A new integrated tool to investigate genetic differentiation and hybridization between hare species

Chiara Mengoni[1], Valter Trocchi[2], Francesco Riga[1], Nadia Mucci[1]

[1]Italian Institute for Environmental Protection and Research (ISPRA)
[2]Italian Hunting Federation (FIdC),

A - Research concept and design, B - Collection and/or assembly of data, C - Data analysis and interpretation, D - Writing the article, E - Critical revision of the article, F - Final approval of the article

Chiara Mengoni - 0000-0002-2270-8849
Francesco Riga - 0000-0003-2569-2413
Nadia Mucci - 0000-0002-7522-7213

Abstract:
Biomolecular tools are widely employed in conservation genetics and wildlife management, as they provide essential information for assessing population status, guiding conservation strategies, and supporting management decisions. These tools are particularly effective in addressing a range of research questions, including the assessment of genetic variability, the characterization of population structure, individual identification, and the detection of hybridization events.
In this study, we applied a panel of 4,472 high-quality genome-wide Single Nucleotide Polymorphisms (SNPs) in 78 samples belonging to the species *Lepus corsicanus*, *Lepus timidus* and *Lepus europaeus*, with the aim of evaluating their diagnostic efficiency in species assignment and hybridization detection compared to a previously tested set of 13 microsatellite loci (STRs).
The results revealed strong concordance between SNPs and STRs, with no significant differences in their overall discriminatory power for species identification or detection of recent (F1) hybridization events. However, SNPs showed greater sensitivity in identifying recent backcrosses, a context in which STRs proved ineffective, making SNPs more suitable for detecting signals of introgression.
These findings confirm that, a relatively low-density panel of SNPs can enhance the robustness and precision of genetic assessments, particularly in taxa with complex evolutionary histories or in anthropogenically managed populations.

Keywords: Single Nucleotide Polymorphism, hybridization, *Lepus* spp, genotype by sequencing, microsatellite loci.

Received: 2025-10-23
Revised: 2026-05-18
Accepted: 2026-05-24
Final review: 2026-04-09

Short title
Genetic differentiation and hybridization among hare species

Corresponding author
Nadia Mucci
Italian Institute for Environmental Protection and Research (ISPRA); email: nadia.mucci@isprambiente.it

HYSTRIX
the Italian Journal of Mammalogy

A new integrated tool to investigate genetic differentiation and hybridization between hare species

Chiara Mengoni[1], Valter Trocchi[2], Francesco Riga[3] and Nadia Mucci[1]

[1] Unit for Conservation Genetics (BIO-CGE), Department for the Monitoring and Protection of the Environment and for Biodiversity Conservation, Italian Institute for Environmental protection and Research (ISPRA), 40064 Ozzano dell'Emilia, Italy
[2] Italian Hunting Federation (FIdC), 00198 Roma, Italy
[3] Unit for Technical Advice and Strategies for Conservation and Management of National Wildlife and Mitigation of Damages and Impacts (BIO-CFN), Department for the Monitoring and Protection of the Environment and for Biodiversity Conservation, Italian Institute for Environmental Protection and Research (ISPRA), 40064 Ozzano dell'Emilia, Italy

Corresponding Author:
Nadia Mucci[1]
Via Cà Fornacetta 9, 40064 Ozzano dell'Emilia, Italy
Email address: nadia.mucci@isprambiente.it

Running title: Investigating genetic differentiation and hybridization among hare species.

HYSTRIX
the Italian Journal of Mammalogy

Abstract

Biomolecular tools are widely employed in conservation genetics and wildlife management, as they provide essential information for assessing population status, guiding conservation strategies, and supporting management decisions. These tools are particularly effective in addressing a range of research questions, including the assessment of genetic variability, the characterization of population structure, individual identification, and the detection of hybridization events.

In this study, we applied a panel of 4,472 high-quality genome-wide Single Nucleotide Polymorphisms (SNPs) in 78 samples belonging to the species *Lepus corsicanus*, *Lepus timidus* and *Lepus europaeus*, with the aim of evaluating their diagnostic efficiency in species assignment and hybridization detection compared to a previously tested set of 13 microsatellite loci (STRs).

The results revealed strong concordance between SNPs and STRs, with no significant differences in their overall discriminatory power for species identification or detection of recent (F1) hybridization events. However, SNPs showed greater sensitivity in identifying recent backcrosses, a context in which STRs proved ineffective, making SNPs more suitable for detecting signals of introgression.

These findings confirm that, a relatively low-density panel of SNPs can enhance the robustness and precision of genetic assessments, particularly in taxa with complex evolutionary histories or in anthropogenically managed populations.

Keywords: *Lepus* spp.; Genotype by Sequencing; Single Nucleotide Polymorphism; Hybridization, Microsatellite loci.

Introduction

Biomolecular analyses have been routinely employed in wildlife studies to gather insights into phylogeny and phylogeography (Fritz et al. 2022; Lavergne et al. 2010; Riddle and Jezkova 2019), population structure (Lohay et al. 2020; Venables et al. 2021; Vonholdt et al. 2010), hybridization (Iacolina et al. 2019; Leonard et al. 2014; Moroni et al. 2022), as well as parentage and individual identification (Åkesson et al. 2022; Jan and Fumagalli 2016; Moran et al. 2021). The advent of Next Generation Sequencing (NGS) has enabled researchers to analyse genomes more cost-effectively compared to the traditional Sanger sequencing and has facilitated the discovery of new polymorphisms to utilize in the study of natural populations and species. Microsatellite loci are commonly used markers in forensic and conservation genetics due to their high resolution in individual and parentage identification, as well as their strength in discovering population substructure, admixture, or hybridization events (Padula et al. 2023; Russell et al. 2021; Štohlová Putnová et al. 2021). Although fragment analysis protocols on first-generation automated sequencers are relatively straightforward and produce ready-to-use data, they are prone to several limitations, such as ambiguities in raw data interpretation and the necessity for a calibration to ensure consistency across different laboratories. Unlike STRs, Single Nucleotide Polymorphisms (SNPs), isolated after the genome-wide analysis, allow for reliable comparison of data generated across different time periods and operators; they are widely distributed in both coding and non-coding DNA regions and provide greater resolution for detecting admixture events or fine-scale genetic structures (Andrews et al. 2021; Pérez-González et al. 2023; Zimmerman et al. 2020).

Three hare species inhabit the Italian mainland: the Italian hare (*Lepus corsicanus*), the mountain hare (*Lepus timidus*), and the European brown hare (*Lepus europaeus*). Unlike the first two species, which are respectively restricted to southern and northern Italy, the European hare is widely distributed across the entire peninsula (Pierpaoli et al. 2003).

Hybridization between mountain and European hares in nature has been widely documented using a variety of genetic markers (Acevedo et al. 2015; Alves et al. 2003; Alves et al. 2006; Jansson et al. 2007; Levänen et al. 2018; Liu et al. 2011; Marques et al. 2017b; Melo-Ferreira et al. 2007). In contrast, no evidence of admixture between Italian and European hares has been reported in the wild, despite their overlapping geographic ranges. An exception was observed in Corsica, where three hare *species (Lepus corsicanus, L. europaeus, and L. granatensis*) coexist

due to extensive human-mediated reintroductions. Analysis of the transferrin gene in hunted individuals (Pietri et al. 2011) identified a single *L. corsicanus* × *L. europaeus* hybrid. Given the high degree of population management, this case was attributed to repeated large-scale releases for hunting, which may have facilitated interspecific mating. No further analyses using additional genetic markers (e.g., STRs or SNPs) have been conducted to verify the hybrid's genomic composition. Using a STR panel, Mengoni et al. (2015) first reported a discordance between mitochondrial and nuclear markers in two European hares collected in the Sila Mountains, southern Italy, where brown hare releases have been documented. The mitochondrial DNA (mtDNA) control region showed a haplotype typical of *Lepus corsicanus*, whereas the 13 microsatellite loci assigned both individuals to *L. europaeus* and showed no evidence of admixture. As this pattern may reflect a non-recent hybridization event, the analysis of genome-wide markers or functional loci could provide greater power to detect traces of introgression from non-native lineages, including historical admixture events.

Most studies on hares relied on mtDNA, STRs and nuclear gene markers (Alves et al. 2008; Fickel et al. 2005; Pietri et al. 2011; Pohjoismäki et al. 2021; Reid et al. 2022; Schai-Braun et al. 2024), whereas only a limited number have employed SNP-based approaches (Thulin et al. 2006; Levänen et al. 2018; Michell et al. 2022). To date, no SNP panel has been specifically developed and applied simultaneously to the three hare species occurring in Italy (mountain, brown, and Italian hares) to jointly assess genetic differentiation and hybridization. Moreover, no comparison of the efficiency of results obtained from SNPs and STRs has yet been performed for these species.

