hare species and their hybrids. We fitted models for the following response variables: relative frequency of Alpine mountain hares ($n = 20$); European hares ($n = 20$); and hybrids ($n = 20$), including the fixed effect predictors study winter and dwarf shrub heaths, Alpine grasslands or forest (see Table S3). *P*-values for all linear models were extracted by likelihood ratio tests (Faraway, 2006). Residuals of the models were checked for normal distribution by QQ-plots and histograms. The homogeneity of variances and goodness of fit were examined by plotting residuals versus fitted values (Faraway, 2006). The alpha level for determining statistical significance was 0.05.

Results

A total of 525 faecal samples were collected during the three winters 2016/17, 2017/18 and 2018/19, of which 398 faecal samples were genotyped successfully (76%, see Table S4). Individuals were recaptured on average 2.18 times per winter (see Table S5), and faecal pellets were collected within an altitudinal range of 1642–2489 m a.s.l. (median = 2.112 m, SD = 179.5 m).

Frequency of genotypes

The STRUCTURE analysis revealed that the four study areas differed greatly regarding the number of European hare and Alpine mountain hare individuals recorded (Table 1, Fig. S2). There were also large temporal differences within the study areas among the three winters. The fewest European hares were recorded in Durnholz, whereas in Reinswald the highest number of European hares were recorded (i.e. in winter 2017/18). The ratio of European hares to Alpine mountain hares ranged from 1:0.9 up to 1:18 within study areas and years (Table 1). This varied greatly within some study areas between years as can be seen by comparing ratios in Reinswald between the winters 2017/18 and 2018/19.

The NEWHYBRIDS analysis revealed evidence for 14 individuals having a hybrid status. No hybrids were detected in Durnholz (Fig. 1), nine hybrids were recorded in Reinswald (16.7%, one recapture), six in Ulten (9.0%, 2 recaptures) and 2 hybrids in Mühlwald (10.5%, no recaptures, Table 2). No hybrids were determined as F1 hybrids, whereas four of the 14 hybrids were classified as F2 hybrids with the following posterior probabilities: 54%, 69%, 98% and 99%. The other 10 hybrids were characterised by an older interspecific gene flow. For 12 hybrids (85.7%), including the individuals classified as F2 hybrids, the next highest posterior probability was for European hares, whereas for two hybrids (14.3%) the next highest posterior probability was for Alpine mountain hares.

We recorded 150 individuals within the three study years of which 14 were hybrids, 25 European hares and 111 Alpine mountain hares.

Elevational gradient

The best model, based on the STRUCTURE assignment data, revealed that within the study area (Δ LOO 51.7) there was an association between elevation and genotype of sampled faeces. In particular, Alpine mountain hare faeces were found at higher elevations than European hare faeces (Δ LOO 6.7; Fig. 2a). Overall, there were high levels of variation between

Table 1 Number of individual European hares and Alpine mountain hares determined by STRUCTURE in four study areas in South Tyrol (Italy) during winters 2016/17, 2017/18 and 2018/19

	Winter 2016/17			Winter 2017/18			Winter 2018/19		
	European hare	Alpine mountain hare	Ratio	European hare	Alpine mountain hare	Ratio	European hare	Alpine mountain hare	Ratio
Durnholz	0	12	–	1	18	1:18	–	–	–
Mühlwald	–	–	–	3	11	1:3.7	0	5	–
Reinswald	8	12	1:1.5	10	9	1:0.9	2	14	1:7
Ulten	5	12	1:2.4	5	24	1:4.8	6	17	1:2.8

Ratio refers to the abundance of both hare species (European hare: Alpine mountain hare). STRUCTURE analysed species determination while assuming K = 2 clusters for comparison with a reference dataset for each species. Identification as either Alpine mountain hare or European hare was based on the assignment probability of microsatellites. See text for methodological details.

