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RESEARCH ARTICLE

The genetics of the European polecat in the Iberian Peninsula

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Abstract

Volunteer-based roadkill monitoring schemes, including road carcass sampling, can represent considerable advances with respect to classical methods employed in conservation biology. We studied the genetic diversity, structure, and dynamics of the European polecat (*Mustela putorius*) across the Iberian Peninsula. We used samples of road carcasses collected by volunteers because this carnivore is an elusive species otherwise difficult to monitor with standard field protocols. We gathered 238 samples obtained from 2004 to 2022 from 13 different areas (8–31 samples/area). Using microsatellite loci, we identified 4 genetic units with gene flow among 3 of them in the Iberian Peninsula. The genetic variability was steadily low in 1 of the areas (Girona) for all the parameters evaluated. This area is also genetically isolated from the other studied areas. The inbreeding coefficient was significant in the north- and south-Iberia units, and we did not detect a bottleneck signature in any of the 4 genetic units. Future conservation actions should consider the genetic dissimilarity among detected units and elucidate the ecological factors that have led to the observed genetic patterns.

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citizen science, conservation, genetic isolation, genetic reinforcement, inbreeding, population dynamics, roadkill

Wildlife monitoring is essential for conservation, research, and management of biodiversity, including prompt detection of population declines (ENETWILD Consortium et al. 2020). Most recommended approaches to estimate current abundance such as camera arrays or abundance indices from surveys are based on linear transects (ENETWILD Consortium et al. 2020), which cannot track past oscillations in population numbers or connectivity among population units. To identify such population trends and structure at large scales, genetic-based approaches are more suitable (Aylward et al. 2022, Willi et al. 2022, Horreo et al. 2023).

Population-level studies have used variable markers such as microsatellites for exploring patterns including contemporary gene flow (Rodriguez-Castro et al. 2022, Horreo et al. 2023, Morris et al. 2023) or genetic structure (Thomas et al. 2022, Horreo et al. 2023, Larroque et al. 2022) in wildlife populations. These markers are relatively cheap compared to single nucleotide polymorphisms (SNPs), and suitable when DNA is not perfectly conserved (e.g., feces; Horreo et al. 2014), which can easily happen with carcasses. Large-scale geographical approaches usually do not explore population-level parameters because they commonly sample few individuals from a site or population (Davison et al. 2001, Charruau et al. 2011, Peralta et al. 2021, Suzuki et al. 2023). This limitation is especially relevant for mammalian carnivores because of their low population densities due to heterogeneous distribution of their main resources (e.g., food, habitat) in space and time (Macdonald and Johnson 2015, Aylward et al. 2022, Schepens et al. 2023).

The global road network entails negative effects for biodiversity, especially in developing countries (Meijer et al. 2018). For instance, previous research has documented that road density can limit animal dispersal, promote habitat loss, and cause pollution and human access to natural areas (van der Ree et al. 2015, Ibisch et al. 2016, Ascensão et al. 2018). Despite their generally elusive behavior, some taxa (e.g., carnivores) may approach roads in search of resources such as food when traffic slows down at night (Hill et al. 2021), and some are hit by vehicles and killed. Although road-related mortality is a major conservation problem, it also represents a conservation opportunity to gain wide ecological knowledge, especially for scarce and elusive species such as carnivores. Among road effects, roadkills are the most visible to drivers (Bennett 2017, Pinto et al. 2020), with potential for volunteer-based sampling (Shilling et al. 2020). Roadkill monitoring can be instrumental in contributing to the advancement of knowledge in species distributions, population trends, and population dynamics (Schwartz et al. 2020). For example, roadkill trends anticipated the widespread decline of the European hedgehog (*Erinaceus europaeus*) in the United Kingdom (Wembridge et al. 2016) and proved the spread of invasive red foxes (*Vulpes vulpes*) in Tasmania (Caley et al. 2015). Volunteer-based sampling has also been used in genetic studies (Molbert et al. 2023, Vincent Wildlife Trust 2024).