To fill this gap and better investigate potential differences in assessing genetic differentiation and detecting hybridization between the two marker types, we applied Genotyping-by-Sequencing (GBS) technology to identify a panel of SNPs using a dataset of three hare species (*L. corsicanus*, *L. timidus*, and *L. europaeus*). We then compared these results with those previously obtained using the STR protocol to evaluate the relative efficacy of the two marker panels. Specifically, our aims were to: i) compare the effectiveness of the two marker panels in describing genetic variability and differentiation ; ii) use simulations to evaluate differences between STRs and SNPs in resolving power for detecting hybridization events; iii) using SNP markers, assess the presence of admixture in the two samples found by Mengoni et al. (2015) exhibiting discordance between nuclear and mitochondrial genomes.

Materials & Methods

Sampling

A total of 78 samples belonging to three hare species was selected from the study by Mengoni et al. (2015): *L. corsicanus* (N = 29) from southern Italy, *L. europaeus* (N = 28) from Italy and Austria, and *L. timidus* (N = 21) from Italy, Sweden, Ireland, Finland, and Scotland. Two European hare individuals from that study (EUR547 and EUR549), which exhibited discordant mitochondrial and nuclear DNA, were included in the dataset.

DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen), following the manufacturer's protocol, and analysed at 13 autosomal microsatellite loci: Sat12 (Mougel et al. 1997), Lsa1, Lsa2, Lsa3, Lsa4, Lsa5, Lsa6, Lsa8 (Kryger et al. 2002), Sol33 (Surridge et al. 1997), Sol 30, Ocelamb, Ocls1b, D7utri (Hamill et al. 2006), using the procedures described in the respective references. Genotyping-by-sequencing (GBS) was applied to the sample set to identify polymorphic SNPs. GBS library preparation and sequencing were performed at the Institute for Genomic Diversity (IGD), Cornell University, following the protocol described by Elshire (2011). Full methodological details are provided in Supplementary Material SM1.

Comparison of genetic variability based on STRs and SNPs

Using STR genotypes, GenAlEx 6.5 (Peakall and Smouse 2012) was used to calculate the number of alleles (Na), observed (Ho) and expected heterozygosity (He). Analysis of molecular variance (AMOVA) and pairwise F*st* values were respectively computed using the *poppr* (Kamvar et al. 2014) and the *hierfstat* (Goudet 2005) packages, within the R environment. Deviations from Hardy Weinberg were assessed using Genepop on the web (https://genepop.curtin.edu.au/; Raymond and Rousset 1995).

Actually, the SNP dataset was processed using SNP & Variation Suite (SVS) 8.0.1 (Golden Helix Inc., Bozeman, MT, USA). Only loci and samples with high genotyping rates (>90%) were retained for further analyses. The filtered dataset was then pruned for linkage disequilibrium (LD), removing loci with $r^2 > 0.5$, calculated using sliding windows of 50 SNPs. The resulting dataset was exported from SVS and converted into binary files using PLINK v1.07 (Purcell et al. 2007) for downstream analyses.

Subsequently, the PLINK files were imported into the R environment and converted into *genind* and *genlight* objects using the *data.table* (Lim and Qin 2016) and the *dartR* package (Gruber et al. 2018), enabling compatibility with various population genetics analyses.
Departures from Hardy–Weinberg equilibrium were assessed using PLINK, and average values per population were calculated in R. Observed heterozygosity, analysis of molecular variance (AMOVA), and pairwise F*st* values were computed using the *hierfstat* and *poppr* packages, respectively, within the R environment.

Comparative analysis of STR and SNP power in detecting hare genetic differentiation
For both STRs and SNPs, Discriminant Analyses of Principal Components (DAPC) were conducted using the adegenet package (Jombart, 2008) in the R environment. The optimal number of principal components (PCs) was determined with the xvalDapc() function, considering a training set of 0.6 and 90 repetitions, while the most likely number of genetic clusters was inferred using the find.clusters() function based on the Bayesian Information Criterion (BIC).
Bayesian clustering analysis with STRs was carried out using STRUCTURE (Pritchard et al. 2000; Falush et al. 2007) with 100,000 MCMC iterations following a burn-in of 10,000 iterations. Analyses were conducted under both the admixture and no-admixture models, assuming independent allele frequencies, and testing K values ranging from 1 to 8.
Maximum Likelihood clustering analysis with SNPs was performed using ADMIXTURE version 1.3.0 (Alexander et al. 2009), testing K values from 1 to 8.
For both marker types, the most likely number of clusters was evaluated using StructureSelector (https://lmme.ac.cn/StructureSelector/; Li and Liu 2018), and graphical visualization of clustering results was carried out with CLUMPAK (Kopelman et al. 2015).

F1, BC1, and BC2 hybrid simulations for admixture detection
Using the original SNP and STR datasets of the three species, we simulated 20 individuals for each of the following hybrid categories per species combination: first-generation hybrids (F1), first-generation backcrosses (BC1_A and BC1_B), and second-generation backcrosses (BC2_A and BC2_B), employing two custom Python scripts. Alleles were randomly drawn from the

parental species following Mendelian inheritance, producing simulated *multilocus* genotypes consistent with each hybrid class across all species combinations. The simulated hybrids, comprising a total of 15 genotype clusters, were then combined with the original three parental species and the two individuals showing discordance between mitochondrial and nuclear DNA, to generate separate SNP and STR datasets. These datasets were subsequently analysed using clustering and multivariate analyses to investigate potential signals of admixture. The number of tested K in clustering analyses was increased to 20 to account for the total number of pre-defined groups, following the procedures described in the precedent paragraph. Cluster population membership coefficients (Q) were evaluated to assess differences in cluster assignments. DAPC was conducted retaining the number of discriminant functions suggested by the xvalDapc() function, considering a training set of 0.6 and 90 repetitions.

Results

Generating STR and SNP datasets

A total of 78 samples were genotyped at 13 STRs, with 1.2% of loci remaining unassigned. All loci were polymorphic across the three species, except for *Lsa6*, which was monomorphic in *L. timidus*. Significant departures from Hardy-Weinberg equilibrium were detected within species, likely reflecting heterogeneity in sampling origin and collection periods.

A total of 15,140 SNPs were obtained from the GBS analysis. The raw dataset was subjected to quality filtering, including the removal of SNPs with more than 10% missing data, and pruning for linkage disequilibrium ($r^2 > 0.5$) using sliding windows of 50 SNPs. After these steps, 4,472 high-quality SNPs were retained for downstream analyses. Seven samples (five *L. europaeus* and two *L. corsicanus*) failed to amplify due to low DNA quality, reducing the final dataset to 71 individuals (*L. corsicanus,* N = 26; *L. europaeus,* N = 24; *L. timidus*, N = 21).

Genetic diversity from STR and SNP genotyping

In STRs, the number of alleles (Na) was generally comparable among species (*L. europaeus* = 7,6±1.3, *L. timidus* = 7,3±1.2) although a slight but statistically significant lower value was found in *L. corsicanus* (Na= 5,1±1.0). Allelic richness (Ar), calculated to account for differences in sample size, was significantly lower in *L. corsicanus* compared to both *L. europaeus* and *L.*

timidus. Genetic diversity indices, including observed (Ho) and expected heterozygosity (He), are detailed in Table 1.

F*st* comparisons were all significant, with the lowest differentiation observed between *L. europaeus* and *L. timidus* (F*st* = 0,14). The differentiation between *L. corsicanus* and the other two species was higher, with F*st* values of 0.37 and 0.32, respectively. The AMOVA results, illustrated in the pie chart in Figure 1a, revealed substantial genetic differentiation among species, accounting for 28.5% of the total variance and among individuals within the same species (15.1%). However, the majority of genetic variation was found within individuals (56.4%).

In SNPs, the percentage of polymorphic loci varied among species, with *L. corsicanus* showing the lowest polymorphism (26.5%), *L. europaeus* the highest (80.8%), and *L. timidus* an intermediate value (54.1%). Correspondingly, expected (He) and observed heterozygosity (Ho) were highest in *L. europaeus* (Table 1).

Unlike STR analyses, SNP-based results showed a slightly lower F*st* between *L. corsicanus* and *L. timidus* (F*st* = 0.12), with nearly identical differentiation observed between *L. corsicanus* and *L. europaeus* (F*st* = 0.13) and between *L. europaeus and L. timidus* (F*st* = 0.13). The AMOVA pie chart (Figure 1b) indicated that most genetic variation was found within individuals (67.1%), although a notable proportion (10.6%) was attributed to differences among species.

Genetic differntiation from STR and SNP analysis in parental species

In STRs, Factorial Correspondence Analysis (FCA; Figure SM2) and Discriminant Analysis of Principal Components (DAPC; Figure 2a) yielded concordant results, indicating a closer genetic relationship between *L. timidus* and *L. europaeus*.

The *adegenet* analysis identified three distinct genetic clusters with no signs of admixture or sample misassignment. The lowest Bayesian Information Criterion (BIC) value was obtained for K = 3, although a smaller peak was also observed at K = 2. At this lower cluster number, *L. europaeus* and *L. timidus* grouped together (see Figure SM3).

