

Hares in Corsica: high prevalence of and hybridization with introduced and

Christian Pietri, Paulo Célio Alves, José Melo-Ferreira

► **To cite this version:**

Christian Pietri, Paulo Célio Alves, José Melo-Ferreira. Hares in Corsica: high prevalence of and hybridization with introduced and. *European Journal of Wildlife Research*, Springer Verlag, 2010, 57 (2), pp.313-321. <10.1007/s10344-010-0430-9>. <hal-00625934>

HAL Id: hal-00625934

<https://hal.archives-ouvertes.fr/hal-00625934>

Submitted on 23 Sep 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Hares in Corsica: high prevalence of *Lepus corsicanus* and hybridization with introduced *L. europaeus* and *L. granatensis*

Christian Pietri · Paulo Célio Alves · José Melo-Ferreira

Received: 26 May 2010 / Revised: 31 August 2010 / Accepted: 1 September 2010 / Published online: 23 September 2010
© Springer-Verlag 2010

Abstract The Italian hare, *Lepus corsicanus*, was first described in Corsica more than 100 years ago, but the knowledge on the status of the species in this island remains scarce. Moreover, frequent introductions of thousands of individuals from other hare species, namely *Lepus europaeus* and *Lepus granatensis*, into Corsica are known to have occurred and an updated assessment of the prevalence of *L. corsicanus* in Corsica is therefore of utmost importance. Here, to estimate the relative prevalence of the hare species present in Corsica, we conducted a molecular analysis on 67 samples collected by hunters between 2002 and 2007 in 36 Corsican communes. Sequencing of portions of the nuclear gene transferrin and of the control region of the mitochondrial DNA allowed classifying most of the collected samples as belonging to *L. corsicanus* (70.1%). Of the sampled Corsican communes, 86.1% contained this species, while only in 11.1%, *L. europaeus* was present. Three of the analyzed specimens

showed an inconsistent molecular assignment between markers suggesting a hybrid origin: *L. corsicanus* × *L. europaeus*, *L. corsicanus* × *L. granatensis*, and *L. europaeus* × *L. granatensis*. The first two cases of hybridization had never been described in nature, even in studies focusing on hares from Italy where *L. corsicanus* and *L. europaeus* are often sympatric. These results stress the real risk of corrosion of the native gene pool of *L. corsicanus* via hybridization with introduced species. We highlight the need of urgently rethinking the management plan of hare populations in Corsica.

Keywords Conservation · Corsica · Italian hare · Hybridization · *Lepus* · Introduced species

Introduction

The taxonomic status of the Italian hare, *Lepus corsicanus*, has historically been a source of major controversy. This species was first described in 1898 in Corsica, a French island close to the Italian coast, by De Winton who suggested that the species could also be found in Central and Southern Italy. Later, it was instead considered to be a subspecies of *Lepus europaeus*, the European brown hare (Miller 1912; Ellerman and Morrison-Scott 1951; Toschi 1965). However, recent studies on morphometrics (Palacios 1996) and genetics (Pierpaoli et al. 1999) identified the Italian hare as a distinct entity, re-establishing its specific status despite extensive similarity to the Iberian broom hare, *Lepus castroviejo* (Palacios 1996; Alves et al. 2008a).

Lepus is thought to have been originally brought into Corsica from mainland Italy for hunting purposes before the fifteenth century (Vigne 1999), but the current status of

Communicated by C. Gortázar

C. Pietri
Fédération Départementale des Chasseurs de Haute-Corse
(FDCHC),
Résidence Nouvelle-Corniche, St Joseph,
20600 Bastia, France

P. C. Alves · J. Melo-Ferreira (✉)
CIBIO, Centro de Investigação em Biodiversidade de Recursos
Genéticos, Universidade do Porto,
Campus Agrário de Vairão,
4495-661 Vairão, Portugal
e-mail: jmeloferreira@mail.icav.up.pt

