Mammalian Biology
https://doi.org/10.1007/s42991-026-00595-2

ORIGINAL ARTICLE

Diet ecology of European hares (*Lepus europaeus*), Alpine mountain hares (*L. timidus varronis*), and their hybrids in the Alps: effects of season and elevation in generalist–specialist competition

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Received: 7 December 2025 / Accepted: 3 May 2026

Abstract

The genus *Lepus* is considered a model to investigate ongoing evolutionary processes, as it diverged only 8.61 million years ago. The Alpine mountain hare (*L. timidus varronis*) is a specialist, and the European hare (*L. europaeus*) a generalist. In the European Alps, they occur mostly in parapatry, with reported hybridisation. Hybrids can influence evolutionary trajectories, either promoting genetic homogenisation or contributing to the emergence of novel lineages. Differences in foraging behaviour between the two lagomorph species exist, but resource competition appears to occur in overlapping elevations. We investigated dietary ecology of both species and their hybrids, focusing on seasonal and altitudinal differences, in faecal samples collected along altitudinal gradients in Grisons, Switzerland. Dietary composition was inferred for 90 European hares, 59 Alpine mountain hares and 52 hybrids using a DNA metabarcoding approach. We identified 25 orders, 41 families, 93 genera and 88 plant species. Alpine mountain hares showed a selective feeding behaviour selecting plant species typical of the respective elevation. European hares confirmed a more generalist pattern, feeding on different plant taxa irrespective of the elevation, without evidence for dietary restriction toward the end of the vegetation period. Our findings are consistent with the idea that European hares can successfully inhabit Alpine ecosystems and may compete with Alpine mountain hares. In some cases, hybrids exhibited distinct dietary ecology relative to their parental species, resulting in greater overall variability. Hybrids in the Alps might influence evolutionary processes, especially under climate warming, when the parental species gain advantageous dietary traits through backcrossing.

Keywords Foraging ecology · Brown hare · Lagomorpha · Leporids · Dietary niche breadth · Diet preferences · Dietary plant overlap

Introduction

Evolutionary processes are the drivers of biodiversity, influencing species adaptation and causing speciation (Stearns and Hoekstra 2005). Hybridisation plays a central role in shaping evolutionary processes as hybrids can contribute to both the loss of species, the origin of new species, as well as gaining of adaptive potential (Allendorf et al. 2001; Sakai et al. 2001; Laikre et al. 2010; Ferreira et al. 2023). In this context, hares (*Lepus*) are considered a model genus to investigate evolutionary processes as the genus split only 8.61 million years ago from the other leporids. The split resulted in a broad, yet shallow, diversification within *Lepus* forming 32 different hare species, with several cases of reported ancestral and contemporaneous hybridisation (Schai-Braun and Hackländer 2016).

For example, current hybridisation between the European hare (*Lepus europaeus*), Corsican hare (*L. corsicanus*) and Iberian hare (*L. granatensis*) has been reported in Corsica by Pietri et al. (2011). Moreover, several cases of hybridisation between the European hare and the mountain hare (*L. timidus*) are well documented in various locations in Europe

Handling editor: Heiko G. Rödel.

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(Thulin et al. 2006; Angerbjörn and Schai-Braun 2023). In addition to current hybridisation, ancestral hybridisation events have occurred on the Iberian Peninsula. Introgressed mitochondrial DNA (mtDNA) of mountain hares has successfully remained in all three hare species (European hare, broom hare (*L. castroviejoi*), and Iberian hare) that are present in the colder and mountainous northern part of the Iberian Peninsula, although mountain hares retreated from this region at the end of the last ice age (Melo-Ferreira et al. 2005, 2007). In this case, mtDNA from ancestral hybridisation events seems to affect physiological traits in today's Iberian hares carrying mountain hare mtDNA (Cardoso et al. 2024). A contribution of mountain hare mtDNA towards adaptation to colder climates and higher elevation areas might explain this evolutionary success.

Against this broader evolutionary background, in the Alps, the subspecies Alpine mountain hare (*L. t. varronis*) occurs mostly parapatrically with the European hare, although climatic variation along the elevation gradient provides suitable conditions for partial overlap of their ranges, allowing for hybridisation. Multiple hybrid classes have been recorded in the Alps, including second-generation (F2) hybrids, first backcross (BC1) hybrids, and individuals resulting from older interspecific gene flow (Schai-Braun et al. 2023, 2024a). The distribution and abundance of different hybrid classes in the Alps vary depending on the region, and hybrids from recent hybridisation events are much rarer than hybrids of older interspecific gene flow (Schai-Braun et al. 2023, 2024a). Despite these insights, how hybrids influence ongoing evolutionary processes in the Alps is still unknown.

These patterns of hybridisation occur within a rapidly changing Alpine environment. Analysis of 30 years of hunting bag data from Grisons, Switzerland, indicates that the minimum elevations at which European hares and Alpine mountain hares were harvested in October and November have shifted towards higher elevations (Schai-Braun et al. 2021). As climate data confirm climate warming in the Alps (Wanner et al. 1997), the altered distribution of both hare species may reflect global climate change. According to ecological niche theory, stable coexistence of closely related species in sympatry requires differentiation in ecological niches, thereby reducing interspecific competition through resource partitioning (Hutchinson 1959; Hardin 1960). In the Alps, European hare and Alpine mountain hare exhibit distinct foraging behaviours, yet resource competition seems to be occurring where elevational distributions overlap (Schai-Braun et al. 2024b).

A previous study has shown that Alpine mountain hares used significantly more Ericaceae as food plants than European hares and hybrids in the Alps (Schai-Braun et al. 2025). Although visual microscopy has revealed differences in the use of plant functional types between the three hare groups, it is limited by low taxonomic resolution (Schai-Braun et al. 2025). Advances in DNA metabarcoding, along with improved reference plant DNA barcode libraries (Queirós et al. 2025) and comprehensive local flora inventories, have greatly enhanced the taxonomic resolution and accuracy of trophic niches inferences (Buglione et al. 2018). Consequently, the use of DNA-based methods may allow a more precise discrimination of forage ecology across hare species, as well as across spatial and temporal scales, and shed light on potential competition for food resources between these two hare species and their hybrids.

Dietary diversity and dietary niche breadth are generally higher in generalist species than in specialists (Futuyma and Moreno 1988). The Alpine mountain hare, a specialist, is adapted to cold, mountainous environments (Angerbjörn and Schai-Braun 2023), whereas the European hare, a generalist, inhabits a wide range of different environments (Hackländer 2023). In line with this framework, the Alpine mountain hare feeds on characteristic Alpine vegetation, e.g., Ericaceae (Schai-Braun et al. 2020, 2025), which contain high concentrations of phenolic compounds (Ştefănescu et al. 2019) that Alpine mountain hares tolerate better than European hares (Iason and Palo 1991). In contrast, the European hare prefers a range of weeds and grasses in agricultural landscapes selecting forage for high-energy content and avoiding fibres (Schai-Braun et al. 2015).

The Alpine mountain hare is adapted to the Alpine ecosystem characterised by a short vegetation growing period (Calanca and Holzkämper 2010) and an elevational gradient providing a diversity of available food plants (Körner 2003; Sundqvist et al. 2013) to cover its energy requirements. It is unknown whether the European hare can adjust its feeding behaviour within the Alpine ecosystem —either by consuming alpine-specific plant species, particularly toward the end of the vegetation period, when food becomes scarce, or at high elevations dominated by characteristic Alpine vegetation. A generalist in this context may adopt one of two strategies: enlarge its dietary spectrum to include novel alpine plants, or concentrate on food plants available across elevations. Nevertheless, information on the feeding ecology of hybrids between specialist and generalist species is scarce.

We investigated the diet ecology of Alpine mountain hares, European hares, and their hybrids in the European Alps over space and time, with particular interest in altitudinal and seasonal differences. Our hypotheses were: (1) dietary plant overlap is less between the European hare and the Alpine mountain hare than between hybrids and their parental species. This is because European hares select generalist plant species, e.g., widespread distributed or domesticated, whereas Alpine mountain hares select plant taxa specific to the Alpine environment; (2) dietary plant diversity and dietary niche breadth is larger for the European hare, a generalist, than for the Alpine mountain hare, a specialist, with hybrids close to their parental species; (3) the European hare increases its diet breadth both at the end of the vegetation period and at high elevation ranges to compensate for lower food availability; (4) the Alpine mountain hare shows selective feeding, including plant taxa typical of each elevation. In contrast, the European hare feeds on plant taxa with little variation across elevation gradients. We tested these hypotheses by performing genetic analyses on faecal samples, including DNA-based hare species identification following Schai-Braun et al. (2025) and plant DNA metabarcoding. For the plant DNA metabarcoding, we used two genetic markers (trnL and ITS2) to identify the plants consumed by the hares. We collected the samples along five transects across the altitudinal gradient in the Alps (Grisons) over two years, at the peak and at the end of the vegetation period. All faecal samples analysed in this study have been used previously in Schai-Braun et al. (2024a), with subsets thereof included in Schai-Braun et al. (2025). Using the metabarcoding results together with a ground vegetation inventory (plant availability), we then evaluated diet preferences, dietary plant overlap, dietary plant diversity, and dietary niche breadth of the two hare species and their hybrids.

Materials and methods

Study area

The study area is situated between the villages of Susch and Ramosch in Grisons, Switzerland (Susch 46°44′N, 10°4′E, Ramosch 46°49′N, 10°23′E, Supporting Information 1), covering an elevation range of 1,000–2,600 m a.s.l. The study area is an agroforestry landscape and comprised of forest (39%), grassland (35%), shrubs (19%), alpine habitat types (4%), watercourses (3%), residential area (< 1%), and agricultural cropland (< 1%).

Faeces collection

Faecal samples were collected in 2019 and 2020 along five transects spanning the altitudinal gradient. Each transect was searched twice at peak (beginning of July) and at the end (end of August/beginning of September) of the vegetation period. Faeces with similar freshness and size located within two meters of one another were treated as originating from the same individual and grouped as a single sample. Preference was given to fresh faecal pellets, as older pellets have significantly lower amplification rates (Rehnus and Bollmann 2016). One to six pellets per sample were collected and stored in 50-ml tubes filled with Silica (year 2020) or ethanol (year 2019) for genetic analysis. One pellet per sample was used for DNA extraction and subsequent genetic analysis. Each sample location was recorded with a GPS device (Garmin GPSMAP 60Cx). The faeces were collected with gloves to avoid contamination with human DNA.