The optimal number of clusters identified by the Bayesian analysis was K = 3, regardless of whether admixture or no-admixture models were used (Figure 3d, e). When considering K = 2 (Figure 3a, b), the primary division separated *L. corsicanus* from the other two species.

In SNPs, DAPC and PCA analyses showed concordant separation among individuals of the three species (Figure 2b; Figure SM4). The *adegenet* analysis identified three main genetic clusters corresponding to the species; however, when considering only two clusters, *L. corsicanus* and *L. timidus* grouped together (Figure SM3).

Bayesian analysis also identified three main genetic clusters (Figure 3f). Analysing the first split at K = 2, *L. corsicanus* and *L. timidus* clustered together (Figure 3c).

Analysis of admixture using parental species, F1 BC1 and BC2 hybrids

Bayesian clustering analyses based on STRs confirmed K = 3 as the most likely number of genetic clusters when analysing the combined datasets of parental species, F1, BC1, and BC2 hybrids. Results showed a failure in correctly assign BC2 hybrids, highlighting a low discriminatory power of STR markers for detecting advanced-generation hybrids. The highest component values ranged from 0.864 to 0.941 and the lower from 0.042 to 0.114. In four combinations (BC2A_Lco-Leur, BC2A_Lco-Lti, BC2B_Lco-Lti, and BC2A_Leur-Lti), the minor membership component remained below 10%. Detailed assignment results are reported in Table 2.

Using the SNP dataset, the best clustering was also found at K=3, although the CV value continued to decrease slightly in all subsequent clusters; all parental and hybrids individuals were correctly assigned to their respective clusters, with high resolution and accuracy. No misassignments were recorded.

In particular, BC2 individuals showed values ranging from 0.878 to 0.893 for the greater genetic component, and from 0.107 to 0.121 for the lowest genetic component. Detailed assignment results are reported in Table 2.

In DAPC, the SNP-based scatterplot revealed clear and well-separated clusters for all categories, including parental species and hybrids across F1, BC1, and BC2 generations, demonstrating the high resolution of SNP markers in detecting both recent and older hybridization events (Figure 4). In contrast, the STR-based scatterplot showed substantial overlap between BC2 hybrids and parental species, limiting the ability to accurately identify more advanced-generation hybrids and highlighting the lower discriminatory power of STR markers for detecting non-recent admixture (Figure 4).

In the STR and SNP analyses, the two hares with discordant mitochondrial and nuclear DNA (EUR547 and EUR549, collected in the Sila Mountains, southern Italy) were associated with European hare clusters.

Discussion

In this study, we identified, for the first time, a panel of SNPs using Genotyping-by-Sequencing (GBS) and compared the results obtained from these markers with those previously obtained from STRs (Mengoni et al., 2015) to evaluate their relative power in describing the genetic variability and differentiation among the three hare species occurring in Italy (*L. corsicanus*, *L. europaeus*, and *L. timidus*). Of particular interest was the evaluation of their effectiveness in detecting recent hybrids and interspecific backcrosses, allowing a more detailed investigation of Italian samples identified by Mengoni et al. (2015) that exhibited discordance between mitochondrial and nuclear DNA.

Comparison of SNP and STR markers for detecting species differentiation

Results obtained from Genome-wide SNP and STRs analysis were fully concordant in identifying genetic differentiation among the three hare species.

Fst and AMOVA results were also consistent across marker types, indicating a strong genetic differentiation among species, despite a substantial proportion of genetic variance being attributed to variation within individuals (56.4% for STRs and 67.1% for SNPs).

No differences in genetic differentiation were detected in the Bayesian clustering analyses at the optimal value of K = 3, as all three species were clearly separated. However, differences emerged at K = 2, where the clustering patterns diverged depending on the type of markers. Specifically, the STR-based analysis grouped *L. europaeus* with *L. timidus*, whereas the SNP-based analysis clustered *L. corsicanus* with *L. timidus*.

The phylogenetic relationships proposed by Pierpaoli et al. (1999) and subsequently confirmed by genomic analyses (Ferreira et al. 2021) supported a higher genetic similarity between *L. corsicanus* and *L. timidus*, whereas *L. europaeus* clustered with other *Lepus* species.

The discrepancy observed between STR- and SNP-based analysis may reflect intrinsic differences in marker performance, which can be explained by the distinct mutational dynamics and evolutionary properties of STRs compared to SNPs. Microsatellite loci used in this study

were originally developed and characterized in *Oryctolagus cuniculus* (Korstanje et al. 2001; Mougel et al. 1997; Surridge et al. 1997; Van Haeringen et al. 1996) and, to a lesser extent, in *Lepus saxatilis* (Kryger et al. 2002). As such, their transferability across different *Lepus* spp. may be limited by mutation-driven homoplasy and locus-specific biases (Estoup et al. 2002; Smith et al. 2007).

In contrast, the SNPs analysed in this study were identified through GBS in the target species, ensuring a higher level of informativeness and species-specific relevance. Homoplasy, which has been widely reported in STR analyses due to their high mutation rates and multiallelic nature (Estoup et al. 2002), may contribute to misleading signals of genetic similarity. Moreover, SNPs, given their lower mutation rates and abundance across the genome, are generally more effective in resolving deeper evolutionary relationships (Morin et al. 2004). These factors likely account for the discordant clustering patterns observed between the two marker types at K = 2.

Several studies involving different species support the findings of the present work and reinforce the conclusion that results obtained from STR and SNP markers are often congruent. This concordance has been reported in *Oncorhynchus tshawytscha* (Chinook salmon, Narum et al. 2008), *Salmo salar* (Atlantic salmon, Glover et al. 2010), West African cattle (Álvarez et al. 2021), as well as in various sheep and cattle breeds (Laoun et al. 2020).

Nevertheless, despite this general agreement, there is also growing evidence that SNPs offer superior resolution compared to STRs when differentiating populations at a fine geographic or demographic scale. Notable examples include studies on population of Gunnison sage-grouse (*Centrocercus minimus,* Zimmerman et al. 2020) and northern pike (*Esox lucius,* Sunde et al. 2020), where SNPs outperformed STRs in detecting subtle patterns of genetic structure.

Simulation of hybrids and introgression events between hare species

Using the pure species genotypes, we generated simulated datasets from both SNPs and STRs including 20 individuals for each hybrid class (F1, BC1_A, BC1_B, BC2_A, BC2_B) resulting in 15 groups with a total of 300 individuals. The analysis provided preliminary evidence that SNPs outperform STRs in detecting hybridization, especially in backcrosses with parental species. STRs were unable to identify BC2 hybrids, whereas SNPs provided a clearer signal of non-recent hybridization events. Furthermore, SNP-based assignment membership coefficients (Q) closely reflected the expected genomic proportions across the various hybrid categories.

Microsatellite loci (STRs), although widely used and effective in detecting recent hybridization and admixture events (Barilani et al. 2007; Iacolina et al. 2019; Leo and Millien 2017), are inherently constrained by their relatively low number—typically fewer than 20–30 markers—and their representation of only small genomic regions. As a result, they may lack the sensitivity required to detect older or more cryptic introgression patterns, especially those diluted by successive generations of backcrossing. In contrast, Single Nucleotide Polymorphisms (SNPs) provide a far more comprehensive and fine-scale view of the genome. Being abundant and distributed across both coding and non-coding regions, SNPs have proven more effective in revealing subtle population structure, detecting minor introgressed genomic segments, and identifying signatures of past hybridization (Lavretsky et al. 2015; Spinks et al. 2014).

Hybridization and introgression events between hare species

In this study, we gained further insight into the absence of admixture between sampled *L. corsicanus* and *L. europaeus*. The first compelling evidence supporting the absence of admixture, based on the analysis of 15 microsatellite loci, was reported by Mengoni et al. (2015). Notwithstanding this information, the authors were the first to highlight a notable incongruence between mitochondrial and nuclear DNA in two individuals morphologically attributable to *L. europaeus*. This incongruence—where individuals exhibited skull morphology and nuclear genotypes consistent with *L. europaeus* but carried mitochondrial haplotypes typically associated with *L. corsicanus*—points toward a likely case of mitochondrial introgression. Such introgression events may result from non-recent hybridization episodes followed by repeated backcrossing with the parental species, leading to the long-term retention of mitochondrial DNA from one species within the nuclear genomic background of another. The SNPs data also confirmed the absence of recent hybridization in samples EUR547 and EUR549 and the concordance between the two marker systems reinforces the hypothesis of genetic isolation between *L. corsicanus* and *L. europaeus* at the nuclear level. Nevertheless, the recovery of discordant mitochondrial haplotypes suggests that interspecific hybridization may have occurred in the past and that reproductive incompatibility does not entirely prevent gene flow between these species.

Hybridization and introgression are frequent throughout the genus *Lepus*, involving both past and recent events, and represent important evolutionary drivers for this group (Acevedo et al.