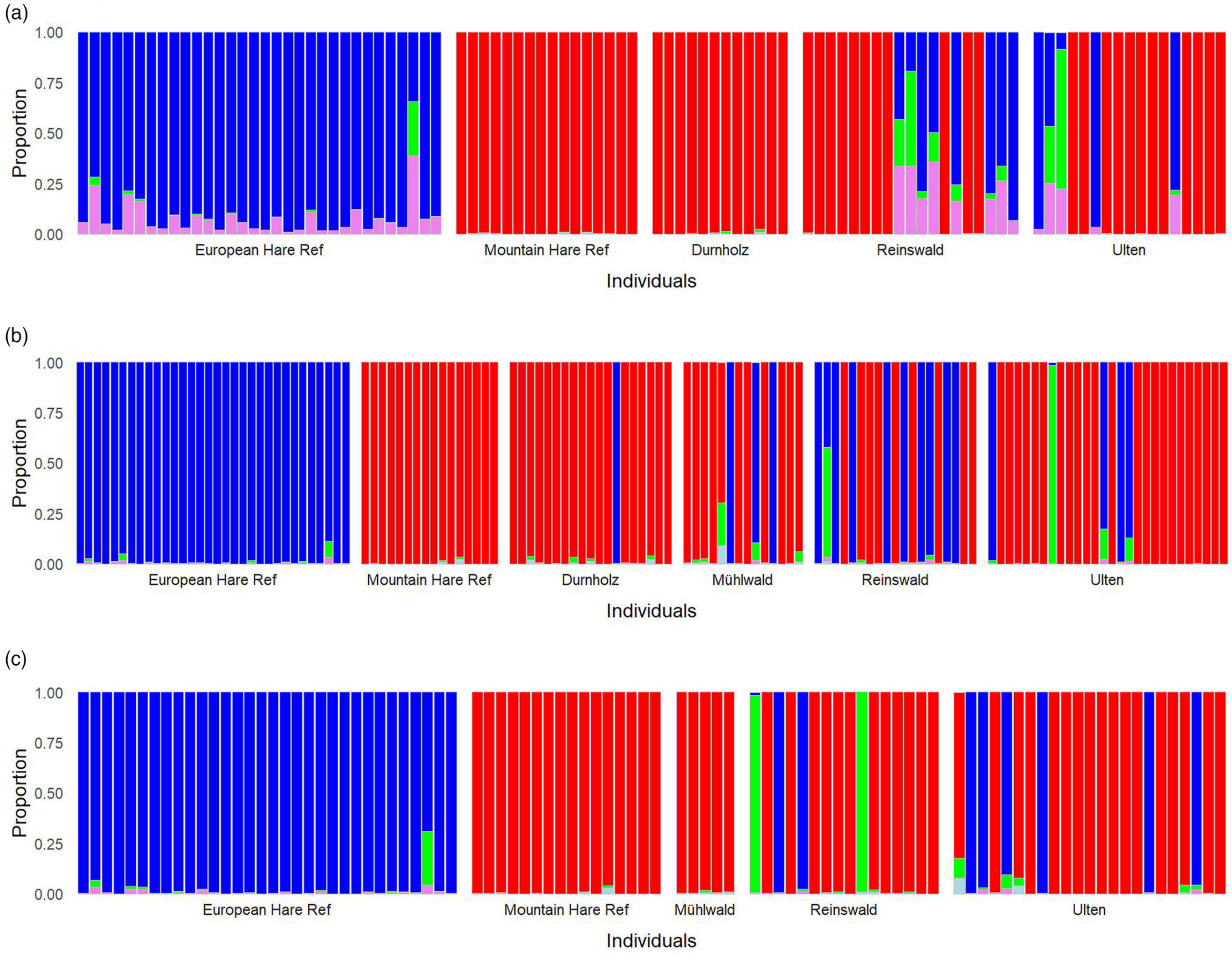

Figure 1 NEWHYBRIDS diagrams based on the analysis of the hare faecal samples' microsatellite composition for the study winters: (a) 2016/17, (b) 2017/18 and (c) 2018/19. Each diagram includes the reference dataset of each hare species. Each column symbolises a hare faecal or tissue (i.e. reference dataset) sample. Each colour corresponds to a genetic proportion of each species, F1 hybrid, F2 hybrid, backcrossed European hare and backcrossed Alpine mountain hare. Red corresponds to the Alpine mountain hare, blue to the European hare, orange to the F1 hybrid, green to the F2 hybrid, violet to the backcrossed European hare and light blue to the backcrossed Alpine mountain hare. Faecal samples were collected in four study areas in South Tyrol (Italy).