Genetic analysis of hare faeces

Hare faeces were analysed for species and hybrid determination following the protocol published in Schai-Braun et al. (2025). Extractions were conducted in a dedicated non-invasive genetics laboratory physically separated from post-PCR areas and regularly decontaminated using ultraviolet (UV) irradiation. Extraction blanks were included in each batch to monitor potential contamination, and extracted DNA was stored at −20 °C until further processing. All faecal samples with a successful species determination based on mtDNA and nDNA (Structural Maintenance of Chromosomes X (SMCX) and Albumin genes, microsatellites) and, hence, assigned the species identity including the detection of hybrids and older introgression were used for analysis of hares' diet preferences, dietary plant overlap, dietary plant diversity and dietary niche breadth. In addition, the microsatellites were used to assess the degree of genetic admixture between species and to determine the probable generation of recent hybrids (F1, F2, BC1, or BC2), according to the protocol described in Schai-Braun et al. (2024a). The determination of the optimal number of genetic clusters ($\Delta K = 2$) based on the Evanno method was previously reported in Schai-Braun et al. (2024a) and is not reassessed here. PCR negative controls were included in all amplification runs to detect potential contamination. Amplification success was assessed by electrophoresis on 2% agarose gels and confirmed by the presence of clear bands of the expected fragment size. All faecal samples analysed in this study were previously used in Schai-Braun et al. (2024a), and subsets were included in Schai-Braun et al. (2025). However, the present study addresses distinct research questions and includes additional plant DNA metabarcoding analyses generating previously unpublished data. Reused data were restricted to species/hybrid identification of faeces, and the prior use of each sample is documented in Supporting Information 2. The reuse of samples reflects the long-term nature of the study and allows different ecological hypotheses to be tested using the same material.

Ground vegetation inventory

Given that hares may exploit a feeding area of roughly 25 ha within a 24-hour period (Smith et al. 2004), an inventory of all plant species present during faecal sample collection in 2019 (1.–13.7. and 2.–11.9.2019) was carried out within a 282 m buffer around the transects, corresponding to the radius of a 25 ha area. The habitat types present in the buffer of each transect were mapped and then typical plant species and their frequency per habitat type were aggregated for each transect separately. Note that due to the methodological limitations of the conducted inventory, we recorded a potential presence list but not a presence/absence list. A botanist recorded plant species occurring in the ground vegetation layer between 0 and 1 m in height, applying the identification criteria of Fischer et al. (2008), and using plant nomenclature as defined by Lauber et al. (2018). When uncertainties arose in the field, we collected samples and verified them under a microscope. The ground vegetation inventory of this study was previously used in Schai-Braun et al. (2025).

Plant availability

Around each point where a faecal sample of a hare was mapped, a circle with the size of 10 ha was drawn. 24-hour home-range sizes of European hares in small-field agricultural landscapes have been reported to be up to 10 ha, with mean daily distances between home-range centres of 122.7 m (Schai-Braun and Hackländer 2014). We used 10 ha circles, as our study area is also characterised by fine-scale spatial heterogeneity (median size of landscape elements 1.77 ha ± 0.57 SE). Subsequently, we assessed plant composition within each circle, assuming that the landscape within the circle represented the hare's potential feeding area during the preceding night. We then used the ground vegetation inventory within each circle to calculate plant availability for a hare (see subchapter *Ground vegetation inventory*). We acknowledge that resource selection may be scale-dependent and that alternative multi-scale approaches (e.g. multi-buffer or RSF/SSF frameworks) can provide additional insight into scale sensitivity. However, our analysis was restricted to a single, ecologically informed scale to ensure consistency with published estimates of daily movement and home-range size in European hares.

Genetic analysis of the dietary composition

Two plant DNA markers were selected for diet characterisation: the P6 loop of the plastid DNA trnL (Yoccoz et al. 2012) and the Internal transcribed spacer 2 (ITS2) (Cheng et al. 2016) from the nuclear genome. Amplifications were performed using the primer pairs trnLgA49425-Fw/trnL-hB49466-Rv (Taberlet et al. 2007) and Uniplant-ITS2-Fw/Uniplant-ITS2-Rv (Moorhouse-Gann et al. 2018), both modified with the Illumina overhang adapters. To increase base diversity during sequencing, seven forward and seven reverse primers were used for each marker, differing in the number of random "N" bases. PCR reactions were performed using 5 µL QIAGEN Multiplex PCR Master Mix, 3,4 µL of ultrapure water, 0,3 µL of each primer and 2,5 µL of DNA. Thermal Cycling conditions included an initial denaturation at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s for ITS and 55 °C for 1 min for trnL primers, and extension at 72 °C for 30 s, with a final extension cycle at 72 °C for 10 min. Two replicates were performed for each sample and for all negative controls. PCR replicates for each sample were uniquely indexed and sequenced independently. During bioinformatic processing, read counts from the two PCR replicates were summed at the sample level to derive dietary information. No biological replicates were included in this study. PCR success was verified by loading 2 µL of each PCR product onto a 2% agarose gel. Positive PCR products were then diluted 1:10 and used as templates for a second PCR to attach unique dual P5 and P7 index adapters. The indexing PCR had a total volume of 10 µL, containing 5 µL of 2× KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Cape Town, South Africa), 1 µL of mixed indexing primers (5 µM), 2 µL of ultrapure water, and 2 µL of diluted first-round PCR product. Cycling conditions were an initial denaturation at 95 °C for 3 min, followed by 10 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. The success of the indexing PCR was again confirmed by agarose gel electrophoresis. Indexed amplicons were purified using 1.2× AMPure® XP beads, quantified on an Epoch spectrophotometer (Biotek, USA), and diluted to a final concentration of 15 nM. All libraries were subsequently pooled, quantified by qPCR using the KAPA Library Quantification Kit for Illumina platforms, and normalised to 4 nM. The final pooled library was sequenced on an Illumina NovaSeq 6000 System (2 × 250 bp) using, with an expected average of 30,000 reads paired-end reads per sample and replicate.

Bioinformatic analysis of the dietary composition

Raw reads were processed using OBITools pipeline (Boyer et al. 2016). Paired-end reads were assembled using illuminapairedend (minimum overlap > 40 bp), and non-overlapping reads were discarded with obigrep. Sequences were demultiplexed with ngsfilter, dereplicated within samples using obiuniq, and primer sequences removed. Length filters were applied, retaining fragments of 20–80 bp for trnL and 250–350 bp for ITS2, and singletons were removed. PCR and sequencing artefacts were clustered at 97% similarity using sumaclust (Mercier et al. 2013). To remove potential residual errors, post-clustering curation was performed in R software v4.3.2 (R Core Team 2024) using the package *LULU* (Frøslev et al. 2017) with a minimum similarity threshold of 84%. Read counts from PCR replicates were summed per sample, and contaminant sequences were removed using the decon function of the package *microDecon* (McKnight et al. 2019), based on extraction and PCR blank controls. To improve taxonomic assignment, a curated plant NCBI reference database was built for both markers using CRABS (Jeunen et al. 2023) based on the ground vegetation inventory. Sequences were downloaded from the NCBI nucleotide database (as of 28 November 2023, and updated on 26 March 2024 for *Juncus*, *Polypodium vulgare*, Poaceae, *Luzula luzuloides*, *Luzula sylvatica* and Orchidaceae) using db_download, excluding Metazoa, environmental samples, and unclassified sequences. Amplicons were extracted via in silico PCR (insilico_pcr) using the corresponding primers, allowing up to three mismatches. Missing amplicons were retrieved using Pairwise Global Alignment (PGA), applying thresholds of ≥ 95% identity and coverage, and strict primer-region filtering. Taxonomic lineages were assigned with assign_tax and redundant sequences within species were removed with dereplicate. The final curated reference database was indexed using BLAST+ v2.15.0 (Camacho et al. 2009).

Processed Molecular Operational Taxonomic Units (MOTUs) were compared to the NCBI curated reference database using BLASTn (McGinnis and Madden 2004; Camacho et al. 2009). Taxonomic information was retrieved using the R package *taxonomizr*, and the lowest possible taxonomic rank was assigned based on sequence identity thresholds: ≥ 98% (species), ≥ 95% (genus), ≥ 92% (family), and ≥ 80% (order or higher). MOTUs with fewer than 10 total reads across samples were discarded, as were non-plant (non-Viridiplantae) sequences. Only MOTUs identified to at least the genus or family level were retained for further analyses. For statistical analyses, a taxon was considered present in a sample if it represented ≥ 1% of total reads (Aizpurua et al. 2018). In single cases the MOTU extracted from the hare faeces was replaced with the nearest and most likely locally occurring species based on taxonomic knowledge and morphological similarity (see Supporting Information 3).

The relative read abundance (RRA) of each MOTU was used as a proxy for its dietary contribution. Several studies investigating the correlation of actual herbage mass proportions in experimental diets and the herbage-DNA-sequence proportions in faeces provided favourable support for using DNA barcoding as a proxy for the quantification of plants in faeces (Nichols et al. 2016; Guo et al. 2018; Stapleton et al. 2022). We note that, while RRA provides a useful proxy for plant use, it does not reflect exact biomass intake. As RRAs differ between markers for the same taxon and not all taxa are picked up by every marker (García-Robledo et al. 2013; Hibert et al. 2013; Goldberg et al. 2020), we combined the ITS2 and trnL datasets to obtain more robust dietary estimates. For taxa detected by both markers, the lower frequency was rescaled proportionally to the higher frequency within each sample, and the arithmetic mean of both markers was then calculated to obtain a combined RRA. This procedure helps balance marker-specific over- and underestimation arising from differences in amplification efficiency and taxon detectability, thereby reducing the influence of extreme marker-specific biases. We acknowledge that this is a pragmatic approach and does not fully account for marker-specific amplification biases or differential detectability among taxa. Alternative strategies include marker-specific modelling, hierarchical multi-marker frameworks, occupancy-based models, or calibration using mock communities; however, these approaches require additional calibration data or rely on assumptions of stable marker-specific bias and consistent detectability across taxa that could not be formally validated in this study. While this approach cannot fully eliminate such biases, integrating two markers with complementary properties provides a more robust estimate of dietary contribution than either marker alone. We did not perform a formal sensitivity analysis comparing marker-specific results, but expect broad qualitative consistency due to their complementary taxonomic coverage.