2012; Acevedo et al. 2015; Alves et al. 2003; Ashrafzadeh et al. 2018; Ferreira et al. 2021; Liu et al. 2011; Marques et al. 2017a; Marques et al. 2017b; Melo-Ferreira et al. 2012; Melo-Ferreira et al. 2014; Mengoni et al. 2015; Reid et al. 2022; Seixas et al. 2018).

In the genus *Lepus*, these historical processes also underlie recurrent genetic exchanges—both mitochondrial and nuclear—between closely related taxa and poorly differentiated lineages, resulting in a characteristic reticulated evolutionary pattern (Acevedo et al. 2015; Liu et al. 2011; Reid et al. 2022; Tolesa et al. 2017).

Analysis of whole exome sequences of 61.7 Mb from 15 *Lepus* species across the genus's broad geographic range by Ferreira et al. (2021) revealed repeated introgression events throughout the evolutionary history of the genus, both during its early radiation and more recently, including instances since the Last Glacial Maximum.

Focusing on the genetic differences between the two sister species *L. castroviejoi* and *L. corsicanus*, Meira do Souto (2022) found that their genomic divergence was shaped by gene introgression into *L. castroviejoi* from neighbouring species, particularly *L. granatensis* (the Iberian hare), as well as from *L. europaeus* and *L. timidus*.

Conversely, the values of excess allele sharing, Fb(C), reported by Ferreira (2021), between *L. corsicanus* and *L. europaeus* support the absence of hybridisation also inferred in the present study.

An interesting aspect of hares EUR547 and EUR549—morphologically and genetically identified as *L. europaeus* through STR and SNP genotyping—is that they show no signs of recent hybridization, despite anthropogenic translocations of this taxon in Italy dating back to the last century. Notably, these individuals were collected in a mountainous area in far southern Italy, which may offer habitat conditions more ecologically suitable for *L. europaeus* than the typically Mediterranean environments.

No evidence of introgression was detected in the Italian peninsula also in this study, even when accounting for possible introgression from *L. europaeus* into *L. timidus* populations driven by recent habitat shifts associated with climate warming (Schai-Braun et al. 2024; Schai-Braun et al. 2023).

In the case of *L. europaeus*, this pattern may be partly explained by the extensive importation of individuals from other countries for hunting purposes, which could have contributed to the partial replacement of the native taxon.

In *L. corsicanus*, the absence of introgression may be related to the potential interposition of *L. europaeus* between the distributions of *L. timidus* and *L. corsicanus*. This pattern likely reflects differences in ecological niches, with *L. europaeus* occupying an intermediate position. Despite these general considerations, it is important to emphasize that the sample size in this study was very limited. Although SNPs have been shown to be more powerful in detecting hybridization, a more comprehensive sampling strategy would be essential to confidently confirm the complete absence of hybrids between *L. corsicanus* and *L. europaeus* in Italy.

Conclusion

This study provides new insights into the greater power of SNPs compared to STRs for detecting admixture events and confirms the absence of hybridization between *L. corsicanus* and *L. europaeus*, reinforcing earlier findings based on microsatellite (STR) markers that found no nuclear evidence of admixture. However, the presence of *L. corsicanus* mitochondrial haplotypes in two morphologically identified *L. europaeus* individuals suggests non-recent mitochondrial introgression, likely due to historical hybridization followed by repeated backcrossing. Such events are common within the genus *Lepus*, where both past and recent hybridization episodes have shaped a complex evolutionary history. This is particularly evident in natural contact zones or areas affected by *Lepus* spp. introductions, which have influenced species distributions and the occurrence of hybridization, thereby promoting opportunities for both natural and anthropogenically driven gene flow. Our finding suggests long-term reproductive isolation or the presence of natural ecological or behavioural barriers preventing gene flow between these species. The comparison between STR and SNP markers revealed a high degree of concordance in identifying species boundaries, although preliminary SNPs analysis provided finer resolution for detecting backcross hybridization events. The study is preliminary and was limited by the relatively small number of samples collected across different areas and time periods. Broader sampling, including non-invasive specimens, is therefore required to substantially improve the resolution of the evolutionary histories of these closely related and intensively managed taxa, while also enabling fine-scale monitoring in areas where species hybridization occurs. The development of reduced SNP panels suitable for non-invasive genomics has recently been advocated (Soi et al. 2026) as an effective tool to better characterize population genetic structure and to investigate admixture processes potentially driven by habitat shifts associated with climate

warming. In this context, the present panel may provide a starting point for the definition of an ad hoc tool for non-invasive genetic study aimed at improving our understanding of the short- and medium-term dynamics of these species and their populations in rapidly changing environments.

Acknowledgements

We thank the Park Authorities, regional and provincial administrations who facilitated the collection of samples in their area of responsibility, and also many volunteers and hunters for their valuable cooperation.
We thank the editor and reviewers for their helpful comments and suggestions which greatly contributed to improving the manuscript.

References

Acevedo, P., Melo-Ferreira, J., Real, R., and Alves, P.C. 2012. Past, present and future distributions of an Iberian Endemic, Lepus granatensis: ecological and evolutionary clues from species distribution models. PLoS One **7**(12): e51529.

Acevedo, P., Melo-Ferreira, J., Farelo, L., Beltran-Beck, B., Real, R., Campos, R., and Alves, P.C. 2015. Range dynamics driven by Quaternary climate oscillations explain the distribution of introgressed mt DNA of Lepus timidus origin in hares from the Iberian Peninsula. Journal of Biogeography **42**(9): 1727-1735.

Åkesson, M., Svensson, L., Flagstad, Ø., Wabakken, P., and Frank, J. 2022. Wolf monitoring in Scandinavia: evaluating counts of packs and reproduction events. The Journal of Wildlife Management **86**(4): e22206.

Álvarez, I., Fernández, I., Traoré, A., Menéndez-Arias, N.A., and Goyache, F. 2021. Population structure assessed using microsatellite and SNP data: an empirical comparison in West African cattle. Animals **11**(1): 151.

Alves, P., Ferrand, N., Suchentrunk, F., and Harris, D. 2003. Ancient introgression of Lepus timidus mtDNA into L. granatensis and L. europaeus in the Iberian Peninsula. Molecular Phylogenetics and Evolution **27**(1): 70-80.

Alves, P.C., Melo-Ferreira, J., Branco, M., Suchentrunk, F., Ferrand, N., and Harris, D.J. 2008. Evidence for genetic similarity of two allopatric European hares (Lepus corsicanus and L. castroviejoi) inferred from nuclear DNA sequences. Molecular Phylogenetics and Evolution **46**: 1191-1197. doi:10.1016/j.ympev.2007.11.010.

Alves, P.C., Harris, D.J., Melo-Ferreira, J., Branco, M., Suchentrunk, F., Boursot, P., and Ferrand , N. 2006. Hares on thin ice: Introgression of mitochondrial DNA in hares and its implications for recent phylogenetic analyses. Molecular Phylogenetics and Evolution **40**: 640-641. doi:10.1016/j.ympev.2006.02.016.

Andrews, K.R., De Barba, M., Russello, M.A., and Waits, L.P. 2021. Advances in using non-invasive, archival, and environmental samples for population genomic studies. Population genomics: wildlife: 63-99.

Ashrafzadeh, M.R., Djan, M., Szendrei, L., Paulauskas, A., Scandura, M., Bagi, Z., Ilie, D.E., Kerdikoshvili, N., Marek, P., Soós, N., and Kusza, S. 2018. Large-scale mitochondrial DNA analysis reveals new light on the phylogeography of Central and Eastern-European Brown hare (Lepus europaeus Pallas, 1778). PLOS ONE **13**(10): e0204653. doi:10.1371/journal.pone.0204653.

Barilani, M., Bernard-Laurent, A., Mucci, N., Tabarroni, C., Kark, S., Perez Garrido, J.A., and Randi, E. 2007. Hybridisation with introduced chukars (*Alectoris chukar*) threatens the gene pool integrity of native rock (*A. graeca*) and red-legged (*A. rufa*) partridge populations [doi: DOI: 10.1016/j.biocon.2007.01.014]. Biological Conservation **137**(1): 57-69. Available from http://www.sciencedirect.com/science/article/B6V5X-4N6FVFX-1/2/26479dc6b7bc54c12b6f540611bfd902 [accessed.

do Souto, J.M.M. 2022. Using Genomics to Dissect the History of Divergence and Hybridization in the Sister Broom and Corsican Hares (Lepus Spp). Universidade do Porto (Portugal).

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., and Mitchell, S.E. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PloS one **6**(5): e19379.

Estoup, A., Jarne, P., and Cornuet, J.M. 2002. Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. Molecular Ecology **11**: 1591-1604.

Ferreira, M.S., Jones, M.R., Callahan, C.M., Farelo, L., Tolesa, Z., Suchentrunk, F., Boursot, P., Mills, L.S., Alves, P.C., and Good, J.M. 2021. The legacy of recurrent introgression during the radiation of hares. Systematic biology **70**(3): 593-607.