Among mammalian carnivore species, the European polecat (*Mustela putorius*) has one of the highest incidence of road mortality in the Iberian Peninsula (Quiles et al. 2021). This mustelid species is apparently widespread in Portugal and Spain but occurs discontinuously. The population in northeastern Spain (i.e., Girona) has been depauperated for a long time (Palazón et al. 2010, Salvador et al. 2017). The polecat was categorized as near threatened in 2006 (Virgós et al. 2007). More recently, it has been categorized as endangered in Portugal (Santos-Reis et al. 2023) and regionally in the Province of Girona in Spain (Generalitat de Catalunya 2023), although these decisions have been based on expert suggestions rather than on detailed data on their respective trends. Data on polecats are limited because of their low population density, nocturnal behavior, and lack of distinctive signs in the field, which makes the species difficult to monitor systematically (Croose et al. 2018). All these traits make genetic approaches especially suitable to study this mustelid, primarily when based on programs where a network of volunteers collect the samples from road-killed individuals. Although some genetic studies have been done, population genetics with conservation aims have not been carried out in polecats (Croose et al. 2018) except for phylogeographic approaches (Davison et al. 2001, Pertoldi et al. 2006).

The Iberian Peninsula is a mountainous region and the mountain ranges limit gene flow in other vertebrates (e.g., amphibians, Sánchez-Montes et al. 2018; lizards, Horreo et al. 2019) and mountains structure mustelid populations elsewhere (e.g., American badger [*Taxidea taxus*], Kyle et al. 2004; American mink [*Neovison vison*], Zalewski et al. 2009; Eurasian otter [*Lutra lutra*], Pagacz 2016), but mountains have not been demonstrated as a barrier for polecats. Regarding feeding ecology, polecats mainly forage on rabbits (*Oryctolagus cuniculus*) in Mediterranean habitats (Santos et al. 2009), which can lead to high roadkill rates when polecats visit road embankments where rabbits occur high numbers (Barrientos and Bolonio 2009, Barrientos and Miranda 2012, Carmona et al. 2024). As rabbits are widespread in the Iberian Peninsula, sampling road-killed polecats in road verges where rabbits are abundant very likely provides a good coverage of the current distribution of this predator. Rabbit numbers undergo cyclic oscillations owing to the effect of diseases such as myxomatosis or hemorrhagic disease virus (Queney et al. 2000, Delibes-Mateos et al. 2014, Guerrero-Casado et al. 2016). After population declines of their main prey, polecats undergo population declines (Barrientos and Plaza 2016).

Our objective was to explore the population structure and dynamics of the European polecat in the Iberian Peninsula. We predicted that we would find evidence of large mountain ranges acting as barriers to polecat populations on the Iberian Peninsula, that bottleneck signatures in polecat subpopulations would be mainly related to successive population fluctuations of rabbit populations, and that the individuals in Girona would show the lowest values regarding genetic variability.

STUDY AREA

The studied area covered most of the distribution of the species in the Iberian Peninsula, mainly in Spain (Figure 1). The Iberian Peninsula is a landscape-heterogeneous region (581.353 km^2), with mountainous ranges such as the Central System or Cantabrian Mountains reaching >2,000 m above sea level, and 2 extensive plateaus with a mean altitude of 660 m. Land use is dominated by agriculture, livestock farming, and forestry, and >14% of the surface of inland Spain hosts protected natural areas (Ministerio para la Transición Ecológica [MITECO] 2022). Climate is variable across the Iberian Peninsula, with a typical Mediterranean climate in the most continental-influenced area, a maritime climate in the coast, and a mountain climate in high elevations. The Iberian Peninsula has 4 annual seasons: winter (Dec–Mar), spring (Mar–Jun), summer (Jun–Sep), and autumn (Sep–Dec). The average temperature is around 15°C, ranging between 7°C and 24°C on average. Precipitation is mainly concentrated in autumn and winter, with a mean annual value of about 536 mm (Agencia Española de Metereología 2022). The vegetation is also variable across the study area, from the Mediterranean scrublands and forest of the central and southern Spain dominated by holly oak (*Quercus ilex*) and pine tree (*Pinus* spp.) plantations to the Atlantic forests of the north dominated by European beech (*Fagus sylvatica*) and several species of oaks. Mammal diversity is high in the Iberian Peninsula, especially for the mammal carnivore community, which includes ≥10 species of small and medium-sized carnivores.