Statistical analyses

All statistical analyses were carried out using R 4.3.3 (R Core Team 2024). Note that the analyses of seasonal (at peak and at the end of the vegetation period) and altitudinal variables were conducted independently rather than jointly. This was because sample sizes in some season–altitude combinations were insufficient to provide robust estimates in a joint analysis. This approach may increase the risk of Type I errors, and the results are interpreted with this limitation in mind.

Diet preferences

The hares' relative selections were measured by using Chesson's electivity index ε (Chesson 1983), an index based on Manly's alpha (Manly et al. 1972) which can be used to analyse habitat preferences (Krebs 1989), among others. Although Chesson's electivity index was originally developed for quantitative biomass data, it can be applied to semi-quantitative DNA metabarcoding data because the index compares relative proportions of use versus availability (e.g., Waraniak et al. 2019). We note that RRA provides a proxy for plant consumption, so the resulting electivity values reflect relative selection rather than exact biomass intake. We chose Chesson's electivity index, because it has the advantage that results between cases for which the number of available habitat categories varies are comparable. The Chesson's electivity index ranges between −1 and +1, with negative values showing a negative selection, whereas positive values signify a positive selection. If the index value is zero, the habitat variable concerned is used in the same proportion as it is available. We calculated Chesson's electivity indices at the species, genus, family, and order levels identified in each faecal pellet sample to account for heterogeneous taxonomic resolution inherent to DNA metabarcoding data. Higher-level estimates aggregate lower-level taxa and are not statistically independent, but allow assessment of whether dietary selectivity reflects fine-scale species preferences or broader phylogenetic patterns potentially linked to shared plant traits. Additionally, we examined the Chesson's electivity indices at peak vs. at the end of the vegetation period (Alpine mountain hares: peak $n=33$, end $n=26$; European hares: peak $n=64$, end $n=26$; hybrids: peak $n=40$, end $n=22$), and at low vs. high elevation ranges. The low and high elevation ranges were assessed separately for each hare group by dividing the elevation range symmetrically into two equal halves, i.e., 1800 to 2149 m a.s.l. ($n=29$) vs. 2150 to 2500 m a.s.l. ($n=30$) in Alpine mountain hares, 1200 to 1749 m a.s.l. ($n=53$) vs. 1750 to 2300 m a.s.l. ($n=37$) in European hares, 1100 to 1599 m a.s.l. ($n=53$) vs. 1600 to 2106 m a.s.l. ($n=9$) in hybrids. Note that the high-elevation hybrid sample is small, which may reduce statistical power for this group, and results are interpreted with caution.

The reliability of the electivity indices was tested using the bootstrap method (Dixon 1993). The original ε_i values (ε_i = Chesson's electivity index for the habitat variable i) were resampled 1000 times with replacement and an accelerated bootstrap confidence interval (CI) was calculated. The accelerated bootstrap adjusted the CI for bias and skewness (Efron and Tibshirani 1993). Selection for this habitat variable was considered significant when the 95% CI did not include zero. If the lower and upper boundaries were on opposite sides of zero, the selection of the respective habitat variable was not significant (n.s.). ε_i values for habitat categories were only bootstrapped if they were selected by six or more hares, as smaller sample sizes provide unreliable results. Bootstrapping was done using the R package *boot* (Canty and Ripley 2025).

Dietary plant overlap

The degree of dietary plant overlap between the two hare species and their hybrids was estimated with Pianka's index of niche overlap (Pianka 1973) using the package *EcoSimR* (Gotelli et al. 2015). We tested for significance by comparing used plants with values obtained by randomising the original matrices (1000 iterations), using the default procedure RA3 as randomisation algorithm. Pianka's index of dietary niche overlap was calculated for each plant species, genus, family, and order identified in each faecal pellet sample. Additionally, we examined the Pianka's indices at peak vs. at the end of the vegetation period, and at low vs. high elevation ranges. Pianka's index ranges from 0 (exclusive resource categories) to 1 (similar resource categories). It is considered that values of Pianka's index higher than 0.6 indicate a biologically significant niche overlap for the resources (Wallace 1981).

Dietary plant diversity and dietary niche breadth

We used the Shannon-Wiener diversity index to estimate available and used dietary plant diversity of the hares. Shannon-Wiener index combines species richness (number of species) and evenness (relative abundances) to quantify diversity, with 0 describing no diversity (Spellerberg and Fedor 2003). We calculated Shannon-Wiener index for each plant species, genus, family, and order identified in each faecal pellet sample with the package *vegan* (Oksanen et al. 2022). We used Levin's index to estimate available and used dietary niche breadth of the hares. Levin's index estimates niche breadth by measuring how uniformly resources are used by organisms. A value of 1 indicates generalist taxa that use various resources equally, and 0 indicates specialist taxa that favour specific resources. We calculated Levin's index for each plant species, genus, family, and order identified in each faecal pellet sample (Krebs 2014). Generalised linear mixed-effects models were fitted using the package *lme4* (Bates et al. 2015). The response variables Shannon-Wiener index and Levin's index were investigated with models including the covariate type (4 levels, used by species/hybrids, available). All models included the variable transect as random factor in order to account for the repeated measurements collected from the different transects. As the program R does not directly provide p-values for such models, the p-values were extracted by likelihood ratio tests (Faraway 2006). Post-hoc tests were computed for the covariate type using the Tukey's all-pair comparisons method in the R package *multcomp* (Hothorn et al. 2008). Residuals of the models were visually checked for normal distribution by QQ-plots and histograms. The homogeneity of variances and goodness of fit were examined by plotting residuals vs. fitted values (Faraway 2006). We examined the plant diversity and niche breadth used by the three hare groups and available by comparing peak vs. end of the vegetation period, and low vs. high elevation ranges using generalised linear mixed-effects models. Analyses were conducted separately for each hare group and for available plants. Shannon-Wiener and Levin's indices were used as response variables in models including either season (two levels) or elevation (two levels) as fixed effects, with transect included as a random effect.

Results

Faecal pellet sampling

A total of 522 faecal samples were collected between 2019 and 2020. Of these, 295 were collected at the peak vegetation period (July), and 227 at the end (August/September). From the initially tested faecal pellet samples, a total of 458 (88%) amplified for mtDNA and 211 (40%) amplified successfully for the SMCX and/or Albumin nuclear genes and/or for the microsatellites (see Supporting Information 2). Because individual identity was not available for all faecal samples, analyses reflect population-level patterns, and potential non-independence among samples from the same individual cannot be entirely excluded. Although DNA quality declines in older faeces, we collected all non-decomposing pellets consistently twice on each transect within the same week, minimising potential bias across species, elevation, or season. Among the 211 hare samples (with a successful species determination based on mtDNA and nDNA), 90 were assigned to the European hare, 59 to the Alpine mountain hare, and 62 showed signs of mtDNA introgression, and thus hybridisation (molecular markers gave a discordant species assignment). The great majority of hare samples with mtDNA introgression (61 out of 62) had mtDNA of the Alpine mountain hare and nDNA of the European hare. Only one hybrid sample showed an admixture pattern between species in the nDNA (see Supporting Information 2, Sample ID #115, having as Species ID *L. europaeus* and *L. timidus* based on nuclear microsatellites). The STRUCTURE results of the simulated genotypes for each hybrid class: F1, F2, BC1, BC2 suggested that this hare (qi-EUR = 0.742 and qi-TIM = 0.258) falls within the membership (qi) range values of the cross between F1 hybrid and a European hare (average qi-EUR 0.749, 95% CI 0.715–0.843) or between F2 hybrid and European hare (average 0.721, 95% CI 0.688–0.815). All other hybrids showed discordant species assignment between mtDNA and nDNA, hence, were characterised by an older interspecific hybridisation.

European hare faecal pellets were collected within an altitudinal range of 1,225–2,282 m a.s.l. (on average 1,673 m ± 39 SE), Alpine mountain hare within 1,842–2,420 m a.s.l. (on average 2,149 m ± 19 SE), and hybrids within 1,140–2,065 m a.s.l. (on average 1,467 m ± 26 SE).

Ground vegetation inventory and dietary composition

In total, 245 plant species in 161 genera, 55 families and 28 orders were recorded as being available in the environment (see Supporting Information 4). In total, 88 plant species, 93 genera, 41 families and 25 orders were identified in the faecal samples using the ITS2 and trnL markers (see Supporting Information 3 and 5). Note that taxonomic resolution varies across taxa, depending on marker resolution and the availability of reference barcode databases of plant species. Across both markers, a total of 47.1 million reads were generated. After quality filtering and contamination removal, 14.7 million reads were retained for ITS2 and 22.9 million reads for trnL. Order-level taxonomic resolution was achieved for 94.0% of filtered ITS2 reads and 73.9% of filtered trnL reads (for detailed sequencing depth, filtering performance, and read distribution across taxonomic levels see Supporting Information 6).

Diet preferences

A total of 47 plant species, 56 plant genera, 28 plant families and 21 plant orders were detected in more than six faecal pellet samples and thus yielded reliable Chesson's Electivity indices in the European hare (Figs. 1a, 2a, 3a and 4a; Table 1). Within this set, two species (*Onobrychis montana*, *Silene vulgaris*), two genera (*Onobrychis*, *Silene*), two families (Caryophyllaceae, Fabaceae), and two orders (Caryophyllales, Fabales) were positively selected, while the majority – 41 species, 47 genera, 22 families, and 18 orders – were consistently avoided. Patterns of selection and avoidance at genus, family, and order levels reflect aggregated responses of their constituent species and should therefore be interpreted as summaries of lower-level dietary patterns rather than independent signals of selectivity. For hybrids, 46 plant species, 55 genera, 28 families and 20 orders met the threshold for reliable electivity estimation (Figs. 1b, 2b, 3b and 4b). Among these, one species (*Onobrychis montana*), one genus (*Onobrychis*), two families (Caryophyllaceae, Fabaceae) and two orders (Caryophyllales, Fabales) showed positive selection, whereas 38 species, 48 genera, 24 families, and 17 orders were avoided. In the Alpine mountain hare, 49 species, 59 genera, 30 families and 21 orders were sufficiently represented to calculate Chesson's Electivity indices (Figs. 1c, 2c, 3c and 4c). Selection was restricted to a single plant species (*Vaccinium uliginosum*) and one family (Fabaceae), whereas avoidance was observed for 33 species, 40 genera, 23 families, and 16 orders.