P. C. Alves
Departamento de Biologia da Faculdade de Ciências,
Universidade do Porto,
R. Campo Alegre s/n,
4169-007 Porto, Portugal

the genus in the island is largely unknown (Dubray et al. 1984; Pietri 2007). In the beginning of the twenty-first century, Angelici and Luiselli (2001) assessed the distribution of *L. corsicanus* in peninsular Italy and Sicily, concluding that the species was still present in these regions but stressing that, in Italy, the densities of the species have been strongly declining over the years. No data were however collected from Corsica in this work. Some studies have later identified *L. corsicanus* as the sole species present in Corsica, but were essentially focused in limited areas of the island (Scalera and Angelici 2003; Riga et al. 2003; Suchentrunk et al. 2006). Pierpaoli and Randi (2005) have also identified this species in specimens collected in Corsica between 1999 and 2001, but no details on the location or the number of specimens studied were provided. Although these studies suggested that *L. corsicanus* was the sole hare species present in Corsica, it is known that hunting associations introduced thousands of hares from different species in the region up to this day: *L. europaeus* all over the island, and the Iberian hare, *Lepus granatensis*, in its southern part (Corse-du-Sud). While in northern Corsica, restocking with *L. europaeus* stopped in 1995, these procedures continue in the southern region of the island. Also, the release of several hundreds of *L. granatensis* individuals of Spanish origin in Corse-du-Sud was done over the course of many years, from 1984–2000 (Pietri 2007). Although these practices may be a threat to local populations of *L. corsicanus*, no assessment of the prevalence of each of the hare species occurring in Corsica has been done so far.

In this work, we intend to assess which hare species are present in Corsica and which of these prevail in the island, given the long history of multi-species restocking. We use a genetic approach to identify the species present in hunting bags across Corsica. Our results indicate that *L. corsicanus* may predominate in the island, but that *L. europaeus* and perhaps *L. granatensis* are also present. We furthermore show strong evidences that these species are hybridizing in the region and suggest the urgent development of effective management plans that allow preserving the gene pool of *L. corsicanus*.

Methods

Between 2002 and 2007, hare hunters in Corsica were contacted by hunting federations of Haute-Corse and Corse-du-Sud. For each hare harvested, the hunters kept a piece of muscle along with information about its capture, such as geographic area (commune) and date (Table 1). After collection, tissues were placed in 90% ethanol for storing. In addition, some tissue samples were collected from hare carcasses for which the cause of death was the

European Brown Hare Syndrome virus (EBHS) or road kill. These samples were preserved and stored using the same procedure. Specimens recently introduced in Corsica are tagged, and we did not include these marked individuals in our sample, therefore restricting our analyses to resident specimens occurring in the Island.

Table 1 Origin of the samples used in this study (codes are those in Fig. 1)

Commune	Code	Sample size	Origin
Haute-Corse (2B)			
Aghione	A	1	Hunting
Albertacce	B	2	Hunting
Aleria	C	3	Hunting
Antisanti	D	1	Hunting
Bigorno	E	1	Hunting
Castello di Rostino	F	1	Road kill
Castifao	G	1	EBHS virus
Chiatra	H	1	Hunting
Corte	I	1	Hunting
Erbajolo	J	1	Hunting
Gavignano	K	2	EBHS virus
Gavignano	K	1	Hunting
Ghisonaccia	L	3	Hunting
Giuncaggio	M	1	Hunting
Lento	N	1	Hunting
Linguizzetta	O	2	Hunting
Linguizzetta	O	1	EBHS virus
Lozzi	P	1	Hunting
Lumio	Q	4	Hunting
Moltifao	R	1	Hunting
Morosaglia	S	1	Hunting
Murato	T	2	Hunting
Olmeta di Tuda	U	1	Hunting
Omessa	V	4	Hunting
Pietralba	W	1	Hunting
Pieve	X	1	Hunting
Pioggiola	Y	1	Hunting
Santo Pietro di Tenda	Z	1	Hunting
Sermano	Aa	1	Hunting
Tallone	Ab	1	EBHS virus
Corse-du-Sud (2A)			
Calcatoggio	2	1	Hunting
Cargese	A	1	Hunting
Casaglione	B	2	Hunting
Figari	C	15	Hunting
Sarrola Carcopino	D	1	Hunting
Valle-di-Mezzana	E	1	Hunting
Vico	F	1	Hunting
Zicavo	G	1	Hunting