Table 2 Number of individual European hares, Alpine mountain hares and their hybrids determined by NEWHYBRIDS within four study areas in South Tyrol (Italy) during winters 2016/17, 2017/18 and 2018/19

	Winter 2016/17			Winter 2017/18			Winter 2018/19		
	European hare	Alpine mountain hare	Hybrid	European hare	Alpine mountain hare	Hybrid	European hare	Alpine mountain hare	Hybrid
Durnholz	0	12	0	1	18	0	–	–	–
Mühlwald	–	–	–	2	10	2	0	5	0
Reinswald	2	11	6	9	9	1	2	12	2
Ulten	2	12	3	2	22	3	6	17	0

NEWHYBRIDS quantified the assignment probability to a hybrid class or the parent population in percentages. Hybridisation was classified if the assignment probability of microsatellites was less than 50% in either of the parental classes. See text for methodological details.

study areas and study winters. European hare faeces were found at especially high elevations in Ulten and in the study winter 2017/18 (Table 3a).

When modelling was based on the NEWHYBRIDS data, the best model revealed again that within the study area (Δ LOO 57.5) there was an association between elevation and genotype of sampled faeces. Hybrid faeces (Δ LOO 9.6) were found at lower elevations than Alpine mountain hare faeces but at higher elevations than European hare faeces (Fig. 2b, Table 3b). Hybrids did not differ in their mean elevation depending on whether they were backcrossed European hares or Alpine mountain hares (Fig. 3). However, the number of individual hybrids recorded was low (n = 14, faecal samples = 29).

Population densities

Faecal data provided the means to calculate population estimates by CAPWIRE for hare genotypes pooled (Table 4a), that is, both hare species together, and for Alpine mountain hares (Table 4b) for each winter and each area separately. Population densities differed greatly between winters and study area. Density estimates for hare genotypes pooled fluctuated between winters in each study area (Table 5a). Exceptionally high population densities were estimated for the winter 2016/17 in Reinswald and for the winter 2017/18 in Ulten, whereas for the winter 2018/19 in Mühlwald no population density could be estimated due to insufficient observations. Minimum population densities in all study areas remained rather stable throughout the years but with low minimum population densities in winter 2016/17 in Ulten and in winter 2018/19 in Mühlwald.

Population densities for Alpine mountain hares also displayed fluctuations in the four study areas throughout the three winters (Table 5b). An exceptionally high population density for Alpine mountain hares was estimated for the winter 2017/18 in Ulten, whereas a low population density was estimated

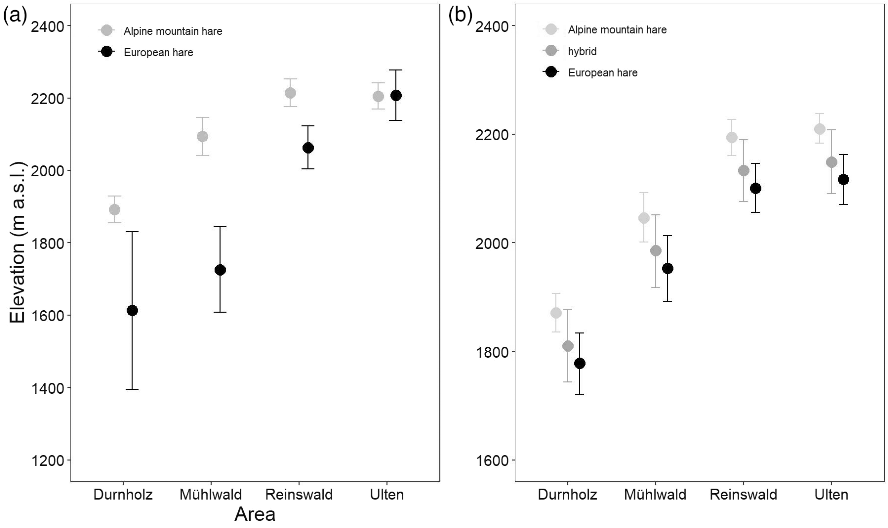

Figure 2 Relationships between study area and elevation (m a.s.l.) using (a) STRUCTURE for species determination in European hares and Alpine mountain hares (number of samples = 398; number of individuals = 153) and (b) NEWHYBRIDS for distinguishing genotypes: European hare, Alpine mountain hare and hybrids (number of samples = 387; number of individuals = 148). The points represent the predicted median values, and the whiskers display the 95% Bayesian credible intervals. Faecal samples were collected in four study areas in South Tyrol (Italy) during winters 2016/17, 2017/18 and 2018/19. See text for statistical details.