METHODS

Sampling

Sampling polecats occurred from 2004 to 2022 by ≥80 volunteers (Table S1, available in Supporting Information) from the project Distribución, Ecología y Conservación del Turón en la Península Ibérica (https://www.facebook.com/profile.php?id=100063562179076, accessed 11 Jan 2024). Since the development of the project, several collaboration requests have been made seeking tissue samples (ear or a tuft of hair with skin) from animals found killed on roads, or simply reporting when road-killed or living polecats were located. Volunteers collected 40% of

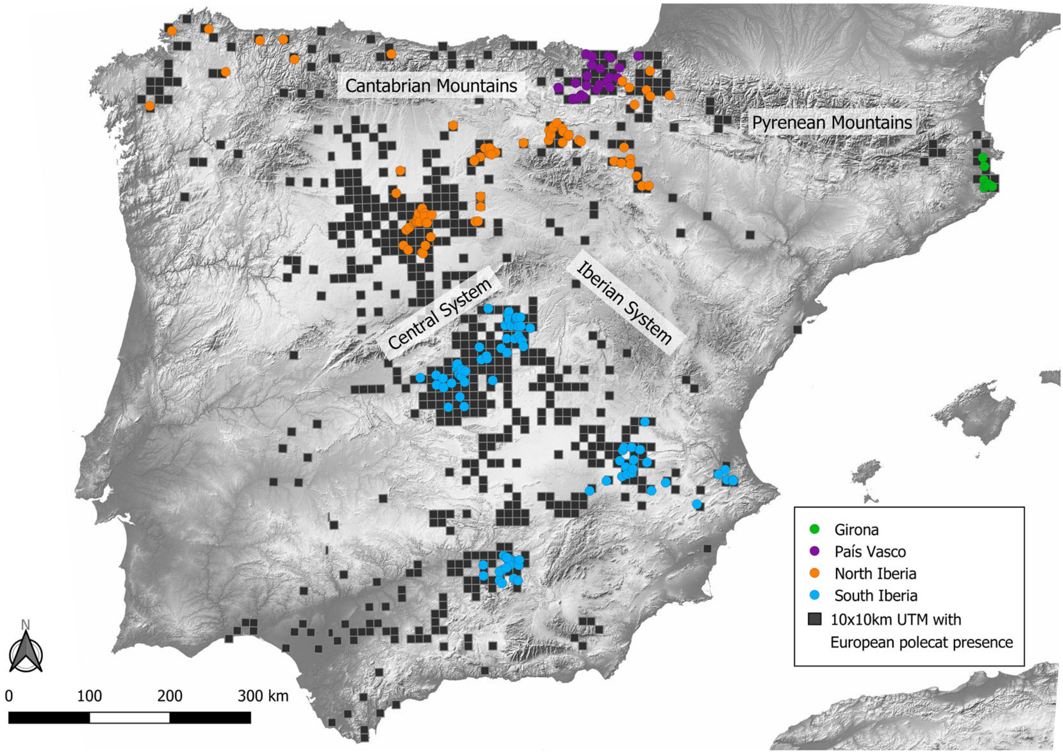

FIGURE 1 Distribution of the sampling points (dots) and inferred genetic units (colored circles) of European polecat individuals ($n = 238$) from the Iberian Peninsula, 2004–2022. The current species distribution, based on the data collected by the project Distribución, Ecología y Conservación del Turón en la Península Ibérica between 2010–2020, is shown 10 × 10-km Universal Transverse Mercator (UTM) squares with presence of the species. Main mountain ranges in the study area are also shown.

the samples (95 out of 238). Other researchers also joined these requests, contributing samples that had been collected for other purposes in the past, amounting for 28% (67 out of 238). We received additional samples from local authorities collected at animal recovery centers, accounting for 10% (23 out of 238). Lastly, we collected some samples ourselves, amounting to 22% (53 out of 238; Table S1).