Total genomic DNA was extracted from the collected tissues using standard high-salt methods similar to those described by Sambrook et al. (1989). Amplification of fragments of the nuclear gene transferrin (TF) and of the mitochondrial DNA control region (CR) was performed using the polymerase chain reaction (PCR) conditions and primers as described by Alves et al. (2003) and Melo-Ferreira et al. (2007), respectively. For each specimen, sequences were obtained following the ABI PRISM BigDye Terminator Cycle Sequencing 3.1 (Applied Biosystems) standard protocol using the forward and/or the reverse PCR primers.

Sequences were visually inspected and then aligned using CLUSTAL W (Thompson et al. 1994). For the CR, assignment of haplotypes to the correspondent specific lineage was done by determining a neighbor-joining phylogeny using Mega v4.0 (Tamura et al. 2007) and the Tamura–Nei model of sequence evolution, which is appropriate for describing the pattern of evolution of mtDNA control region sequences (Tamura and Nei 1993). Sequences previously published in GenBank (Pierpaoli et al. 1999; Melo-Ferreira et al. 2007; Alves et al. 2008a) were

used as controls for identifying specific clades (see Table 2). For the TF, the allelic phase was determined using the program PHASE 2.1.1 (Stephens et al. 2001; Stephens and Scheet 2005). Three replicate runs were performed, consisting of a burn-in of 100 iterations followed by 1,000 main iterations, each consisting of ten steps through the Markov chain. Given the low level of genetic diversity found in this marker, the relationships among the detected alleles were reconstructed using a median-joining network (Bandelt et al. 1999) as implemented in the computer program Network v4.51 (www.fluxus-technology.com). Again, sequences with known species provenance available in GenBank were included (see Table 2). Basic genetic diversity indices were estimated using Arlequin 3.11 (Excoffier et al. 2005).

In order to calculate the 95% confidence intervals (CI) for the frequencies and proportions of each species found in our sample, we assumed binomial and Poisson distributions for proportional data on hares and communes as suggested by Scherrer (1984). The 95% CI estimated with the “binom.exact” and “pois.exact” functions of the “epitools” library (Aragon 2008) in R (R Development Core Team 2008).

Table 2 List of sequences downloaded from GenBank with known species assignment for both the control region and the transferrin fragments

Code	Species	Location	Accession number	Reference
Control region (mtDNA)				
cor1	<i>L. corsicanus</i>	Central Italy	AF157424	Pierpaoli et al. 1999
cor2	<i>L. corsicanus</i>	Central Italy	AF157426	Pierpaoli et al. 1999
cor3	<i>L. corsicanus</i>	South Italy	AF157422	Pierpaoli et al. 1999
cor4	<i>L. corsicanus</i>	South Italy	AF157423	Pierpaoli et al. 1999
cor5	<i>L. corsicanus</i>	Sicily	AF157417	Pierpaoli et al. 1999
cor6	<i>L. corsicanus</i>	Sicily	AF157421	Pierpaoli et al. 1999
eur1	<i>L. europaeus</i>	Macedonia	AY466851	Kasapidis et al. 2005
eur2	<i>L. europaeus</i>	Bulgaria	AY466838	Kasapidis et al. 2005
eur3	<i>L. europaeus</i>	Germany	DQ469661	Stamatis et al. 2009
eur4	<i>L. europaeus</i>	Austria	DQ469658	Stamatis et al. 2009
eur5	<i>L. europaeus</i>	Macedonia	AY466810	Kasapidis et al. 2005
eur6	<i>L. europaeus</i>	Greece	AY466817	Kasapidis et al. 2005
eur7	<i>L. europaeus</i>	Turkey	DQ469704	Stamatis et al. 2009
eur8	<i>L. europaeus</i>	Greece	DQ469690	Stamatis et al. 2009
gra1	<i>L. granatensis</i>	Iberian Peninsula	AY103532	Fickel et al. (unpublished)
gra2	<i>L. granatensis</i>	Iberian Peninsula	AY103533	Fickel et al. (unpublished)
gra3	<i>L. granatensis</i>	Iberian Peninsula	AY103534	Fickel et al. (unpublished)
ocu	<i>O. cuniculus</i>	–	AJ001588	Gissi et al. 1998
Transferrin (nuclear DNA)				
Lcors1	<i>L. corsicanus</i>	Central Italy	AY176270	Alves et al. 2003
Lcors2	<i>L. corsicanus</i>	Corsica	EU196168	Alves et al. 2008a
Lass	<i>L. europaeus</i>	Austria	AY176267	Alves et al. 2003
Nav47	<i>L. europaeus</i>	Spain	AY176264	Alves et al. 2003
Alj107	<i>L. granatensis</i>	Portugal	AY176250	Alves et al. 2003
Alj108	<i>L. granatensis</i>	Portugal	EU196169	Alves et al. 2008a