Table 3 Coefficients of the two best models with elevation of detected faeces as the response variable, where species determinations for each sample were based on (a) STRUCTURE in European hares and Alpine mountain hares (number of individuals = 153; number of samples = 398) and (b) by NEWHYBRIDS in European hares, Alpine mountain hares and their hybrids (number of individuals = 148; number of samples = 387)

Elevation	Estimate	Est. Error	95% Credible interval
(a)			
Intercept	1892.94	18.76	[1855.10, 1929.49]
Area: Mühlwald	201.92	27.78	[146.21, 256.21]
Area: Reinswald	321.64	24.02	[273.48, 368.28]
Area: Ulten	311.95	21.42	[269.47, 353.82]
Species: European hare	−277.85	111.60	[−497.21, −56.19]
Study winter: 2017/18	−31.07	15.64	[−61.61, −0.19]
Study winter: 2018/19	-0.19	21.15	[−42.09, 41.23]
Area: Mühlwald × Species: European hare	−89.65	120.06	[−326.07, 144.57]
Area: Reinswald × Species: European hare	127.61	110.86	[−88.90, 342.35]
Area: Ulten × Species: European hare	280.70	111.08	[63.93, 494.29]
Species: European hare × Study winter: 2017/18	88.34	35.38	[18.35, 159.27]
Species: European hare × Study winter: 2018/19	−63.13	51.24	[−164.38, 38.21]
(b)			
Intercept	1871.99	17.7	[1837.17, 1906.50]
Area: Mühlwald	174.36	29.18	[117.45, 232.07]
Area: Reinswald	322.28	24.29	[274.42, 370.09]
Area: Ulten	338.43	22.26	[294.38, 381.79]
Species/hybrids: Hybrid	−62.02	29.24	[−119.74, −5.13]
Species/hybrids: European hare	−93.18	23.46	[−138.36, −47.07]

for the same winter in Reinswald. No population density could be estimated for the winter 2018/19 in Mühlwald due to insufficient observations. The contemporaneously recorded minimum population densities remained constant within the four study areas throughout the three winters with the exception of a high minimum population density of Alpine mountain hares documented in the winter 2017/18 in Ulten. The average Alpine mountain hare population density for the four study areas and three winters was 7.69 individuals/km^2.

Effect of parental frequencies on the frequency of hybrids

We found no significant effect of the ratio of European hares to Alpine mountain hare frequencies on the frequency of hybrids ($P > 0.10$, $n = 8$) and of F2 hybrids ($P > 0.10$, $n = 4$) although low sample sizes likely reduce the power of these tests.

Effect of vegetation types on the frequencies of genotypes

There was a negative effect on the proportion of Alpine grassland ($\beta = -0.851$, $P = 0.006$, $n = 20$) and a positive effect on the proportion of dwarf shrub heaths ($\beta = 1.294$, $P = 0.020$, $n = 20$) on the frequency of Alpine mountain hares in the study areas (Fig. 4). The proportion of forest had no influence on the frequency of Alpine mountain hares in the study areas ($P > 0.05$, $n = 20$). In comparison, the frequency of European hares and hybrids was not affected by any vegetation type in the study areas ($P > 0.05$, $n = 20$).

Discussion

Genotyping success in our faecal samples was lower compared to a previous mountain hare study conducted in the Alps during winter (Rehnus & Bollmann, 2016: 95–97%). This was presumably due to a very low genotyping success in winter 2016/17 (54%). Recapture rate of individuals per winter in our survey was much higher than in the study by Rehnus & Bollmann (2016; 200 m square grid: 0.39, opportunistic sampling: 0.82).

Frequency of genotypes

We recorded large temporal and spatial differences in numbers of each hare species. We suggest that the differences between the study areas were caused by differing vegetation type composition. The interannual differences were probably caused by large and regular fluctuations in hare densities (mountain hare: Abe & Ota, 1987; Pulliainen & Tunkkari, 1987; European hare: Abildgård et al., 1972; Wasilewski, 1991). However, Alpine mountain hare densities during a 5-year study in the Swiss Alps (Schenker et al., 2020: 4.9–8.3 hares/km^2) fluctuated much less than in our study.