DNA extraction and microsatellite amplification

Hair and tissue (ear) samples were collected from carcasses directly in the field (sometimes it was not possible to know when the animal died). We stored samples frozen at −20°C in zip bags. We also collected some whole carcasses and froze them until necropsy. From these animals, we obtained blood, muscle samples, or both, and stored them immediately in 0.5-mL ethylenediaminetetraacetic acid (EDTA) tubes (blood) or 1-mL 70% ethanol tubes (muscle). We kept samples refrigerated (4°C) until laboratory analysis. The final pool included 238 individuals from 13 different areas (8–31 samples/area; Table 1; Figure 1). Visual determination of sex was possible in 96 individuals (40%): 66 males and 30 females. Although we respected the province (area hereafter) of origin to try to balance the number of samples (at least from those provinces with more specimens), the genetic grouping was only based on the results of genetic units (K) identified by the software STRUCTURE (Falush et al. 2003). Considering the province of origin also allowed comparations among areas.

TABLE 1 Genetic variability of the European polecat in 13 different areas (N-Vasco includes Biscay and Gipuzkoa) of the Iberian Peninsula for the 2004–2022 period. We show the area and the number of samples (*n*), the number of alleles per locus, the mean number of alleles per locus (Mean Na), the effective number of alleles per locus (Ne), the observed (H_O) and expected (H_E) heterozygosities, and the inbreeding coefficient (F_{IS}). We did not detect any deviations from Hardy-Weingberg equilibrium in any loci or area.

Area (*n*)	MLUT25	Mp2.11	Mp3.2	Mp3.1	Mvi111	Mp07	Mp28	Mp1.12	Mp5.11	Mp36	Mp4.14	Mp13	Mean Na	Ne	Ho	He	F_{IS}
Araba (12)	2	2	2	6	5	5	4	3	4	3	4	3	3.58	2.25	0.56	0.54	−0.04
Albacete (19)	4	4	4	3	3	7	4	5	6	4	3	3	4.33	2.48	0.60	0.59	−0.02
GalAst (8)	3	3	3	5	5	7	4	3	4	2	4	4	3.92	2.73	0.62	0.65	0.04
Girona (10)	1	1	1	3	2	4	3	1	2	4	3	3	2.33	1.67	0.33	0.31	−0.05
N-Vasco (16)	2	2	2	4	4	6	3	2	3	7	3	3	3.42	2.43	0.56	0.51	−0.02
Jaén (29)	3	3	3	5	6	6	5	5	4	5	7	3	4.58	2.34	0.49	0.55	0.10*
Rioja (16)	4	2	2	7	5	7	7	5	4	6	3	5	4.75	3.23	0.58	0.66	0.12
Madrid (31)	3	4	3	5	4	5	6	5	4	5	4	4	4.33	2.61	0.54	0.59	0.09
Navarra (13)	2	3	3	4	6	7	4	5	5	6	5	3	4.42	2.82	0.47	0.59	0.20*
Valencia (8)	4	2	3	4	4	2	5	4	4	4	2	3	3.42	2.55	0.63	0.62	−0.00
Burgos (15)	5	4	2	8	6	7	6	4	4	6	3	5	5.00	3.13	0.69	0.70	−0.03
Toledo (31)	4	3	3	6	5	4	7	4	4	5	5	5	4.58	2.75	0.58	0.62	0.07
Valladolid (30)	4	5	3	7	6	6	5	4	4	5	5	5	4.92	3.12	0.65	0.67	0.02

*P-value < 0.05.

We extracted DNA from all the different sources (hair, muscle, blood) using a standard ammonium acetate protocol (Richardson et al. 2001). Afterwards, we amplified 19 microsatellite loci: MP03, MP07, MP13, MP14, MP22, MP28, MP36, MP40 and MP41 (Ciofi et al. 2012), Mp1.12, Mp1.18, Mp2.11, Mp3.1, Mp3.2, Mp3.18 and Mp4.14 (Costa et al. 2012), Mvi111 (O'Connell et al. 1996), MLUT25 (Cabria et al. 2007), and Mn5.11 (Rodrigues et al. 2011).