In summary, the European hare utilised plant taxa in proportion to availability more frequently than the hybrids, and the hybrids utilised them more frequently than the Alpine

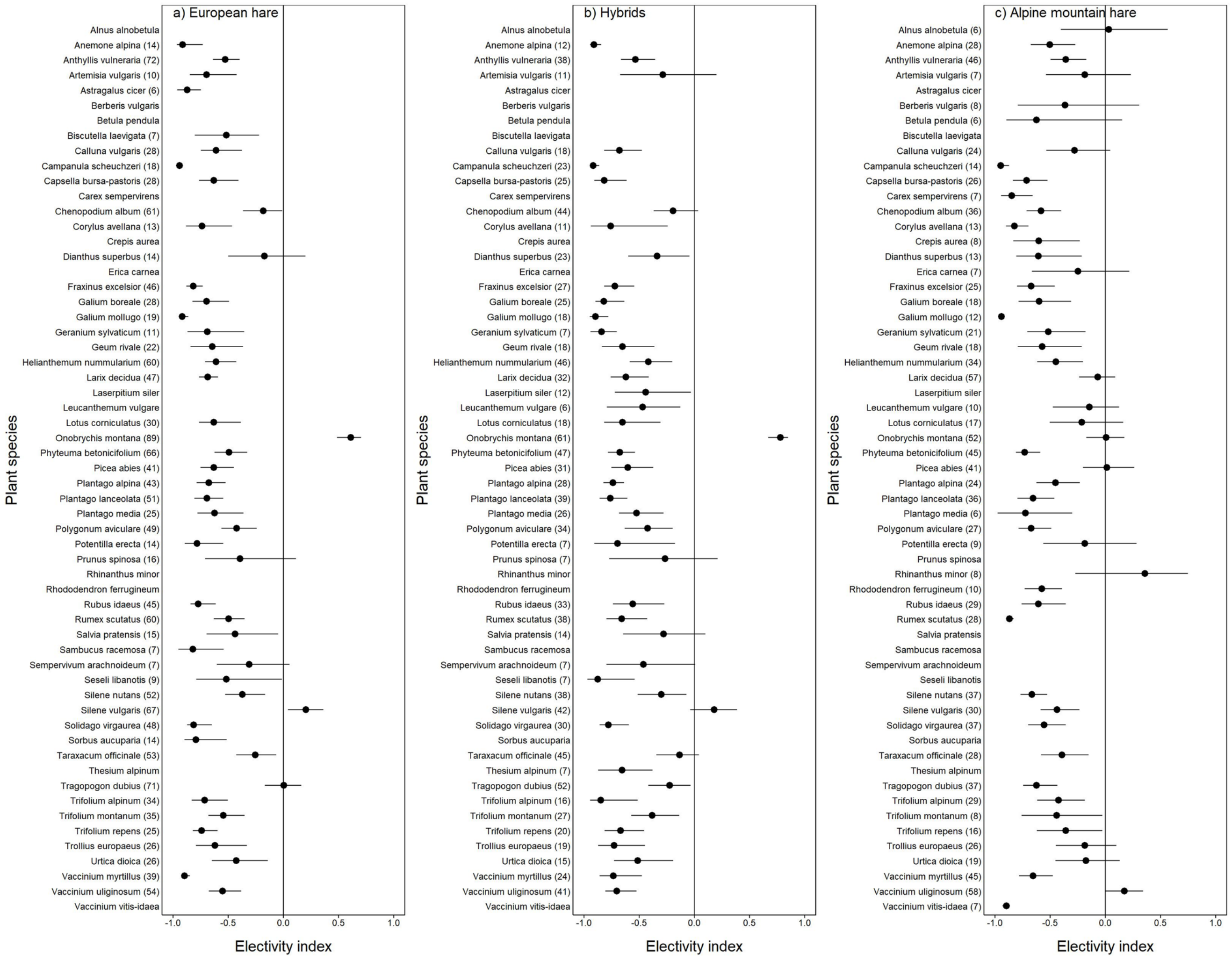

Fig. 1 Chesson's electivity indices in **a**) European hares ($n=90$), **b**) hybrids ($n=62$), and **c**) Alpine mountain hares ($n=59$; species determined by SMCX and Albumin genes, and microsatellites) and their distributions of 1000 bootstrap resamples (mean and 95% confidence interval) for all plant species (determined by two markers, the P6 loop of the plastid DNA trnL and the Internal transcribed spacer 2 (ITS2), from the nuclear genome) which were selected by $n \geq 6$ hares (sample size in brackets is the number of hares selecting the respective plant species). Faecal samples were collected in the European Alpine region in Grisons, Switzerland during the years 2019 and 2020 within an altitudinal range of 1,000–2,600 m a.s.l. Non-significant results cross the vertical line at zero. See text for statistical details

mountain hare, irrespective of the plant taxon level. However, only a few taxa were positively selected, so differences among hare groups were small. In all hare groups, most plant taxa were consumed less than expected based on availability, followed by taxa utilised in proportion to availability, with only very few consumed more than expected, irrespective of the plant taxon level.

Seasonal diet preferences

At peak vegetation period, more plant taxa provided reliable Chesson's Electivity indices in all hare groups than at the end of the vegetation period (European hare: 44 vs. 31 species, 54 vs. 38 genera, 26 vs. 22 families, 21 vs. 18 orders; hybrids: 39 vs. 30 species, 50 vs. 32 genera, 25 vs. 20 families, 19 vs. 17 orders; Alpine mountain hare: 37 v. 35 species, 48 vs. 42 genera, 26 vs. 23 families, 21 vs. 18 orders, Table 2). The European hare selected two species (*Onobrychis montana*, *Silene vulgaris*), two genera (*Onobrychis*, *Silene*), two families (Caryophyllaceae, Fabaceae), and two orders (Caryophyllales, Fabales) at peak, and one species (*Onobrychis montana*), one genus (*Onobrychis*), one family (Fabaceae), and two orders (Caryophyllales, Fabales) at the end of the vegetation period. Hybrids selected one species (*Onobrychis montana*), one genus (*Onobrychis*), two families (Caryophyllaceae, Fabaceae), and one order (Caryophyllales) at peak, and one species (*Onobrychis montana*), one genus (*Onobrychis*), two families (Caryophyllaceae, Fabaceae), and two orders (Caryophyllales, Fabales) at the end of the vegetation period. Alpine mountain hares selected

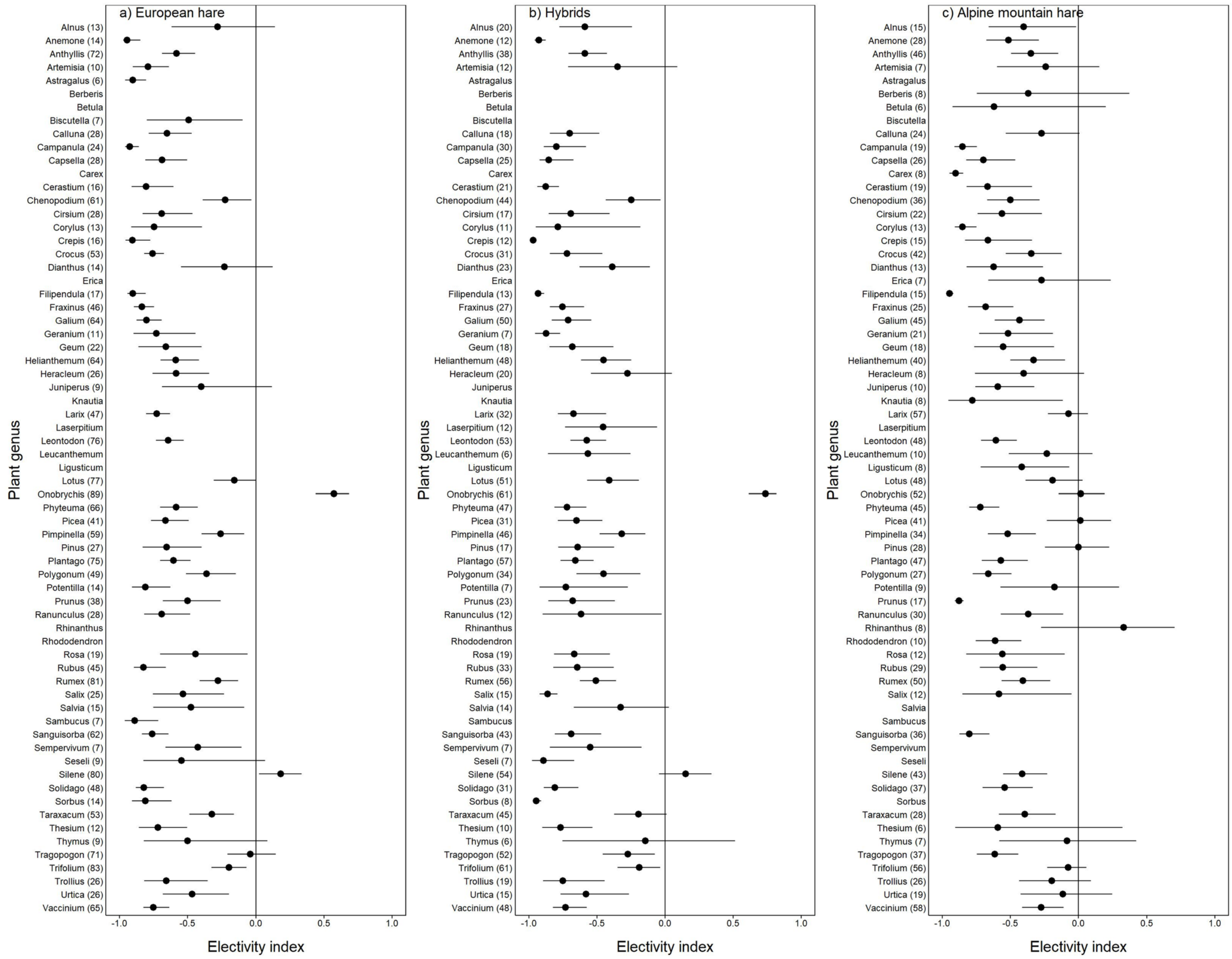

Fig. 2 Chesson's electivity indices in **a**) European hares ($n=90$), **b**) hybrids ($n=62$), and **c**) Alpine mountain hares ($n=59$; species determined by SMCX and Albumin genes, and microsatellites) and their distributions of 1000 bootstrap resamples (mean and 95% confidence interval) for all plant genera (determined by two markers, the P6 loop of the plastid DNA trnL and the Internal transcribed spacer 2 (ITS2), from the nuclear genome) which were selected by $n \geq 6$ hares (sample size in brackets is the number of hares selecting the respective plant genus). Faecal samples were collected in the European Alpine region in Grisons, Switzerland during the years 2019 and 2020 within an altitudinal range of 1,000–2,600 m a.s.l. Non-significant results cross the vertical line at zero. See text for statistical details

one single species (*Vaccinium uliginosum*) at peak and one order (Caryophyllales) at the end of the vegetation period.