Results

Hunters provided muscular tissues from 61 non-marked hares during the hunting season, 38 of which came from Haute-Corse and 23 from Corse-du-Sud. Six more samples were obtained from hares that died of EBHS or were killed on the road. The geographic distribution of sampled specimens was heterogeneous (Fig. 1 and Table 1): it concerned 28 communes in Haute-Corse and eight in Corse-du-Sud, where the commune of Figari represents 65% ($n=23$) of collected samples for the department.

The CR sequences produced an alignment of 474 bp of which 105 were polymorphic forming 15 different haplotypes (CR1 to CR15). One haplotype (CR1) was clearly predominated over the others, containing more than half of the sequenced specimens (found in 37 individuals). The nucleotide diversity of all obtained CR sequences was high (5.3%), suggesting that different specific lineages might have been sampled in this study. For the reconstruction of the phylogenetic relationships among the obtained CR

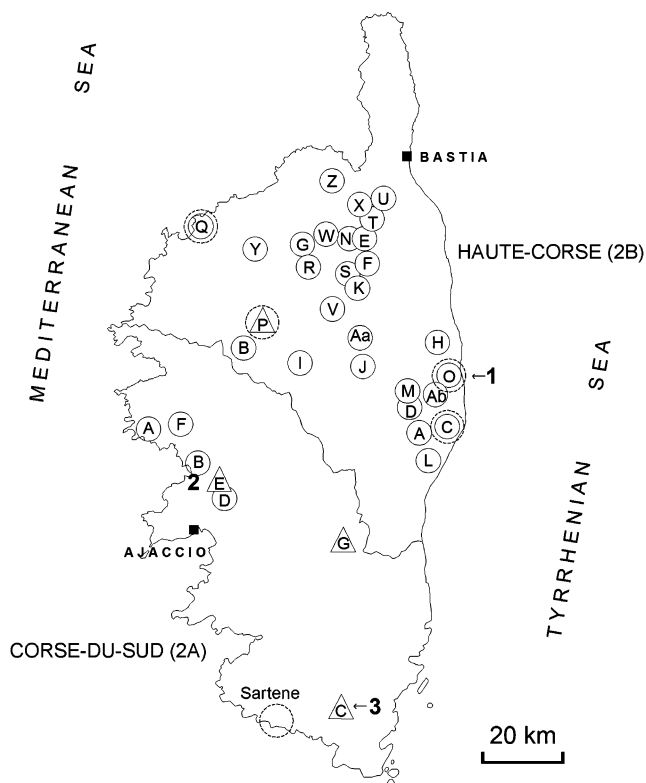


Fig. 1 Corsican communes with *Lepus* sampled in this work. Species identification based on the sequencing of mtDNA control region and nuclear transferrin is indicated (each symbol is placed in the center of the commune, the circle represents *L. corsicanus*, and the triangle represents *L. europaeus*). See Fig. 2 for the assignment of haplotypes to species and Table 1 for commune names and sample sizes. The hybrids found were 1, *L. corsicanus* × *L. europaeus*, 2, *L. corsicanus* × *L. granatensis*, and 3, *L. europaeus* × *L. granatensis*. *L. corsicanus* was already known to be present in areas with spotted line circles (Riga et al. 2003; Scalera and Angelici 2003; Suchentrunk et al. 2005).