Fickel, J., Schmidt, A., Putze, M., Spittler, H., Ludwig, A., Streich, W.J., and Pitra, C. 2005. Genetic structure of populations of european brown hare: implications for management. Journal of wildlife management **69(2)**: 760-770.

Fritz, U., Tolley, K.A., Vamberger, M., and Ihlow, F. 2022. Phylogeny and phylogeography of chelonians from sub-Saharan Africa—A review of current knowledge in tribute to Margaretha D. Hofmeyr. Vertebrate Zoology **72**: 951-969.

Glover, K.A., Hansen, M.M., Lien, S., Als, T.D., Høyheim, B., and Skaala, Ø. 2010. A comparison of SNP and STR loci for delineating population structure and performing individual genetic assignment. BMC genetics **11**(1): 2.

Goudet, J. 2005. Hierfstat, a package for R to compute and test hierarchical F-statistics. Molecular ecology notes **5**(1): 184-186.

Gruber, B., Unmack, P.J., Berry, O.F., and Georges, A. 2018. dartr: An r package to facilitate analysis of SNP data generated from reduced representation genome sequencing. Molecular ecology resources **18**(3): 691-699.

Hamill, R.M., Doyle, D., and Duke, E.J. 2006. Spatial patterns of genetic diversity across European subspecies of the mountain hare, Lepus timidus L. Heredity **97**: 355-365. doi:doi:10.1038/sj.hdy.6800880.

Iacolina, L., Corlatti, L., Buzan, E., Safner, T., and Šprem, N. 2019. Hybridisation in European ungulates: an overview of the current status, causes, and consequences. Mammal Review **49**(1): 45-59.

Jan, C., and Fumagalli, L. 2016. Polymorphic DNA microsatellite markers for forensic individual identification and parentage analyses of seven threatened species of parrots (family Psittacidae). PeerJ **4**: e2416.

Jansson, G., Thulin, C.G., and Pehrson, Å. 2007. Factors related to the occurrence of hybrids between brown hares Lepus europaeus and mountain hares L. timidus in Sweden. Ecography **30**(5): 709-715.

Kamvar, Z.N., Tabima, J.F., and Grünwald, N.J. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ **2**: e281.

Korstanje, R., Gillissen, G., Kodde, L., Den Bieman, M., Lankhorst, A., Van Zutphen, L., and Van Lith, H. 2001. Mapping of microsatellite loci and association of aorta atherosclerosis with LG VI markers in the rabbit. Physiological Genomics **6**(1): 11-18.

Kryger, U., Robinson, T.J., and Bloomer, P. 2002. Isolation and characterization of six polymorphic microsatellite loci in South African hares (Lepus saxatilisF. Cuvier, 1823 and Lepus capensis Linnaeus, 1758). Molecular Ecology Notes **2**: 422-242. doi:10.1046/j.1471-8278 .2002.00265.x.

Laoun, A., Harkat, S., Lafri, M., Gaouar, S.B.S., Belabdi, I., Ciani, E., De Groot, M., Blanquet, V., Leroy, G., and Rognon, X. 2020. Inference of breed structure in farm animals: Empirical comparison between SNP and microsatellite performance. Genes **11**(1): 57.

Lavergne, A., Ruiz-García, M., Catzeflis, F., Lacote, S., Contamin, H., Mercereau-Puijalon, O., Lacoste, V., and de Thoisy, B. 2010. Phylogeny and phylogeography of squirrel monkeys (genus Saimiri) based on cytochrome b genetic analysis. American Journal of Primatology: Official Journal of the American Society of Primatologists **72**(3): 242-253.

Lavretsky, P., Engilis, A., Jr, Eadie, J.M., and Peters, J.L. 2015. Genetic admixture supports an ancient hybrid origin of the endangered Hawaiian duck. Journal of Evolutionary Biology **28**(5): 1005-1015. doi:10.1111/jeb.12637.

Leo, S.S.T., and Millien, V. 2017. Microsatellite markers reveal low frequency of natural hybridization between the white-footed mouse (Peromyscus leucopus) and deer mouse (Peromyscus maniculatus) in southern Quebec, Canada. Genome **60**(5): 454-463. doi:10.1139/gen-2016-0163 %M 28177836.

Leonard, J.A., Echegaray, J., Randi, E., Vilà, C., and Gompper, M. 2014. Impact of hybridization with domestic dogs on the conservation of wild canids. Free-ranging dogs and wildlife conservation: 170-184.

Levänen, R., Thulin, C.-G., Spong, G., and Pohjoismäki, J.L. 2018. Widespread introgression of mountain hare genes into Fennoscandian brown hare populations. PLoS One **13**(1): e0191790.

Lim, B., and Qin, H. 2016. The data. table Package for Data Cleaning and Preparation in R.

Liu, J., Yu, L., Arnold, M.L., Wu, C.-H., Wu, S.-F., Lu, X., and Zhang, Y.-P. 2011. Reticulate evolution: frequent introgressive hybridization among Chinese hares (genus Lepus) revealed by analyses of multiple mitochondrial and nuclear DNA loci. BMC evolutionary biology **11**(1): 223.

Lohay, G.G., Weathers, T.C., Estes, A.B., McGrath, B.C., and Cavener, D.R. 2020. Genetic connectivity and population structure of African savanna elephants (Loxodonta africana) in Tanzania. Ecology and evolution **10**(20): 11069-11089.

Luu, K., Bazin, E., and Blum, M.G. 2017. pcadapt: an R package to perform genome scans for selection based on principal component analysis. Molecular ecology resources **17**(1): 67-77.

Marques, J.P., Farelo, L., Vilela, J., Vanderpool, D., Alves, P.C., Good, J.M., Boursot, P., and Melo-Ferreira, J. 2017a. Range expansion underlies historical introgressive hybridization in the Iberian hare. Scientific Reports **7**(1): 40788.

Marques, J.P., Ferreira, M.S., Farelo, L., Callahan, C.M., Hackländer, K., Jenny, H., Montgomery, W.I., Reid, N., Good, J.M., and Alves, P.C. 2017b. Mountain hare transcriptome and diagnostic markers as resources to monitor hybridization with European hares. Scientific data **4**(1): 1-11.

Melo-Ferreira, J., Boursot, P., Carneiro, M., Esteves, P.J., Farelo, L., and Alves, P.C. 2012. Recurrent introgression of mitochondrial DNA among hares (Lepus spp.) revealed by species-tree inference and coalescent simulations. Systematic biology **61**(3): 367.

Melo-Ferreira, J., Farelo, L., Freitas, H., Suchentrunk, F., Boursot, P., and Alves, P.C. 2014. Home-loving boreal hare mitochondria survived several invasions in Iberia: the relative roles of recurrent hybridisation and allele surfing. Heredity **112**(3): 265-273.

Melo-Ferreira, J., Boursot, P., Randi, E., Kryukov, A., Suchentrunk, F., Ferrand, N., and Alves, P.C. 2007. The rise and fall of the mountain hare (Lepus timidus)during Pleistocene glaciations: expansion and retreat with hybridization in the Iberian Peninsula. Molecular Ecology **16**: 605-618. doi:10.1111/j.1365-294X.2006.03166.x.

Mengoni, C., Mucci, N., and Randi, E. 2015. Genetic diversity and no evidences of recent hybridization in the endemic Italian hare (Lepus corsicanus). Conservation Genetics **16**(2): 477-489. doi:10.1007/s10592-014-0674-0.

Moran, B.M., Thomas, S.M., Judson, J.M., Navarro, A., Davis, H., Sidak-Loftis, L., Korody, M., Mace, M., Ralls, K., and Callicrate, T. 2021. Correcting parentage relationships in the endangered California Condor: Improving mean kinship estimates for conservation management. The Condor **123**(3): duab017.

Morin, P.A., Luikart, G., and Wayne, R.K. 2004. SNPs in ecology, evolution and conservation. Trends in Ecology and Evolution **19**: 208-216. doi:10.1016/j.tree.2004.01.009.

Moroni, B., Brambilla, A., Rossi, L., Meneguz, P.G., Bassano, B., and Tizzani, P. 2022. Hybridization between Alpine ibex and domestic goat in the Alps: a sporadic and localized phenomenon? Animals **12**(6): 751.

Mougel, F., Mounolou, J.C., and Monnerot, M. 1997. Molecular genetic markers: Nine polymorphic microsatellite loci in the rabbit, Oryctolagus cuniculus. Animal Genetics **28**: 58-71.

Narum, S., Banks, M., Beacham, T., Bellinger, M., Campbell, M., Dekoning, J., Elz, A., Guthrie Iii, C., Kozfkay, C., and Miller, K. 2008. Differentiating salmon populations at broad and fine geographical scales with microsatellites and single nucleotide polymorphisms. Molecular Ecology **17**(15): 3464-3477.

Padula, A., Buono, V., Giangregorio, P., Mengoni, C., and Mucci, N. 2023. Cross-amplification in falcons and their hybrids: A new im-plemented multi-locus panel for conservation and forensic purposes. JOURNAL OF FORENSICS RESEARCH **14**(6).