We found a lower proportion of hybrids than have been recorded in Sweden (Thulin & Tegelström, 2002: in European hares about 15% hybrids) or Ireland (Reid et al., 2022: 34% hybrids between native Irish hare *Lepus timidus hibernicus* and non-native European hare) but more hybrids than in the Swiss Alps (Zachos et al., 2010: in Alpine mountain hares about 4% hybrids). The lower proportion of backcrossed European hares in our study may be explained by our study areas having very low abundance of European hares. The much lower proportion

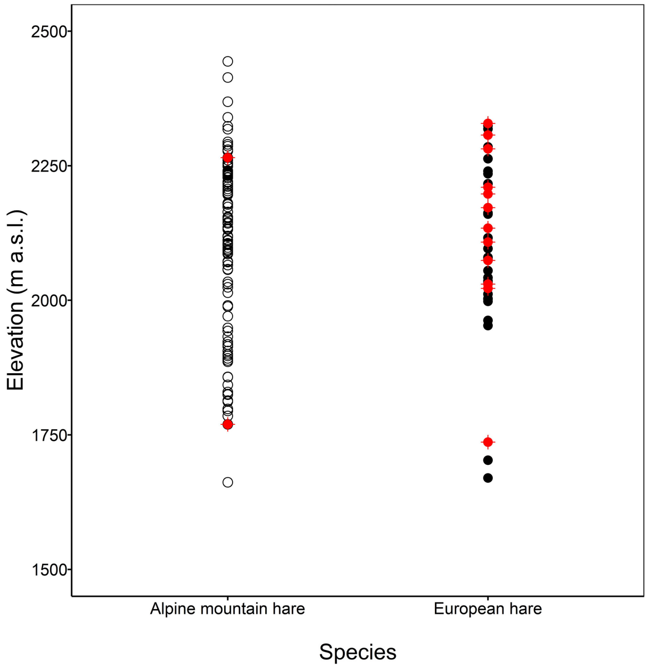

Figure 3 Relationship between the elevation (m a.s.l.) where successfully genotyped faecal samples were found and the hare species determined using STRUCTURE and NEWHYBRIDS (number of samples = 398; number of individuals = 153). Points represent mean elevations of individual hares. Red symbols represent hybrids backcrossed with either Alpine mountain hares (left) or European hares (right). Faecal samples were collected in four study areas in South Tyrol (Italy) during winters 2016/17, 2017/18 and 2018/19.

Table 4 Estimated population size using CAPWIRE for (a) hare genotypes pooled and (b) for Alpine mountain hares for each winter and each area separately

	Estimated population size (±95% CI)		
	Winter 2016/17	Winter 2017/18	Winter 2018/19
(a)			
Durnholz	15 (12, 20)	25 (19, 27)	–
Mühlwald	–	16 (14, 20)	insuf. obs.
Reinswald	67 (40, 72)	28 (23, 43)	26 (16, 38)
Ulten	26 (17, 41)	53 (44, 80)	28 (23, 32)
(b)			
Durnholz	15 (12, 20)	23 (18, 25)	–
Mühlwald	–	12 (11, 14)	insuf. obs.
Reinswald	24 (16, 43)	10 (9, 12)	21 (14, 31)
Ulten	15 (12, 21)	40 (33, 57)	19 (17, 21)

insuf. obs., insufficient observations.

of backcrossed Alpine mountain hares is likely due to the prevalence of European hares in each study and availability of preferred habitat types. The proportion of backcrossed Alpine mountain hares was much lower than the proportion of backcrossed European hares in our study, which is in line with other studies showing that hybridisation is mostly unidirectional and a result of the front wave of expansion of the invading European hares (Levänen, Kunnasranta, & Pohjoismäki, 2018; Levänen, Thulin, et al., 2018). In contrast, a high prevalence of bidirectional hybridisation was recorded in Ireland (Reid et al., 2022). This might be explained by local adaptations (e.g. variation in body size) characteristic of the unique lineages in mountain hares (Giska et al., 2022).