haplotypes, we restricted the analysis to 257 bp to allow the alignment with sequences with known specific source we obtained from GenBank (Table 2). The obtained neighbor-joining tree clearly splits the 15 haplotypes into three clades, each corresponding to the lineage of three distinct species: CR1 to CR5 in the clade of *L. corsicanus*, CR6 to CR13 in that of *L. europaeus*, and CR14 and CR15 in that of *L. granatensis* (Fig. 2a).

For the TF, 357 bp were sequenced. The application of the Bayesian statistical method implemented in PHASE allowed identification of seven different alleles (TF1 to TF7; Fig. 2b). Again, one allele was found to be predominant over the others (TF1 was present in 49 of the 67 analyzed specimens). The allelic pairs were all determined with 100% of probability, except in one case in which the most probable pair was determined with 64% (since it was later shown that the position with doubtful phase determination was polymorphic intraspecifically—only in *L. europaeus*—and thus, did not influence the assignment of the alleles to the species, this phase call was kept in the remaining analyses). The organization of the haplotypes in the median-joining network that included sequences with known specific provenance (Table 2) revealed that the seven alleles could easily be assigned to each of three species: TF1 grouped with *L. corsicanus*, TF2 to TF6 with *L. europaeus*, and TF7 with *L. granatensis* (Fig. 2b). All sequences obtained in this study were deposited in GenBank (accession numbers HQ174270–HQ174291).

The cross-examination of the results allowed verifying that in 64 out of the 67 analyzed individuals, the species assignment determined using the CR was confirmed by the sequencing of the TF (Table 3). *L. corsicanus* was the most frequent among the sampled hares, with 70.1% (95% CI, 57.7–80.7%; $n=67$) in the total sample, and 95.5% (95% CI, 68.8–100.0%; $n=44$) in the sample of Haute-Corse department. The predominance of *L. corsicanus* in these samples is even more visible in the commune distribution: the species is present in 86.1% (95% CI, 58.5–100%; $n=36$) of the communes studied as opposed to only 11.1% (95% CI, 3.0–28.4%; $n=36$) of communes with *L. europaeus* present. The high prevalence of *L. corsicanus* in an overwhelming majority of communes is quite visible in Haute-Corse where 96.4% (95% CI, 63.5–100.0%; $n=28$) of communes with hares have the species. The 31 communes where the presence of the Italian hare was demonstrated are detailed in Fig. 1 and Table 1. The brown hare was identified in the communes of Lozzi (2B in Fig. 1) as well as Figari, Valle-di-Mezzana, and Zicavo (2A in Fig. 1).

In three cases, an incongruence between the identification based on each molecular marker was observed suggesting a hybrid origin for those individuals: (1) CR assigned to *L. granatensis* and the TF to *L. europaeus* in