Peakall, R., and Smouse, P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics **28**(19): 2537-2539. doi:10.1093/bioinformatics/bts460.

Pérez-González, J., Carranza, J., Anaya, G., Broggini, C., Vedel, G., de la Peña, E., and Membrillo, A. 2023. Comparative analysis of microsatellite and SNP markers for genetic management of red deer. Animals **13**(21): 3374.

Pierpaoli, M., Riga, F., Trocchi, V., and Randi, E. 1999. Species distinction and evolutionary relationships of the Italian hare (*Lepus corsicanus*) as described by mitochondrial DNA sequencing. Molecular Ecology **8**(11): 1805-1817.

Pierpaoli, M., Riga, F., Trocchi, V., and Randi, E. 2003. Hare populations in Europe: intra and interspecific analysis of mtDNA variation. Comptes rendus. Biologies **326**(S1): 80-84.

Pietri, C., Alves, P.C., and Melo-Ferreira, J. 2011. Hares in Corsica: high prevalence of Lepus corsicanus and hybridization with introduced L. europaeus and L. granatensis. European journal of wildlife research **57**: 313-321.

Pohjoismäki, J.L., Michell, C., Levänen, R., and Smith, S. 2021. Hybridization with mountain hares increases the functional allelic repertoire in brown hares. Scientific Reports **11**(1): 15771.

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

Reid, N., Hughes, M.F., Hynes, R.A., Montgomery, W.I., and Prodöhl, P.A. 2022. Bidirectional hybridisation and introgression between introduced European brown hare, Lepus europaeus and the endemic Irish hare, L. timidus hibernicus. Conservation Genetics **23**(6): 1053-1062.

Riddle, B.R., and Jezkova, T. 2019. How is phylogeography shaping our understanding of the geography of diversity, diversification, and range dynamics in mammals? Journal of Mammalogy **100**(3): 872-893.

Russell, T., Cullingham, C., Ball, M., Pybus, M., and Coltman, D. 2021. Extent and direction of introgressive hybridization of mule and white-tailed deer in western Canada. Evolutionary Applications **14**(7): 1914-1925.

Schai-Braun, S.C., Cybulska, N., Filli, F., Jenny, H., Queirós, J., Alves, P.C., and Hackländer, K. 2024. Competition between sympatric hare species in the Alps is boostered by climate change and hybridisation. European Journal of Wildlife Research **70**(4): 83.

Schai-Braun, S., Schwienbacher, S., Smith, S., and Hackländer, K. 2023. Coexistence of European hares and Alpine mountain hares in the Alps: what drives the occurrence and frequency of their hybrids? Journal of Zoology **320**(3): 214-225.

Seixas, F.A., Boursot, P., and Melo-Ferreira, J. 2018. The genomic impact of historical hybridization with massive mitochondrial DNA introgression. Genome Biology **19**(1): 91.

Smith, C.T., Antonovich, A., Templin, W.D., Elfstrom, C.M., Narum, S.R., and Seeb, L.W. 2007. Impacts of Marker Class Bias Relative to Locus-Specific Variability on Population Inferences in Chinook Salmon: A Comparison of Single-Nucleotide Polymorphisms with Short Tandem Repeats and Allozymes. Transactions of the American Fisheries Society **136**(6): 1674-1687. doi:10.1577/t06-227.1.

Soi, E., Iacolina, L., and Scandura, M. 2026. Faecal Pellets as a Non-Invasive Source for DNA-Based Monitoring of Lagomorphs. Mammal Review **56**(1): e70030.

Spinks, P.Q., Thomson, R.C., and Shaffer, H.B. 2014. The advantages of going large: genome-wide SNPs clarify the complex population history and systematics of the threatened western pond turtle. Molecular Ecology **23**(9): 2228-2241. doi:https://doi.org/10.1111/mec.12736.

Štohlová Putnová, L., Štohl, R., Ernst, M., and Svobodová, K. 2021. A microsatellite genotyping-based genetic study of interspecific hybridization between the red and sika deer in the Western Czech Republic. Animals **11**(6): 1701.

Sunde, J., Yıldırım, Y., Tibblin, P., and Forsman, A. 2020. Comparing the Performance of Microsatellites and RADseq in Population Genetic Studies: Analysis of Data for Pike (Esox lucius) and a

Synthesis of Previous Studies [Original Research]. Frontiers in Genetics **Volume 11 - 2020**. doi:10.3389/fgene.2020.00218.

Surridge, A.K., Bell, D.J., Rico, C., and Hewitt, G.M. 1997. Polymorphic microsatellite loci in the European rabbit (Oryctolagus cuniculus) are also amplified in other lagomorph species. Animal Genetics **28**: 302-305.

Tolesa, Z., Bekele, E., Tesfaye, K., Ben Slimen, H., Valqui, J., Getahun, A., Hartl, G.B., and Suchentrunk, F. 2017. Mitochondrial and nuclear DNA reveals reticulate evolution in hares (Lepus spp., Lagomorpha, Mammalia) from Ethiopia. PLoS One **12**(8): e0180137.

Van Haeringen, W., Den Bieman, M., van Zutphen, L., and Van Lith, H. 1996. Polymorphic microsatellite DNA markers in the rabbit (Oryctolagus cuniculus). Journal of experimental animal science **38**(2): 49-57.

Venables, S.K., Marshall, A.D., Armstrong, A.J., Tomkins, J.L., and Kennington, W.J. 2021. Genome-wide SNPs detect no evidence of genetic population structure for reef manta rays (Mobula alfredi) in southern Mozambique. Heredity **126**(2): 308-319.

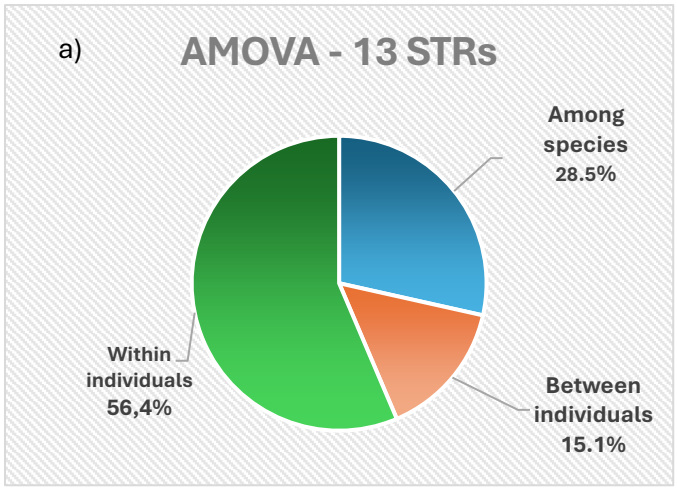
Vonholdt, B.M., Stahler, D.R., Bangs, E.E., Smith, D.W., Jimenez, M.D., Mack, C.M., Niemeyer, C.C., Pollinger, J.P., and Wayne, R.K. 2010. A novel assessment of population structure and gene flow in grey wolf populations of the Northern Rocky Mountains of the United States. Molecular ecology **19**(20): 4412-4427.

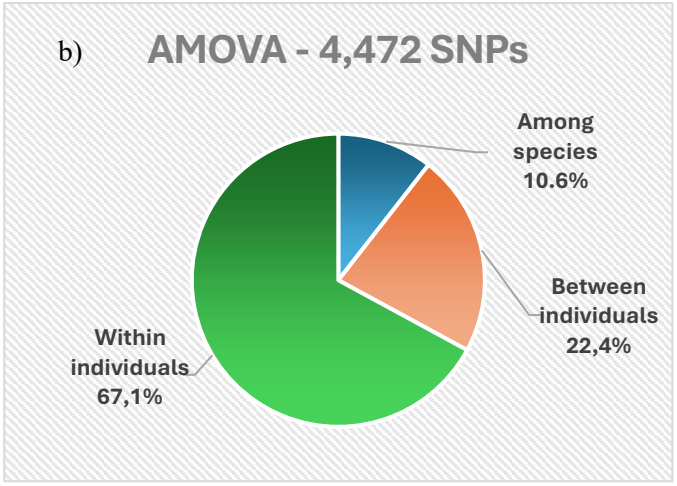
Zimmerman, S.J., Aldridge, C.L., and Oyler-McCance, S.J. 2020. An empirical comparison of population genetic analyses using microsatellite and SNP data for a species of conservation concern. BMC genomics **21**: 1-16.