In summary, European hares selected more plant taxa at peak than at the end of the vegetation period at the species, genus and family level. In hybrids, the number of selected plant taxa was similar between the two seasons at these taxonomic levels. In Alpine mountain hares, more plant species were selected at peak while genus- and family-level selection was similar in both seasons. Furthermore, in European hares and hybrids, the composition of selected plant taxa was highly consistent between the two seasons.

Altitudinal diet preferences

The European hare selected one species (*Onobrychis montana*), two genera (*Onobrychis*, *Silene*), two families (Caryophyllaceae, Fabaceae), and two orders (Caryophyllales, Fabales) at lower elevations, and two species (*Onobrychis montana*, *Silene vulgaris*), one genus (*Onobrychis*), one family (Fabaceae), and two orders (Caryophyllales, Fabales) at higher elevation. Hybrids selected one species (*Onobrychis montana*), one genus (*Onobrychis*), two families (Caryophyllaceae, Fabaceae), and two orders (Caryophyllales, Fabales) at lower elevations, and one species (*Onobrychis montana*), one genus (*Onobrychis*), and one plant family (Fabaceae) at higher elevation (note: this group was represented by a small sample, $n=9$, which may reduce

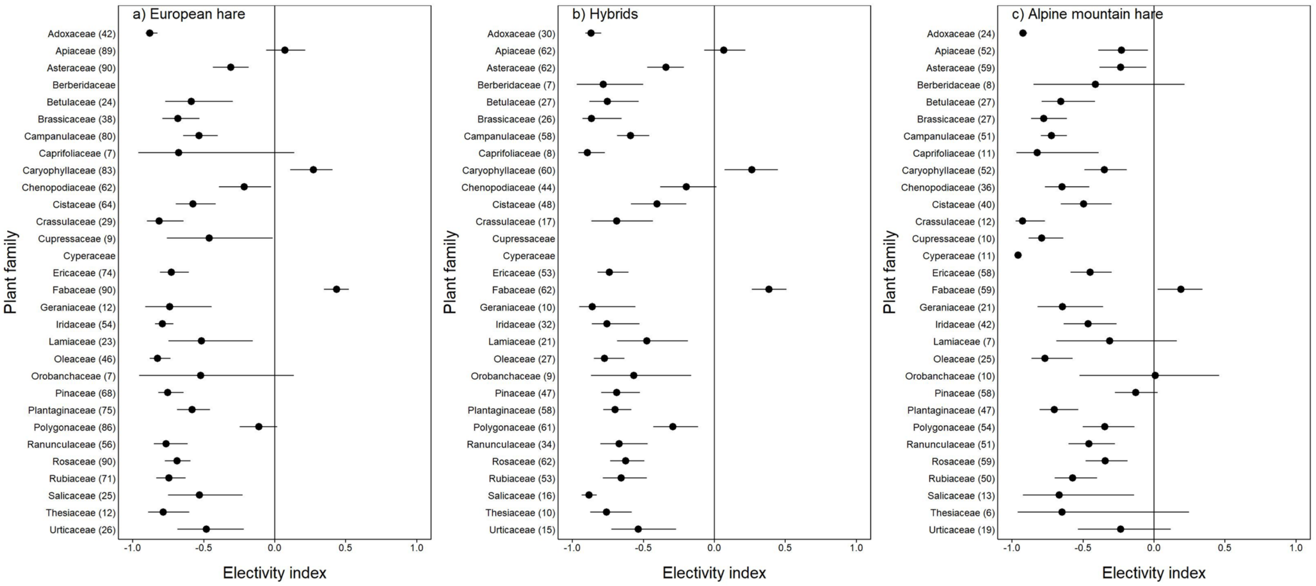

Fig. 3 Chesson's electivity indices in **a**) European hares ($n=90$), **b**) hybrids ($n=62$), and **c**) Alpine mountain hares ($n=59$; species determined by SMCX and Albumin genes, and microsatellites) and their distributions of 1000 bootstrap resamples (mean and 95% confidence interval) for all plant families (determined by two markers, the P6 loop of the plastid DNA trnL and the Internal transcribed spacer 2 (ITS2), from the nuclear genome) which were selected by $n \geq 6$ hares (sample size in brackets is the number of hares selecting the respective plant family). Faecal samples were collected in the European Alpine region in Grisons, Switzerland, during the years 2019 and 2020 within an altitudinal range of 1,000–2,600 m a.s.l. Non-significant results cross the vertical line at zero. See text for statistical details

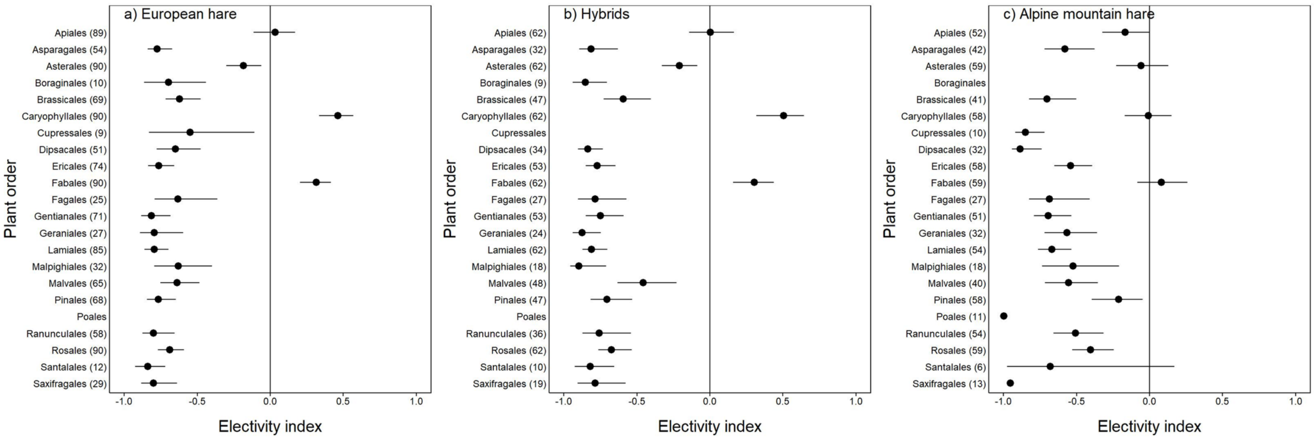

Fig. 4 Chesson's electivity indices in **a**) European hares ($n=90$), **b**) hybrids ($n=62$), and **c**) Alpine mountain hares ($n=59$; species determined by SMCX and Albumin genes, and microsatellites) and their distributions of 1000 bootstrap resamples (mean and 95% confidence interval) for all plant orders (determined by two markers, the P6 loop of the plastid DNA trnL and the Internal transcribed spacer 2 (ITS2), from the nuclear genome) which were selected by $n \geq 6$ hares (sample size in brackets is the number of hares selecting the respective plant order). Faecal samples were collected in the European Alpine region in Grisons, Switzerland during the years 2019 and 2020 within an altitudinal range of 1,000–2,600 m a.s.l. Non-significant results cross the vertical line at zero. See text for statistical details

reliability). Alpine mountain hares selected one species at lower elevations (*Picea abies*), and one species (*Vaccinium uliginosum*) and one family (Fabaceae) at higher elevation. In all three hare groups, avoidances exceeded neutral selections of plant taxa in both elevation ranges (Table 3). An exception was hybrids at higher elevations, which showed an equal number of avoidances and neutral selections at the species level (4 species) and more neutral selections than avoidances at the genus level (10 vs. 6 genera). Overall, the composition of selected plant taxa was similar between elevation ranges in European hares and hybrids, but differed in Alpine mountain hares.

Table 1 Number of plant taxa consumed above expected levels, in proportion to availability, below expected levels, and total for European hares, hybrids, and Alpine mountain hares at the levels of (a) species, (b) genus, (c) family, and (d) order

a) Species	+	*n*.s.	-	Total
European hare	2	4	41	47
Hybrids	1	7	38	46
Alpine mountain hare	1	15	33	49
b) Genus	+	n.s.	-	Total
European hare	2	7	47	56
Hybrids	1	6	48	55
Alpine mountain hare	0	19	40	59
c) Family	+	n.s.	-	Total
European hare	2	4	22	28
Hybrids	2	2	24	28
Alpine mountain hare	1	6	23	30
c) Order	+	n.s.	-	Total
European hare	2	1	18	21
Hybrids	2	1	17	20
Alpine mountain hare	0	5	16	21

Dietary plant overlap

The overlap between European hares and hybrids was significantly larger than between Alpine mountain hares and hybrids or between Alpine mountain hares and European hares at all plant taxa levels (Fig. 5). While the dietary overlap between European hares and hybrids was almost equally high across the different plant taxa levels (species level = 0.98, genus level = 0.99, family level = 0.99, order level = 0.99), the overlap between Alpine mountain hares and hybrids/European hares was almost the same and increased from the species to the order level (species level = 0.47/0.45, genus level = 0.47/0.45, family level = 0.62/0.60, order level = 0.66/0.64). This increase in overlap at higher taxonomic levels is expected, due to the reduced number of categories, and likely does not reflect a biological change in diet similarity.

Seasonal dietary plant overlap

Dietary plant overlap changed from peak to the end of the vegetation period for European hares vs. Alpine mountain hares and hybrids vs. Alpine mountain hares but not for European hare vs. hybrids (species: 0.96/0.98, genus: 0.97/0.98, family: 0.99/0.99, order: 0.99/0.99). Dietary plant overlap was consistently smaller at peak and higher at the end of the vegetation period irrespective of the taxon level (European hare vs. Alpine mountain hares: species: 0.21/0.66, genus: 0.25/0.65, family: 0.49/0.70, order: 0.51/0.74; Alpine mountain hare vs. hybrids: species: 0.23/0.67, genus: 0.27/0.67, family: 0.51/0.71, order: 0.54/0.75).