We conducted polymerase chain reactions (PCRs) using the DNA amplitools master mix kit (Biotools S.A., Madrid, Spain) following the manufacturer's protocols (final concentrations: MasterMix x1, primers 0.5 μM, DNA between 4 and 40 ng), and with PCR cycling conditions as follows: 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds (54°C for Mvi111, Mp1.12, Mn5.11, Mp1.18 and Mp4.14), 72°C for 30 seconds, and a final extension of 72°C for 10 minutes. We checked PCR amplifications in 2% agarose gels before sequencing with an ABI PRISM 3130XL sequencer in the Genomic Unit of the Universidad Complutense de Madrid. We genotyped microsatellite peaks using GeneMapper 4.0 (Applied Biosystems, Waltham, MA, USA) and binned alleles using tandem 1.09 (Matschiner and Salzburger 2009).

Statistical analyses

We used Genepop on the Web (Raymond and Rousset 1995, Rousset 2008) to test linkage disequilibrium (LD) among loci to discard linked loci before subsequent analyses. We measured the genetic variability of the unlinked loci per area (number of alleles per locus, effective number of alleles per locus, and observed and expected heterozygosities), deviations from Hardy–Weinberg equilibrium and fixation indexes, and their associated *P*-values (with sequential Bonferroni correction for multiple testing; Rice 1989) with GenoDive 2.0b25 (Meirmans and Van Tienderen 2004). We estimated allelic richness and observed and expected heterozygosities using 1,000 permutations to compare them among the inferred genetic units independently of sample sizes with FSTAT version 2.9.4 software (Goudet 2002).

We estimated the number of genetic units (*K*) present in the Iberian Peninsula with the Bayesian approach implemented in STRUCTURE version 2.3.4 (Falush et al. 2003), using a model of admixture, correlated allele frequencies, and the LOCPRIOR model because it detects the presence of weak genetic population structure better than other models (Hubisz et al. 2009). We conducted 10 different runs of 10^5 steps (10% burn-in) for each number of genetic units (*K* range = 1–13). We employed StructureSelector (Li and Liu 2018) for choosing the correct number of genetic units with the model of Puechmaille (2016), the estimators MedMeaK and MedMedK, and a threshold of mean or median membership at which a subpopulation is defined as belonging to a cluster of 0.65 as an intermediate value (author recommendation ranged 0.5–0.8).

We estimated contemporary gene flow with the number of migrants per generation (*N*m) among the inferred genetic units with Genepop on the Web (Raymond and Rousset 1995, Rousset 2008), and the presence of inbreeding and genetic bottlenecks using Arlequin version 3.11 (Excoffier et al. 2005), and BOTTLENECK version 1.2.02 (Piry et al. 1999), respectively. Settings for BOTTLENECK included the 2-phase model (TPM, variance = 30, proportion of stepwise mutation model [SMM] = 70%) and Wilcoxon signed-rank test (10,000 interactions), as commonly accepted for microsatellite data (Horreo et al. 2023).

RESULTS

With the DNA extracted from all samples, from the 19 amplified loci, only 12 resulted in correct PCR amplification and consistent peak patterns, with a 90% success in PCR amplification plus genotyping. The Mp14 locus did not amplify correctly by PCR, Mp02 and Mp14 were monomorphic, and Mp40, Mp1.18, Mp3.18, and Mp22 had inconsistent peak patterns. We did not detect linkage disequilibrium (thus loci giving the same information) in global

TABLE 2 Genetic diversity in European polecat males (*n* = 66) and females (*n* = 30) sampled in the Iberia Peninsula, 2004–2022. We show the number of alleles per locus (Na), the effective number of alleles per locus (Ne), and the observed (H_O) and expected (H_E) heterozygosity.

	Na		Ne		H_O		H_E	
Locus	Female	Male	Female	Male	Female	Male	Female	Male
MLUT25	3	5	2.58	2.72	0.57	0.58	0.62	0.64
Mp2.11	4	4	1.91	2.21	0.44	0.49	0.49	0.55
Mp3.2	3	4	1.63	1.60	0.26	0.29	0.40	0.38
Mp3.1	7	8	2.93	4.84	0.63	0.72	0.67	0.80
Mvi111	6	7	3.99	4.27	0.69	0.72	0.76	0.77
Mp07	8	7	3.61	5.97	0.62	0.62	0.74	0.84
Mp28	8	6	3.56	3.49	0.69	0.60	0.73	0.72
Mp1.12	4	6	3.27	3.91	0.61	0.55	0.71	0.75
Mn5.11	4	5	2.56	2.61	0.59	0.40	0.62	0.62
Mp36	6	7	2.79	2.53	0.52	0.61	0.66	0.61
Mp4.14	7	11	2.69	2.44	0.62	0.55	0.64	0.59
Mp13	5	5	2.91	3.07	0.55	0.57	0.67	0.68
Overall	5.42	6.25	2.87	3.31	0.57	0.56	0.64	0.66