Almost one-third of the hybrids in our study were recent events of hybridisation (F2 hybrids), whereas the others were resulting from older hybridisation events. Surprisingly, we discovered no F1 hybrids even though F2 hybrids were identified with high probabilities. It is possible that the F2 classification resulted from a statistical artefact related to reduced sensitivity of our marker set, and these individuals represent a more complex history of introgression. Nevertheless, in a study of 48 hares from Sweden and Finland, only one F1 hybrid (2.1%) and no F2 hybrids were detected. The proportion of recent hybridisation events in Sweden and Finland (Levänen, Thulin, et al., 2018: 2.1%) was comparable to our study in the Italian Alps (2.7%).

Elevational gradient

Our finding that European hares inhabit lower elevations than Alpine mountain hares and that the study area affected the elevation range of both hare species concur with analyses of hunting bags from Grisons (Schai-Braun et al., 2021). However, we found that European hares can inhabit higher elevations than Alpine mountain hares in some areas (e.g. Ulten) or winters (e.g. 2017/18). This divergence might be explained by altitudinal migration, differential food preferences, or a displacement that took place temporarily. These situations might be endorsed by climate warming and/or competition between the two species.

Table 5 Minimum population density (Min. Ind/km^2) and population density (Ind/km^2) estimated using CAPWIRE for (a) hare genotypes pooled and (b) for Alpine mountain hares for each winter and each area separately

	Winter 2016/17		Winter 2017/18		Winter 2018/19	
	Minimum population density (Min. Ind/km^2)	Population density (Ind/km^2)	Minimum population density (Min. Ind/km^2)	Population density (Ind/km^2)	Minimum population density (Min. Ind/km^2)	Population density (Ind/km^2)
(a)						
Durnholz	4.51	5.64	5.54	7.29	–	–
Mühlwald	–	–	5.07	5.80	1.85	insuf. obs.
Reinswald	7.66	25.67	7.17	10.57	6.15	10.00
Ulten	6.69	10.24	12.18	22.27	10.22	12.44
(b)						
Durnholz	4.51	5.64	5.25	6.71	–	–
Mühlwald	–	–	3.99	4.35	1.85	insuf. obs.
Reinswald	4.60	9.20	3.40	3.77	5.38	8.08
Ulten	4.72	5.91	10.08	16.81	7.56	8.44

Minimum population densities were calculated using the actual number of individuals recorded.
insuf. obs., insufficient observations.

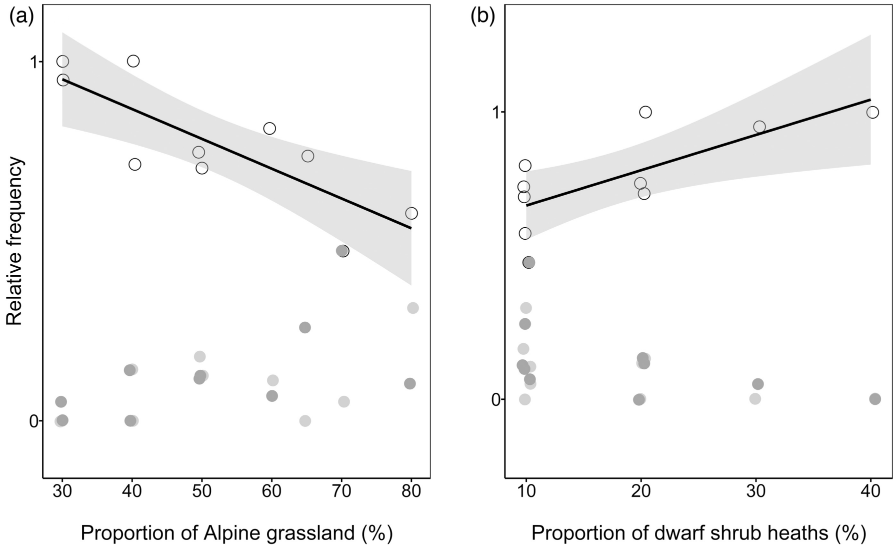

Figure 4 Relationships between relative frequency of genotypes (i.e, Alpine mountain hares (white points), hybrids (light grey points) and European hares (dark grey points)) and the proportion of (a) Alpine grassland (%) and (b) dwarf shrub heaths (%) in the four study areas. Faecal samples were collected in South Tyrol (Italy) during winters 2016/17, 2017/18 and 2018/19. Statistically significant regression lines are black, and the grey area represents the confidence intervals. See text for statistical details.