Altitudinal dietary plant overlap

Dietary plant overlap differed between hare groups inhabiting low and high elevations. The dietary plant overlap of European hares vs. Alpine mountain hares and hybrids vs. Alpine mountain hares increased from low to high elevations irrespective of the taxon level (European hare vs. Alpine mountain hares: species: 0.20/0.54, genus: 0.22/0.54, family: 0.39/0.70, order: 0.48/0.70; Alpine mountain hare vs. hybrids: species: 0.26/0.61, genus: 0.27/0.68, family: 0.42/0.81, order: 0.51/0.82). In contrast, the dietary plant overlap of European hare vs. hybrids decreased slightly from low to high elevations (species: 0.97/0.89, genus: 0.98/0.89, family: 0.99/0.95, order: 0.99/0.95).

Table 2 Number of plant taxa consumed above expected levels, in proportion to availability, below expected levels, and total for European hares, hybrids, and Alpine mountain hares at the levels of (a) species, (b) genus, (c) family, and (d) order at peak (beginning of July) and at the end (end of August/beginning of September) of the vegetation period

	Peak of the vegetation period				End of the vegetation period			
a) Species	+	*n*.s.	-	Total	+	*n*.s.	-	Total
European hare	2	4	38	44	1	6	24	31
Hybrids	1	9	29	39	1	8	21	30
Alpine mountain hare	1	10	26	37	0	12	23	35
b) Genus	+	n.s.	-	Total	+	n.s.	-	Total
European hare	2	6	46	54	1	11	26	38
Hybrids	1	9	40	50	1	9	22	32
Alpine mountain hare	0	16	32	48	0	15	27	42
c) Family	+	n.s.	-	Total	+	n.s.	-	Total
European hare	2	3	21	26	1	6	15	22
Hybrids	2	2	21	25	2	3	15	20
Alpine mountain hare	0	8	18	26	0	5	18	23
c) Order	+	n.s.	-	Total	+	n.s.	-	Total
European hare	2	2	17	21	2	2	14	18
Hybrids	1	2	16	19	2	2	13	17
Alpine mountain hare	0	6	15	21	1	3	14	18

Table 3 Number of plant taxa consumed above expected levels, in proportion to availability, below expected levels, and total for European hares, hybrids, and Alpine mountain hares at the levels of (a) species, (b) genus, (c) family, and (d) order at low and high elevation ranges

	Low elevation ranges				High elevation ranges			
a) Species	+	*n*.s.	-	Total	+	*n*.s.	-	Total
European hare	1	6	35	42	2	6	29	37
Hybrids	1	8	33	42	1	4	4	9
Alpine mountain hare	1	10	24	35	1	8	25	34
b) Genus	+	n.s.	-	Total	+	n.s.	-	Total
European hare	2	4	42	48	1	13	33	47
Hybrids	1	7	44	52	1	10	6	17
Alpine mountain hare	0	18	26	44	0	16	28	44
c) Family	+	n.s.	-	Total	+	n.s.	-	Total
European hare	2	4	19	25	1	8	16	25
Hybrids	2	2	24	28	1	6	6	13
Alpine mountain hare	0	4	20	24	1	6	18	25
c) Order	+	n.s.	-	Total	+	n.s.	-	Total
European hare	2	1	17	20	2	4	14	20
Hybrids	2	1	17	20	0	6	7	13
Alpine mountain hare	0	5	14	19	0	5	13	18

Dietary plant diversity

The plant diversity for each plant taxon was significantly higher in the available plants compared to those actually used by the hares ($p<0.001$, Fig. 6). Alpine mountain hares showed a significantly greater dietary plant diversity than European hares and hybrids, irrespective of taxon level (for an overview of all p-values see Supporting Information 7).

Seasonal dietary plant diversity

Available plant diversity was significantly higher at peak vegetation period at the plant species and genus level, whereas at the family and order levels no significant difference was observed ($p>0.05$, for an overview of all p-values, means, and standard deviations see Supporting Information 8). European hares used a significantly higher dietary plant diversity at peak than at the end of the vegetation period across all plant taxon levels except the family level. In Alpine mountain hares and hybrids, no significant difference in dietary plant diversity between the two plant seasons was detected, except for Alpine mountain hares, which used a significantly higher dietary plant diversity at peak than at the end of the vegetation period at the plant order level.

Altitudinal dietary plant diversity

Available plant diversity was significantly higher at lower elevations compared to higher elevations, irrespective of plant taxon level (for an overview of all p-values, means, and standard deviations see Supporting Information 9). European hares used a significantly higher dietary plant diversity at higher than at lower elevations, but only at the plant order level. No significant difference in dietary plant diversity between the two altitudinal ranges was observed in hybrids. Alpine mountain hares had significantly higher dietary plant diversity at lower compared to higher elevations at the plant species and genus levels, whereas no significant difference was noted at the plant family and order levels.

Dietary niche breadth

Niche breadth of available plants was significantly larger than dietary niche breadth of the European hare, Alpine mountain hare and their hybrids at all plant taxon levels ($p<0.001$). Alpine mountain hares had a significantly larger dietary niche breadth than hybrids at the plant species level (for an overview of post-hoc test results see Supporting Information 10). At all other plant taxon levels, Alpine mountain hares showed a significantly larger dietary niche breadth than both hybrids and European hares.

Seasonal dietary niche breadth

Niche breadth of available plants did not significantly differ between peak and the end of the vegetation period across all plant taxon levels ($p>0.05$) except the family level (peak: mean = 0.340, ± 0.110 SE, end: mean = 0.361, ± 0.102 SE, $\chi^2(1)=4.017$, $p=0.045$). Dietary niche breadth at peak and at the end of the vegetation period did not significantly differ for the European hare, Alpine mountain hare and their hybrids at the species, genus and family level ($p>0.05$). However, Alpine mountain hares had a significantly larger niche breadth at peak than at the end of the vegetation period at the order level ($\chi^2(1)=5.403$, $p=0.020$, Supporting

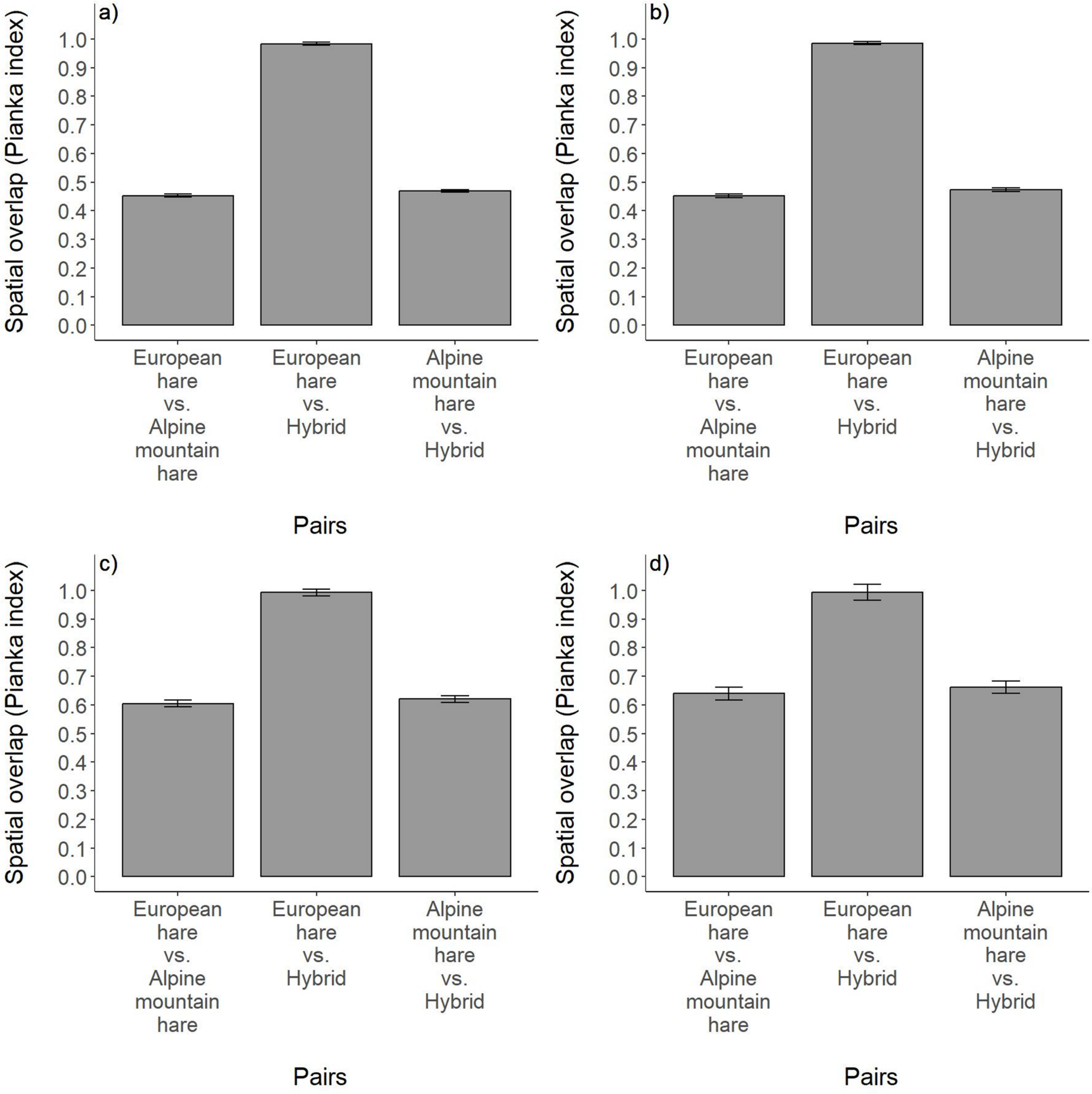

Fig. 5 Dietary plant overlap at the **a**) species, **b**) genus, **c**) family, and **d**) order level based on Pianka's index of niche overlap. Arrows represent variance of simulated index. Faecal samples of European hares (*n*=90), hybrids (*n*=62), and Alpine mountain hares (*n*=59; species determined by SMCX and Albumin genes, and microsatellites) were collected in the European Alpine region in Grisons, Switzerland, during the years 2019 and 2020 within an altitudinal range of 1,000–2,600 m a.s.l. See text for statistical details

Information 11). Consequently, dietary niche breadth at peak vegetation period was significantly higher for Alpine mountain hares than for European hares (β = -0.076, ± 0.019 SE, $p<0.001$) and hybrids (β = -0.103, ± 0.022 SE, $p<0.001$) at the order level, while no significant differences between the three hare groups were apparent at the end of the vegetation period ($p>0.05$).