analyses or across areas. Thus, the final dataset included MLUT25, Mp2.11, Mp3.2, Mp3.1, Mvi111, Mp07, Mp28, Mp1.12, Mp5.11, Mp36, Mp4.14, and Mp13. None of these 12 loci deviated from Hardy–Weinberg equilibrium in any of the areas (Table 1). The genetic variability of the studied areas included a mean number of alleles per locus (Na) between 2.33 (Girona) and 5.00 (Burgos), an effective number of alleles per locus (Ne) ranged from 1.67 (Girona) – 3.23 (Rioja), an observed heterozygosity (Ho) ranged from 0.33 (Girona) – 0.69 (Burgos), and an expected heterozygosity (He) between 0.31 (Girona) and 0.70 (Burgos). These values were similar between sexes (Table 2), with no differences in Ne (analysis of variance [ANOVA] $F = 1.12$, $P = 0.31$), Ho ($F = 0.01$, $P = 0.92$), or He (ANOVA $F = 0.20$, $P = 0.66$). Inbreeding coefficients (F_{IS}) were significant in Jaén and Navarra (Table 1).

The number of genetic units (*K*) identified by STRUCTURE was 4 using both estimators (MedMeaK and MedMedK) of StructureSelector (Figure 2; Figure S1, available in Supporting Information). These units were N-Vasco and Araba (País Vasco genetic unit hereafter); Girona; GalAst, La Rioja, Navarra, Burgos, and Valladolid (north-Iberia); and Madrid, Toledo, Valencia, Albacete, and Jaen (south-Iberia). Genetic variability was lower in Girona than in the other genetic clusters for all parameters, and the inbreeding coefficient was significant in the north- and south-Iberia clusters, with no bottleneck presence detected in any of the clusters (Table 3). We detected significant genetic differences (F_{ST} values) among clusters and evidence of gene flow (*N*m), except in the case of Girona, with *N*m values always <1 (Table 4).

DISCUSSION

We provide information on the genetic variability, population structure, and dynamics of the European polecat in the Iberian Peninsula. Some potential biases inherent to roadkill sampling could be present including if bold animals or males with larger home ranges (males were twice as abundant in our sample; Table 2) cross the road more frequently, or if slower animals are easily killed. None of these potential biases seem to affect population genetics

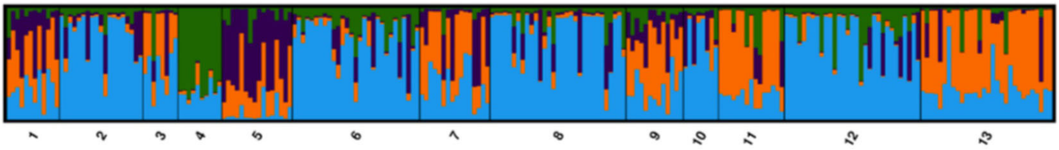

FIGURE 2 Structure plot ($K = 4$) of European polecat individuals ($n = 238$) from the Iberian Peninsula in the 2004 to 2022 period. Areas: 1 = Araba, 2 = Albacete, 3 = GalAst, 4 = Girona, 5 = N-Vasco, 6 = Jaén, 7 = Rioja, 8 = Madrid, 9 = Navarra, 10 = Valencia, 11 = Burgos, 12 = Toledo, and 13 = Valladolid. Colors of the genetic clusters are as follows: orange for north-Iberia, light blue for south-Iberia, green for Girona, and violet for País Vasco.