	13 STRs					4,472 SNPs			
Species	**Na**	**Ar**	**Ho**	**He**	**P (%)**	**Na**	**Ho**	**He**	**P (%)**
L. corsicanus	5.1±1.0	4.0±0.2	0,331±0,053	0,457±0,074	100	1,26±0.01	0,034±0,001	0,09±0,001	26,5
L. europaeus	7,6±1.3	6.6±0.2	0,587±0,058	0,671±0,056	100	1,81±0.01	0,182±0,002	0,200±0,001	80,8
L. timidus	7,3±1.2	6.9±0.2	0,486±0,067	0,702±0,067	92.3	1,54±0.01	0,103±0,002	0,130±,001	54,1

	STRs – STRUCTURE software (K=3)			SNPs – ADMIXTURE software (K=3)		
	L. corsicanus	*L. europaeus*	*L. timidus*	*L. corsicanus*	*L. europaeus*	*L. timidus*
L. corsicanus	**0.978**	0.011	0.011	**1.000**	0.000	0.000
L. europaeus	0.019	**0.958**	0.023	0.005	**0.992**	0.003
L. timidus	0.017	0.079	**0.905**	0.001	0.006	**0.993**
F1_Lco_Leu	**0.526**	**0.450**	0.024	**0.499**	**0.501**	0.000
F1_Lco_Lti	**0.539**	0.044	**0.417**	**0.497**	0.000	**0.503**
F1_Leu_Lti	0.020	**0.543**	**0.436**	0.000	**0.490**	**0.510**
BC1A_Lco_Leur	**0.832**	**0.148**	0.020	**0.753**	**0.247**	0.000
BC1B_Lco_Leur	**0.240**	**0.732**	0.027	**0.247**	**0.753**	0.000
BC1A_Lco_Lti	**0.791**	0.031	**0.178**	**0.743**	0.000	**0.257**
BC1B_Lco_Lti	**0.234**	0.022	**0.744**	**0.228**	0.000	**0.772**
BC1A_Leur_Lti	0.023	**0.852**	**0.125**	0.000	**0.754**	**0.246**
BC1B_Leur_Lti	0.016	**0.254**	**0.730**	0.000	**0.239**	**0.761**
BC2A_Lco_Leur	**0.938**	0.042	0.020	**0.119**	**0.881**	0.000
BC2B_Lco_Leur	**0.114**	**0.864**	0.022	**0.885**	**0.115**	0.000
BC2A_Lco_Lti	**0.941**	0.016	0.043	**0.107**	0.000	**0.893**
BC2B_Lco_Lti	0.090	0.022	**0.887**	**0.883**	0.000	**0.117**
BC2A_Leur_Lti	0.018	**0.937**	0.045	0.000	**0.878**	**0.121**
BC2B_Leur_Lti	0.014	**0.113**	**0.873**	0.000	**0.118**	**0.882**

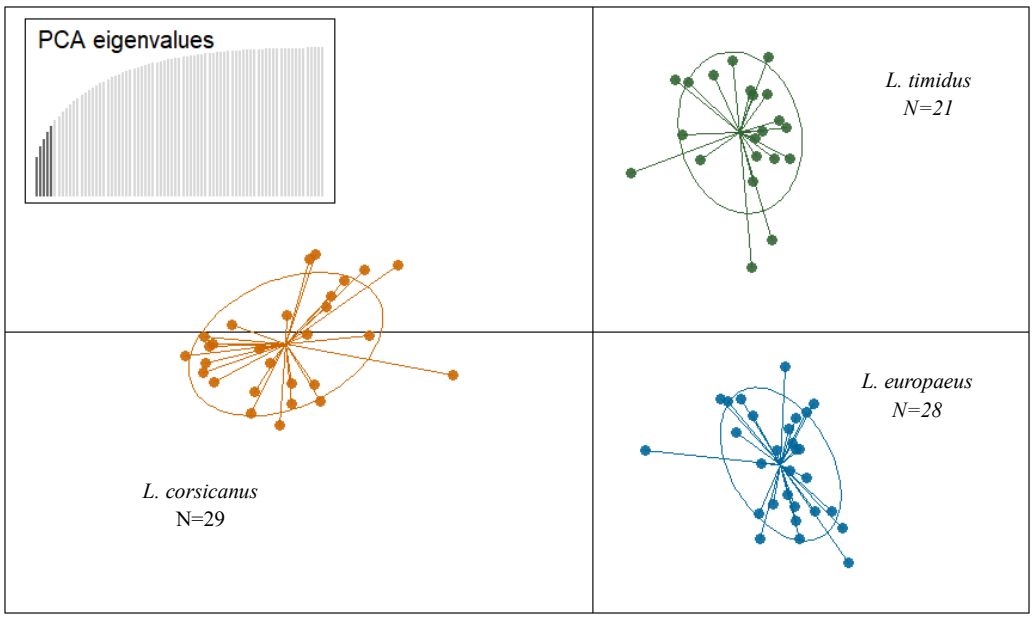
HYSTRIX
the Italian Journal of Mammalogy



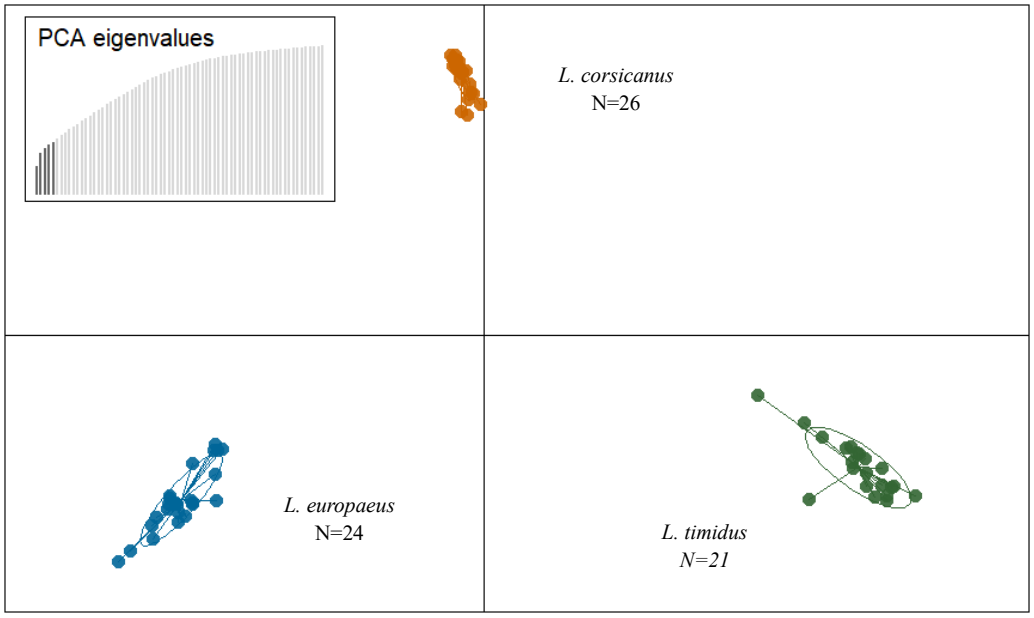


HYSTRIX
the Italian Journal of Mammalogy

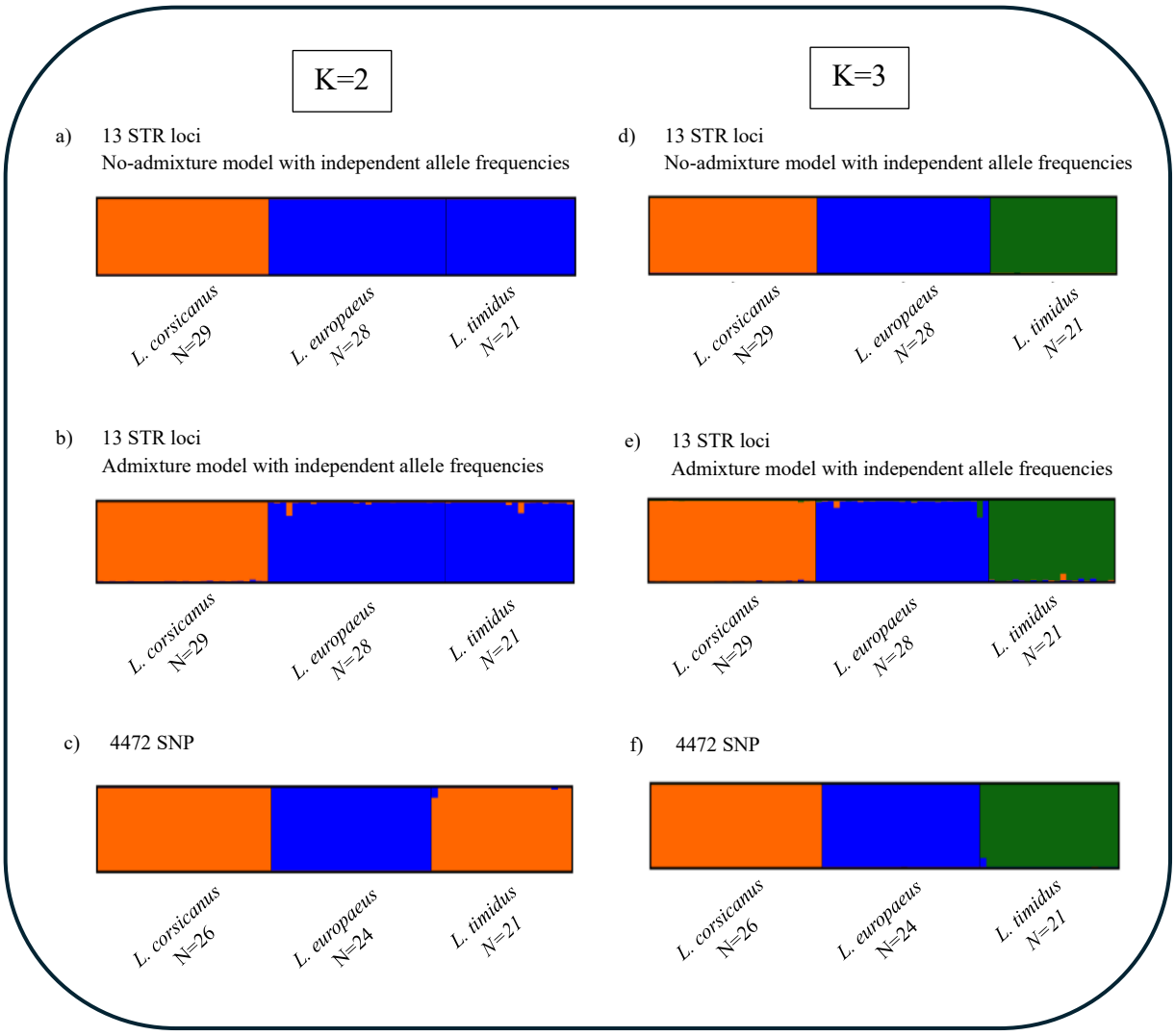
a)