Altitudinal dietary niche breadth

Niche breadth of available plants was significantly larger at lower elevations than at higher elevations, but only at the plant order level ($\chi^2(1)=9.393$, $p=0.002$). Nevertheless, dietary niche breadth at lower and higher elevations did not differ significantly for the European hare, Alpine mountain

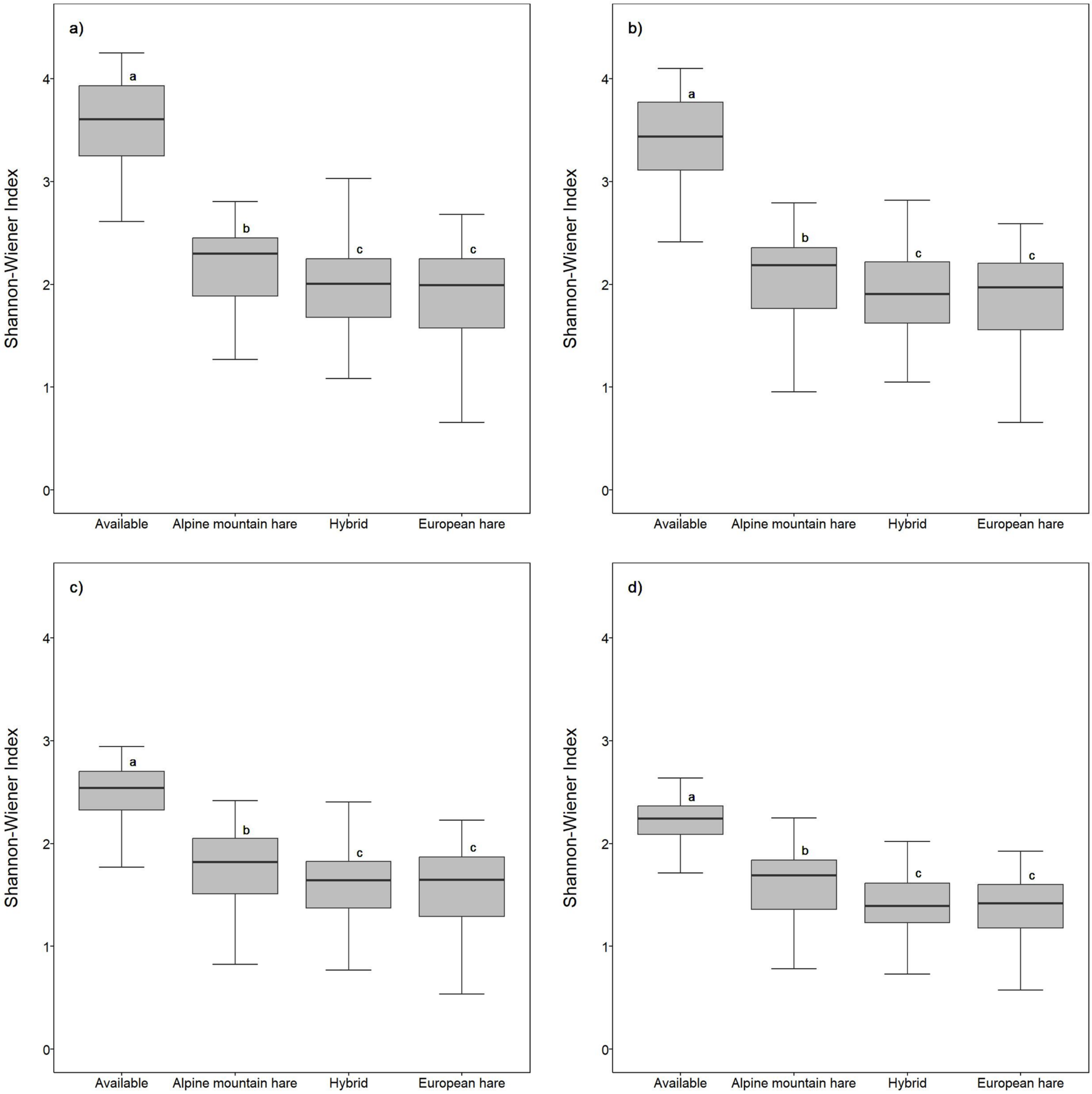

Fig. 6 Dietary plant diversity at the **a**) species, **b**) genus, **c**) family, and **d**) order level measured by the Shannon-Wiener index (medians with 25th /75th and 10th /90th percentiles). Faecal samples of European hares (*n* = 90), hybrids (*n* = 62), and Alpine mountain hares (*n* = 59; species determined by SMCX and Albumin genes, and microsatellites) were collected in the European Alpine region in Grisons, Switzerland, during the years 2019 and 2020 within an altitudinal range of 1,000–2,600 m a.s.l. See text for statistical details

hare, or their hybrids at the species, genus, family, and order levels ($p > 0.05$).

Discussion

Diet preferences

In our study, both hare species and their hybrids mostly consumed plant taxa less than expected based on availability, followed by taxa utilised in proportion to availability, with only very few taxa consumed more than expected. This pattern was consistent across taxonomic levels. This aligns with the diet preferences of Alpine mountain hares in another Alpine region (Schai-Braun et al. 2020), but contrasts with patterns observed in European hares in arable landscapes, which consumed a greater number of taxa than expected (Reichlin et al. 2006). Alpine habitats are characterised by high plant diversity (Spehn and Körner 2005), whereas arable landscapes generally exhibit much lower plant diversity (Carmona et al. 2020). Accordingly, all hares in our Alpine study area had access to a wide range of potential food plants. We therefore interpret the mostly neutral consumption of plant taxa, i.e. consumption in proportion to availability, as an indication that sufficient nutritious food plants were available and selective foraging was mostly unnecessary.

Despite the overall neutral consumption pattern, hare groups showed preferences for particular plant taxa. All hare groups preferred plant species specialised in Alpine environments (European hares and hybrids: *Onobrychis montana*, Alpine mountain hares: *Vaccinium uliginosum*) but European hares also preferred a generalist plant species (*Silene vulgaris*). However, only the Alpine mountain hare preferred a plant species rich in phenols, i.e., *Vaccinium uliginosum* (Ștefănescu et al. 2019). This may reflect the stronger specialisation of the Alpine mountain hare on Alpine vegetation. Some Alpine-specialised plant species were consumed neutrally by all hare groups, though Alpine mountain hares did so more frequently. These results partially support our hypothesis that diet selection of the European hare, a generalist, includes generalist plant taxa, e.g., widespread or domesticated, whereas the Alpine mountain hare selects plant taxa specialised in Alpine environments. Furthermore, the fact that the same plant lineages were among the few selected taxa at different taxonomic levels suggests that dietary selectivity may partly reflect traits shared among related plants, such as nutritional value or secondary chemistry, rather than responses to individual plant species alone.

Dietary plant overlap

We could confirm our hypotheses that dietary plant overlap is lower between the European hare and the Alpine mountain hare but higher between hybrids and their parental species. Hybrids and European hares had a very high dietary plant overlap, in line with the finding that all but one hybrid were backcrossed with the European hare. Dietary plant overlap of European hares and hybrids vs. Alpine mountain hares was higher at the end of the vegetation period and at high elevations irrespective of plant taxon. This pattern may reflect the reduced availability of food at the end of the vegetation period (Pavelek and Kocourkova 1994), combined with lower available plant species diversity at the end of the vegetation period and at high elevations. Under such conditions, hares might have fewer options to meet their daily energy requirements, potentially contributing to the larger dietary plant overlap compared to the peak vegetation period and at lower elevations. A study investigating dietary plant overlap during the winter could clarify whether overlap might be greater during the time of year when food is least available.

Dietary plant diversity and dietary niche breadth

All hares included much less plant diversity in their diet than was available in their environment. In addition, niche breadth of available plants was also larger than dietary niche breadth of the hares. We explain these results by the fact that hares inhabiting the Alps have a high food plant variety available, as plant composition changes along the elevation gradient (Körner 2003; Sundqvist et al. 2013). The high diversity of available plant species may exceed what is required for hares to meet their daily energy requirements, and thus does not need to be fully utilised. Additionally, energy expenditure associated with moving along the elevation gradient might be larger than the benefit of increasing dietary plant variability. Contrary to our hypothesis, Alpine mountain hares included a higher plant diversity in their diet than European hares, and both hare species had the same dietary niche breadth at the species level although one is a generalist and the other a specialist species. However, these findings should be interpreted with caution due to potential non-independence among faecal samples, which may reduce the effective sample size and lead to some degree of pseudoreplication, as well as uneven subgroup sample sizes. Given the observational nature of our data, these results are interpreted in terms of dietary patterns and associations rather than causal dietary mechanisms. It has been shown that large herbivores increase their niche breadth in high-diversity grasslands to reduce niche overlap and thereby facilitate dietary partitioning between them (Xu et al. 2024).

It may be possible that Alpine mountain hares increased their niche breadth due to competition with the European hare in the Alpine ecosystem characterised by high plant diversity (Spehn and Körner 2005). An alternative explanation is that the mountain hare may be less specialised than previously assumed. In Scandinavia, it appears to function as a generalist, occupying habitats ranging from alpine and arctic regions to boreal and temperate forests and agricultural landscapes (Angerbjörn and Schai-Braun 2023), suggesting that such classifications may be perspective-dependent. A high dietary plant diversity seems to be advantageous for the Alpine mountain hare. Mammalian herbivores that are capable of detoxifying high doses of similar secondary compounds present need to increase their dietary diversity (Dearing et al. 2000). It might be possible that the Alpine hare's high adaptation to the Alpine environment including its specificity to feed on Ericaceae with high phenol content requires the inclusion of a higher plant diversity in the diet in order to mitigate negative effects of single food plant species. These patterns should be interpreted with caution given potential scale dependence in resource selection and residual marker-specific biases, which may influence effect sizes but are unlikely to alter the overall ecological patterns observed.