TABLE 3 Genetic variability of the inferred genetic units of European polecat in the Iberian Peninsula, 2004–2022. We show the number of samples (*n*), allelic richness (AR), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), and the *P*-value for bottleneck analyses (*B*).

Genetic unit (*n*)	AR	H_O	H_E	F_{IS}	*B*
País Vasco (28)	2.45	0.54	0.53	0.05	0.18
Girona (10)	1.80	0.33	0.29	−0.05	0.64
North-Iberia (82)	3.02	0.62	0.67	0.08*	0.52
South-Iberia (118)	2.72	0.56	0.61	0.10*	0.73

*P-value < 0.05.

TABLE 4 Differentiation index (F_{ST}; below diagonal) and gene flow (*N*m; above diagonal) values between the European polecat genetic units of the Iberian Peninsula, 2004–2022.

F_{ST}/*N*m	País Vasco	Girona	North-Iberia	South-Iberia
País Vasco	–	0.22	2.72	1.61
Girona	0.27*	–	0.61	0.40
North-Iberia	0.06*	0.23*	–	4.91
South-Iberia	0.09*	0.25*	0.05*	–

*P-value < 0.001.

studies, so this approach was indeed suitable for exploring genetic structure and identifying which population units deserve special conservation efforts because of their reduced genetic variability.

The genetic variability of European polecats was not sex-dependent, and was similar to that found in northern and western European (Na = 2–10; Pertoldi et al. 2006), central and northern European (Na = 2–8; Cabria et al. 2011) or eastern European (Na = 4–6; Szatmári et al. 2021) populations but higher than those in France (Lodé 2001), Denmark (Moller et al. 2004), Portugal, and Great Britain (Costa et al. 2012, 2013). This suggests this species is not genetically threatened in the Iberian Peninsula at present (except in Girona).

We identified 4 major genetic units in the Iberian Peninsula: País Vasco, Girona, north-Iberia, and south-Iberia (Figure 1). These units could be structured by the 2 central mountain systems of the Iberian Peninsula: the Central System (dividing north-Iberia and south-Iberia units) and the Iberian System (despite not exceeding 2,000 m, it divides Girona from the other 3 Iberian units). Several considerations led us to reject this hypothesis of isolation by mountain systems. First, we observed a high rate of gene flow (*N*m = 4.91) through the first mountain range, dividing north- and south-Iberia units. Second, other mountain ranges in our study region do not seem to produce division within such units, even with a similar altitude (e.g., the Cantabrian Mountains located within the north-Iberia unit). Finally, European polecats are not scarce in montane pinewoods (<2,000 m) in the Iberian System (Virgós 2003). Thus, the complete isolation of the northeastern unit (gene flow between Girona and the other 3 units is <1) might be due to other factors beyond those merely orographic. These factors (climatic, land uses, historical events, or any other) remain unstudied. In addition, the north-Iberia and País Vasco units are genetically distinct, although they are very close to each other, with no major geographical barriers that would prevent gene flow (Figure 1). This could potentially be explained by gene flow between País Vasco and France or even hybridization with European mink (*Mustela lutreola*) because such hybrids have been reported in the area (Ceña et al. 2003). Unfortunately, French populations or European mink individuals have not been sampled in this study, so we cannot confirm or rule out these explanations, and more studies would be necessary in this regard.

The polecat genetic unit of Girona shows not only the lowest genetic variability values of the 4 units (Table 3) but also extremely reduced values (Table 1), likely because it has been a depauperate population for a long time (Palazón et al. 2010, Salvador et al. 2017). Our results also show that its isolation from the rest of the Iberian genetic units (*N*m very low; Table 4) prevents a potential natural genetic rescue from the other Iberian genetic units. Contrary to what is expected in these cases of reduced genetic variability, inbreeding depression has not been detected in Girona; thus, current individuals may not be close relatives. Again, potential gene flow from France could be acting here, avoiding inbreeding in this area.