Our study revealed a sympatric zone comprising hybrids between the allopatric populations of mountain hares and populations of European hares, which is similar to findings from the study in southern Sweden (Thulin, 2003). However, in our study, the zone of overlap separated populations based on altitude rather than latitude. In contrast, Irish hares and European hares are not separated by any gradient, perhaps because they have overlapping habitat niches (Caravaggi et al., 2015, 2017). Interestingly, introgressed individuals in our study did not inhabit similar elevation ranges as the genetically closer hare species. This may be reflective of the fact that there were only 14 individual hybrids sampled.

Population densities

The differences in population estimates were based on the large temporal and spatial variation in numbers of each hare species. Population densities fluctuated in a similar fashion compared to other mountain hare subspecies (see Angerbjörn & Schai-Braun, 2023) and in other hare species (see Schai-Braun & Hackländer, 2016). As minimum population densities were more stable than population densities, we recommend the estimation of sustainable harvest rates of Alpine mountain hares based on minimum population estimates for areas where hares are hunted.

Frequency of European hares, Alpine mountain hares and their hybrids

We did not find any effect of the ratio of the two hare species on the frequency of hybrids in contrast to a Swedish study (Jansson et al., 2007). However, hybrid sample size was low, which might explain the absence of an effect.

The positive effect of dwarf shrub heaths and the negative effect of Alpine grassland on the frequency of Alpine mountain hares is in line with the subspecies' preference of dwarf mountain-pine habitat and avoidance of most open habitats (Bisi et al., 2013). We did not find any effect of forest, which was in contrast to the preference of densely forested habitats recorded by Bisi et al. (2013). However, the type of forest might be decisive. Our results confirm that the European hare is a generalist (Hackländer, 2023) that inhabits diverse vegetation types also in the Alps. Hybrids seem to be generalists as well, as there was no association with vegetation types in our study.

Conclusion

Our results support the widely raised concerns that the European hare is a strong competitor for the Alpine mountain hare in the Alpine ecosystem in the time of global climate change. Our findings suggest that the European hare might be outcompeting the Alpine mountain hare in certain areas of the Alps or certain winters. Hence, our findings support the concerns that the European hare might displace the Alpine mountain hare from its traditional range in the Alps. We suggest that more detailed information on habitat selection of these two lagomorph species is collected in the Alps. This could help inform management interventions to maintain the Alpine mountain hare subspecies within its native range. In conclusion, we emphasise that the Alpine mountain hare represents an ideal model species to demonstrate the effect of global warming on biodiversity in the Alps.

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Author contributions

SSch, SSm and KH conceived the ideas and designed methodology. SSch collected the data. SS-B, SSch and SSm analysed the data. SS-B led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Map of the four study areas in the province of South Tyrol (Italy).

Figure S2. STRUCTURE diagrams based on the analysis of the hare faecal samples' microsatellite composition for the study winters: (a) 2016/17, (b) 2017/18 and (c) 2018/19 assuming K = 2 clusters (species level). Each diagram includes the reference dataset of each hare species. Each column symbolises a hare faecal or tissue (i.e. reference dataset) sample. Each colour corresponds to the genetic proportion of a given species. Red corresponds to the Alpine mountain hare, blue to the European hare. Faecal samples were collected in four study areas in South Tyrol (Italy).

Table S1. Sizes of the four study areas in South Tyrol (Italy) during winters 2016/17, 2017/18 and 2018/19.

Table S2. Proportions of vegetation types in the four study areas in South Tyrol (Italy) during winters 2016/17, 1017/18 and 2018/19.

Table S3. Description of all generalised linear models and linear models used in the analyses.

Table S4. Description of the genotypes of all hare faecal samples collected in the four study areas in South Tyrol (Italy) during winters 2016/17, 1017/18 and 2018/19.

Table S5. Description of the number of samples, number of successfully genotyped samples, number of successfully genotyped samples[%], individual hares, averaged number of recaptured individuals for the four study areas in South Tyrol (Italy) during winters 2016/17, 1017/18 and 2018/19.