Seasonal effects on the hares' diet

The Alps have a much shorter vegetation period than lowland areas (Calanca and Holzkämper 2010), and in our study a significant reduction in available plant diversity was already evident in August. Although the vegetation period differs across elevations, plant availability was assessed locally at each faecal sample location, so dietary comparisons reflect hare selectivity relative to the plants actually present and should be interpreted in this context. The availability of certain food plant taxa might be reduced additionally towards the end of the vegetation period, as pastures are grazed and meadows are mown in the agricultural landscape. Thus, the availability of forage taxa for the hares is also influenced by competition with farmers and their stock and, additionally, some wild herbivores. It remains to be seen whether this type of competition is directing the availability of forage plants more than competition with the respective other hare species. We could not find indications that European hares had to compensate for their lack of adaptation to Alpine environments by increasing their dietary plant diversity or dietary niche breadth at the end of the vegetation period. It seems as if the European hare's diet and, hence, its energy uptake were not limited by the short vegetation period in the Alps. Nevertheless, European hares decreased their number of preferred plant taxa at the end of the vegetation period at the species, genus, and family level, whereas in Alpine mountain hares this was only noticeable at the species level but not at the genus and family level. This indicates that European hares changed their feeding behaviour at the end of the vegetation period. Generalists change their food plants according to the season more distinctly than specialists (Carron et al. 1990) but this does not necessarily include an increase in dietary niche breadth or dietary plant diversity. Our findings are consistent with the idea that the European hare can successfully inhabit Alpine ecosystems and may compete with the congeneric Alpine mountain hare.

Altitudinal effects on the hares' diet

In line with our hypothesis, Alpine mountain hares showed selective feeding behaviour by selecting plant species typical for the respective elevation, whereas European hares selected plant taxa with little difference along elevation gradients. It seems as if Alpine mountain hares inhabiting high elevations, where specific plant species adapted to the harsher climate at high elevations occur, change their feeding behaviour to select for plant species that are specific to high elevations. This is consistent with patterns observed in another herbivorous alpine specialist, the American pika (*Ochotona princeps*), whose populations shifted their dietary composition in response to lower-elevation habitats (Varner and Dearing 2014). Dietary plasticity may be critical for specialists to tolerate changes in temperature, precipitation, and resource availability associated with ongoing climate change. On the other hand, European hares exhibited generalist behaviour and fed on generalist plant taxa irrespective of the inhabited elevation. Additionally, Alpine mountain hares increased their dietary plant diversity at low elevations where plant diversity was higher. This might indicate that the Alpine mountain hare, a specialist, adapts its feeding behaviour in relation to elevation and to available plant diversity. We did not analyse the effects of elevation and season jointly. Therefore, there might be interactions between the two covariates that we did not detect.

Hybrids feeding behaviour

Hybrids often exhibited dietary ecological patterns comparable to those of European hares, which can be explained by the fact that all but one hybrid were backcrossed with European hares. For example, the plant taxa preferred by hybrids were similar to the ones preferred by the European hare and the dietary overlap was by far the strongest between European hares and hybrids. Nevertheless, when comparing the number of selected plant taxa, the dietary plant diversity, the two vegetation growing seasons or the two elevation ranges, the hybrids showed an inconsistent dietary ecology being in some cases similar to the Alpine mountain hare, to the European hare or in-between the two species. This is contrary to the habitat preferences of hybrids in the Alps that resembled mostly those of European hares (Schai-Braun et al. 2024a). In addition, hybrids showed a dietary ecology that was not related to any of their parental species. As an example, hybrids at higher elevations showed little diet avoidance irrespective of the taxon level. It should be noted that the number of hybrids sampled at high elevations was low. Consequently, some patterns observed in this subset, such as reduced diet avoidance or intermediate feeding behaviour, should be interpreted cautiously, as they are based on limited sample size and may not be robust to additional sampling. Research on feeding ecology and hybridisation indicates that when parental lineages possess similar foraging traits, hybrids typically show feeding ecologies that overlap with those of one or both parental species (Vamosi 2000; Sas et al. 2005; Peters and Kleindorfer 2015). Hybrids might also be intermediate between parents on average, but with much more variability (Barton 2001; Mallet 2007; Rieseberg et al. 2007). Our findings on the diet ecology of the hybrids reflect all of the above mentioned feeding traits characteristics with respect to their relatedness to the parental species.

Influence of hybrids on evolutionary processes in the Alps

Our findings on hybrids' diet ecology underline that hare hybrids in the Alps may play a role in evolutionary processes, especially under ongoing climate warming. European hares could potentially acquire advantageous behavioural traits facilitating the use of mountainous habitats through backcrossed individuals. Although rare, backcrossed Alpine mountain hares may also introduce more generalist traits into the Alpine specialist, which could be advantageous in a changing environment due to climate warming. Our results indicate that hybrids in some cases show a different diet ecology than their parental species. This difference may point to increased variability compared to parental species (Rieseberg et al. 1999; Stelkens et al. 2009; Harrison and Larson 2014). The dietary ecology of the hybrids was less closely associated with a single parental species than their spatial ecology, where hybrids showed strong similarities in habitat preferences to one parental species (Schai-Braun et al. 2024a). This highlights the importance of investigating hybrids' ecology holistically across different ecological domains to better understand their potential influence on evolutionary processes. The hybrids in our study were mostly characterised by older interspecific gene flow. The very low incidence of recent hybridisation events could suggest reduced survival of recent hybrids, given that both species occur in proximity in the study area and probably meet regularly. However, this interpretation remains speculative, as survival was not directly assessed. Nevertheless, the relatively high proportion of backcrossed hybrids suggests that hybrids that survive may be relevant to ecological and evolutionary processes. From an ecological perspective, hybrids may also influence ecosystem functioning. By foraging differently than their parental species, they could alter plant community composition (Poe et al. 2019), affect nutrient cycling (Bakker et al. 2004), and modify the availability of prey for predators (Barbar and Lambertucci 2023). Thus, the presence of hybrids (and European hares) may slightly shift the functional roles that Alpine mountain hares provide in Alpine ecosystems, potentially affecting other herbivores, e.g., Alpine marmots (*Marmota marmota*), by altering plant community composition, which may change the availability and quality of forage, and potentially increase competition for preferred food plants.

Comparing DNA metabarcoding and visual microscopy

We combined a traditional method — ground vegetation inventory — for recording available plant taxa with an innovative DNA metabarcoding approach using two genetic markers to determine the dietary plant composition in hare faeces. To benchmark the performance of DNA metabarcoding, we compared our results with our previous studies using visual microscopy. In Vorarlberg, Austria, visual microscopy of hare faeces was used to record dietary plant composition (Schai-Braun et al. 2020). Compared to visual microscopy, DNA metabarcoding in the current study identified more plant taxa at all taxonomic levels (species: +15.8%, genus: +36.8%, family: +10.8%, order: +8.7%), even though plant availability measured by ground vegetation inventories was higher in the Vorarlberg study (species: +7.9%, genus: +6.9%, family: +12.7%, order: +22.2%, Schai-Braun et al. 2020). In addition, in the same study area as the current work, visual microscopy applied to the 2019 faecal samples was unable to identify plant epidermal fragments to genus or species level, likely due to the high diversity of plants occurring across multiple habitat types, which limited the ability to assign fragments to specific taxa based on habitat context (Schai-Braun et al. 2025). This demonstrates the greater taxonomic resolution achievable with DNA metabarcoding, although it remains dependent on reference sequences in nucleotide databases, which are still incomplete for many Alpine plant species.

Conclusion

The feeding behaviour of both hare species and their hybrids indicates that hares had access to sufficient nutritious food plants, likely due to the high plant diversity in the Alps. Alpine mountain hares exhibited greater dietary diversity and elevation-specific selectivity, whereas European hares maintained a generalist diet across seasons and elevations. High dietary diversity may be advantageous for the Alpine mountain hare in specialised habitats, for example by facilitating the detoxification of secondary compounds. In addition, dietary plasticity may be critical for specialists to tolerate environmental changes associated with ongoing climate change. In contrast, the European hare appears to be well adapted to Alpine ecosystems and may represent a strong competitor to the congeneric Alpine mountain hare. The variable and occasionally distinct feeding ecology of hybrids suggests that hybridisation may enhance ecological flexibility and influence evolutionary processes under ongoing climate change. In conclusion, our results support the presence of competition between the two hare species with respect to their dietary ecology. Both lagomorph species appear to be strongly adapted through their specific evolutionary strategies, whether characterised by generalist or specialist behaviour. Although the outcome of this competition under global warming cannot yet be assessed, hybrids may play a crucial role in shaping future population dynamics, species interactions, and distribution patterns of the two hare species.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42991-026-00595-2.

Acknowledgements We are grateful to Nicolas Cybulska, Julia Virgolini and Tobias Schiller for data collection, and to Lucas Costa for his contribution to the DNA metabarcoding analysis. We thank the hunters of Grison as licence holders and the municipalities of Scuol and Valsot for their cooperation, and Urs Monstein for his valuable support. The study was funded by the following foundations or organisations: Basler Stiftung für biologische Forschung, Swiss Delegation of the International Council for Game and Wildlife Conservation CIC, German Society for Mammalian Biology, International Rotary Fellowship of Hunters, Stiftung Temperatio, Graf Fabrice, von Gundlach und Payne Smith-Stiftung, Verein Grünes Kreuz, Legat Dr. Joachim de Giacomi, Amt für Jagd und Fischerei Graubünden, Swiss National Park, Institute of Wildlife Biology and Game Management, BOKU University. We are grateful to the German Wildlife Foundation for a grant to SS. JQ was supported by the Invited Research Chair in Hunting and Biodiversity, funded by a consortium of private and public entities, including Fundação para a Ciência e Tecnologia, Portugal.

Author contributions SS and KH conceived the ideas and designed methodology. FF, HJ, SW, and SS helped collecting data. SS, JQ, and PCA analysed the data. SS led the writing of the manuscript. SS, FF, HJ, JQ, KH, PCA, and SW contributed critically to the drafts and gave final approval for publication.

Data availability Most of the data generated or analysed during this study are included in this published article and its supplementary information files. The data from the ground vegetation inventory and the raw sequencing data are available from the corresponding author upon request, as their extensive nature precludes their inclusion in the article or supplementary information files.

Declarations

Ethical approval The study complies with the current laws of Switzerland and was conducted in accordance with the ethical principles set out in the 1964 Declaration of Helsinki and subsequent amendments, or comparable ethical standards.

Competing interests SS is a subject editor for Mammalian Biology.

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