We did not detect bottlenecks in any of the genetic units (Table 3). Thus, population crashes of the polecat's main prey due to diseases (Queney et al. 2000, Delibes-Mateos et al. 2014, Guerrero-Casado et al. 2016) or any other historical events leading to reductions in polecat abundance and fitness seem not to have affected the population dynamics of this carnivore in the Iberian Peninsula. This could be due to 4 non-exclusive causes: these crashes were short-lived (Keller et al. 2001, McEachern et al. 2011); numbers of individuals stayed high enough during population reductions (if any; Queney et al. 2000); the units are structured in a meta-population system where subpopulations undergoing crashes receive rescuers from other areas (Keller et al. 2001, McEachern et al. 2011); or hybridization with ferrets, as proposed in Great Britain (Costa et al. 2013), reducing polecat fitness. Despite the lack of bottleneck evidence, we detected inbreeding signals in the north-Iberia and south-Iberia units (Table 3), corresponding to Navarra and Jaén areas, respectively (Table 1). In both cases, we did not detect a reduction of genetic variability, and thus these areas do not seem to be threatened; however, we recommend continuous monitoring of both.

Our results indicate that the northeastern genetic unit of the Iberian Peninsula (i.e., Girona) is potentially threatened because reduced genetic variability is among the major threats for species conservation (Horreo et al. 2011*a*,*b*, 2023). Given this concern, the Girona unit should be protected, and specific conservation measures implemented. This unit showed no evidence of bottleneck or inbreeding, which could be, as commented above, due to potential introgression from French polecats arriving from the north (Figure 1). Polecats in France are still trapped as a perceived pest species (Croose et al. 2018). Population reinforcement (Taylor et al. 2017), if aimed to increase gene flow, may not be effective (Pertoldi et al. 2006), and genetic rescue via translocations can hinder local adaptation or produce maladaptation of those populations that are intended to be protected if the latter genetically differ from donor populations (e.g., other Iberian units), which could potentially lead to reduced population fitness (Kirkpatrick and Barton 1997, Lenormand 2002). European polecats are adapted to different land cover types across their range (Croose et al. 2018); thus, their genetic characteristics must be different. They usually occur in marshlands and flooded meadows in Girona (Palazón et al. 2010) and neighboring France (Lodé 1993, 1994), where they mainly feed on voles and anurans (Lodé 1993, 1994). In contrast, in Mediterranean areas of Spain and Portugal, the species selects dry crops with a high diversity of habitat characteristics, scrublands of yellow broom (*Retama sphaerocarpa*) and alfa grass (*Stipa tenacissima*), and riparian vegetation, often in non-permanent water courses (Mestre et al. 2007, Barrientos and Bolonio 2009, Barrientos and Miranda 2012, Carmona et al. 2024), where rabbits, its main prey here (Santos et al. 2009), are particularly abundant. Before accomplishing any translocation or stocking (which can also encompass artificial selection; Horreo et al. 2008), the causes leading to population decline must be clearly identified and reversed or population reinforcement will fail (Fischer and Lindenmayer 2000). In this sense, several potential factors affecting polecat populations have been suggested, from habitat alteration, prey scarcity, poisoning or human-related mortality to hybridization and competition with other mustelids (Barrientos 2015, Croose et al. 2018, Sainsbury et al. 2018). To the best of our knowledge, there is no scientific evidence on how changes in such factors could potentially contribute to increasing the viability of the polecat units in the Iberian Peninsula. Thus, more ecological studies are needed before appropriate conservation measures can be suggested and implemented.

CONSERVATION IMPLICATIONS

This work, describing population genetics of the European polecat in the Iberian Peninsula, shows this species is structured in 4 genetic units covering a broad geographical area, with high gene flow except for Girona. We recommend that these units should be treated independently, and gene flow among them conserved. The genetic variability of the species is like other regions throughout its range. In relation to its conservation in the Iberian Peninsula, Girona must be a priority for conservation because of its genetic erosion and isolation from the other units, but population reinforcement with animals from other Iberian genetic units is discouraged.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

ETHICS STATEMENT

Samplings were in their majority taken from animals found killed on roads. Other samples, which came from researchers contributing samples that had been collected for other purposes, or from authorities (collected at animal recovery centers) were authorized and conducted under regional authorities’ permissions and comply with the Spanish (RD 53/2013) and European (2010/63/UE) guidelines for animal wildlife research.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the authors upon request.

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SUPPORTING INFORMATION

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