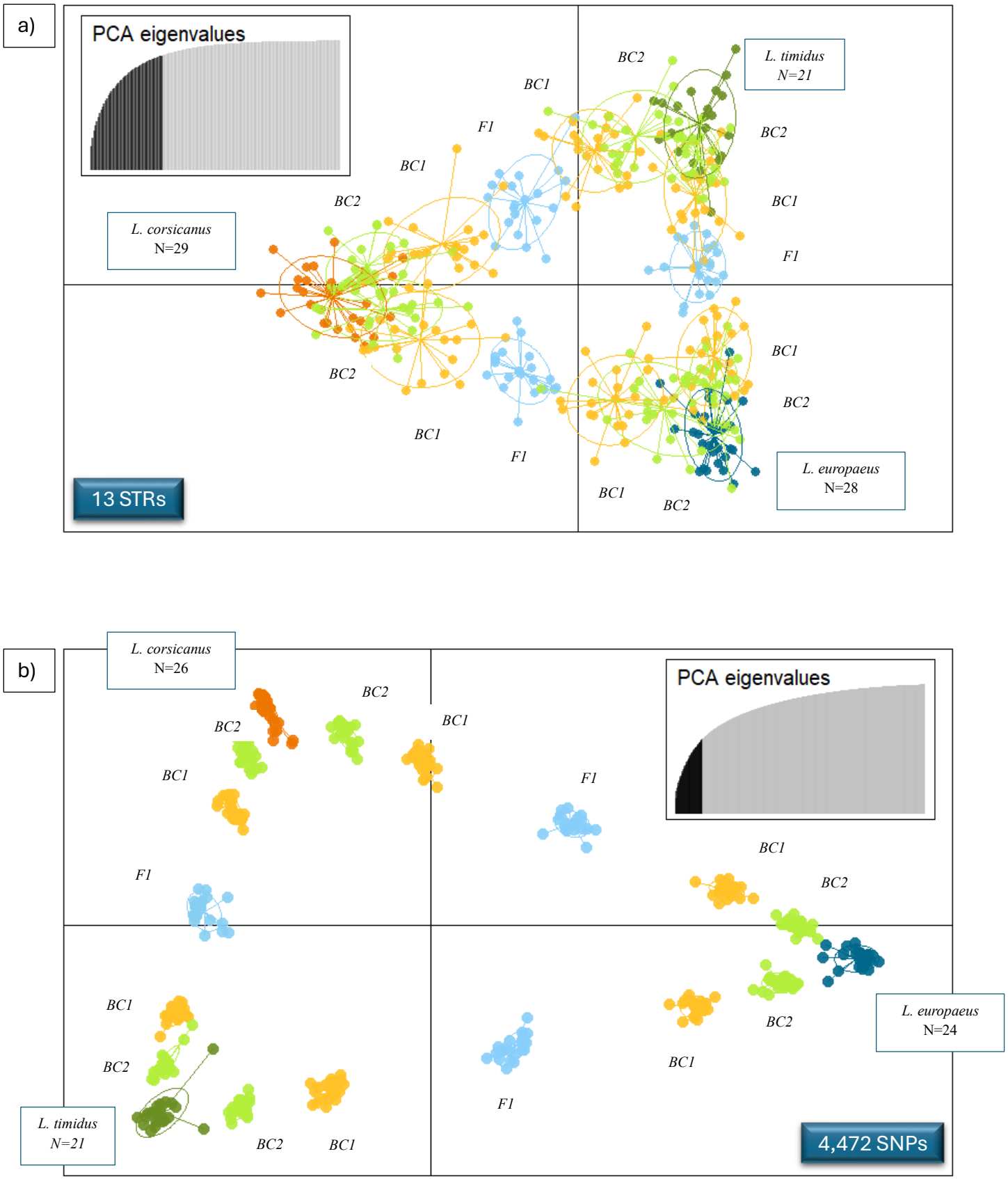
b)



HYSTRIX
the Italian Journal of Mammalogy



HYSTRIX
the Italian Journal of Mammalogy



Manuscript body

Download source file (102.22 kB)

Tables

Table 1 - Download source file (15.76 kB)
Table 1. Indices of genetic variability in STRs and SNPs.
Na= Number of Alleles; Ar= Allelic richness; Ho= Observed heterozygosity; He= Expected heterozygosity; P%= Polymorphic loci; Standard errors are reported for Na, Ar, Ho, He. Differences between observed (Ho) and expected (He) heterozygosity were significant after a t-test.

Table 2 - Download source file (20.61 kB)
Table 2. Membership coefficients (Q values) inferred from STRs and SNPs
Membership coefficients (Q) of parental and hybrid classes inferred from STRUCTURE (STRs) and ADMIXTURE (SNPs) analyses at K=3. Highest membership coefficients for each cluster are shown in bold, whereas BC2 backcrosses lacking a membership component higher than 10% for either of the two species are underlined.

Figures

Figure 1 - Download source file (74.53 kB)
Figure 1. Analysis of Molecular Variance (AMOVA) based on two marker types.
a) AMOVA results for 78 individuals genotyped at 13 STR loci. b) AMOVA results for 71 individuals genotyped at 4,472 SNPs. The pie charts show the proportion of genetic variance partitioned among species (blue), between individuals within species (orange), and within individuals (green).

Figure 2 - Download source file (92.38 kB)
Figure 2. Comparison of genetic differentiation inferred from STR and SNP datasets.
a) Discriminant Analysis of Principal Components (DAPC) based on 78 individuals genotyped at 13 STR loci; b) Discriminant Analysis of Principal Components (DAPC) based on 71 individuals genotyped at 4,472 SNPs. In both analyses, three genetic clusters corresponding to Lepus corsicanus, L. europaeus, and L. timidus are identified. DAPC plots are based on 40 retained principal components and 2 discriminant functions. The percentage of explained variance is indicated on the axes.

Figure 3 - Download source file (209.74 kB)
Figure 3. Identification of the best genetic clustering. Visualization using StructureSelector.
a) STR-based analysis using STRUCTURE, assuming K = 2, under a no-admixture model with independent allele frequencies; b) STR-based analysis using STRUCTURE, assuming K = 2, under an admixture model with independent allele frequencies; c) SNP-based analysis using ADMIXTURE, assuming K = 2; d) STR-based analysis using STRUCTURE, assuming K = 3, under a no-admixture model with independent allele frequencies; e) STR-based analysis using STRUCTURE, assuming K = 3, under an admixture model with independent allele frequencies; f) SNP-based analysis using ADMIXTURE, assuming K = 3.

Figure 4 - Download source file (115.38 kB)
Figure 4. DAPC of genotypes from the three original species and their simulated counterparts.
Discriminant Analysis of Principal Components (DAPC) based on genotypes from three species and 15 classes of simulated hybrids (including F1, BC1, and BC2 clusters), using a) 13 STR loci and b) 4,472 SNPs. In both analyses, three genetic clusters corresponding to Lepus corsicanus, L. europaeus, and L. timidus, as well as clusters corresponding to the F1, BC1, and BC2 hybrid classes, are identified. The number of individuals for each parental species is indicated in brackets; no indication of sample size is provided for the simulated genotypes, as a total of 20 admixed individuals were generated for each hybrid class and combination. The percentage of explained variance is indicated on the axes.

Supplementary Online Material

HYSTRIX
the Italian Journal of Mammalogy

Download source file (134.89 kB)