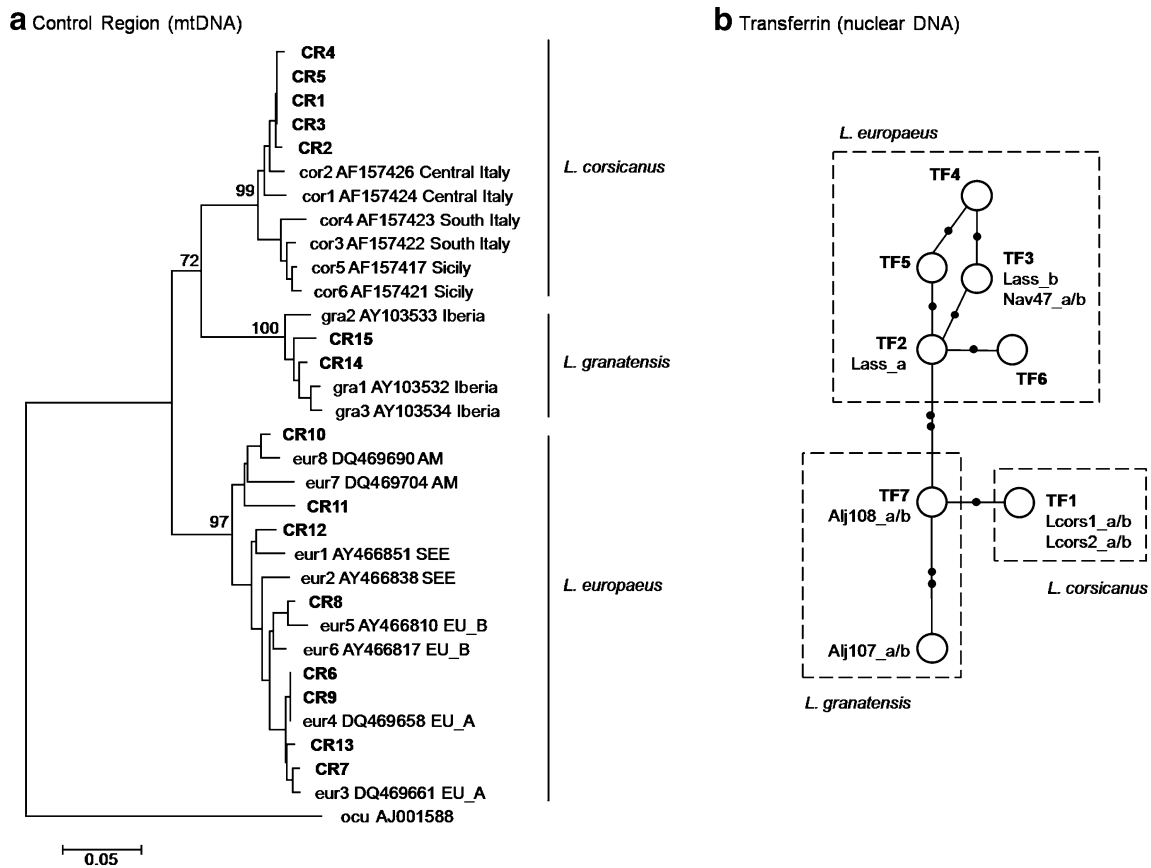


Fig. 2 a Phylogenetic relationships derived from a neighbor-joining analysis of the mtDNA control region haplotypes sequenced the studied hare specimens from Corsica (CR1 to CR15) and including sequences downloaded from GenBank with known specific source: sample location and Accession Numbers are shown next to the tips of the tree, except for the *L. europaeus* sequences for which the mtDNA haplogroup is indicated. *AM* Anatolian/Middle Eastern type, *EU*

European type, *SEE* Southeastern European type (from Stamatis et al. 2009). **b** Median-joining network reconstructed using the obtained transferrin allelic sequences (TF1 to TF7) including some with known species assignment downloaded from GenBank (from Alves et al. 2003 and 2008a, b); dots indicate mutations. See Table 2 for a detailed description of the sequences obtained from the GenBank database and Table 3 for sample sizes

one hare from Figari, (2) CR assigned to *L. europaeus* and TF to *L. corsicanus* in one specimen from Linguizzetta, and (3) CR assigned to *L. granatensis* and the TF presenting two different alleles, one assigned to *L. granatensis* and the second assigned to *L. corsicanus* in an individual from Calcatoggio (see Fig. 1 and Table 3).

Discussion

The current knowledge on the species of hares occurring in Corsica is very scarce and somewhat controversial. This lack of knowledge, coupled with data supporting recent introductions of thousands on individuals from two non-native hare species into the island for hunting purposes, motivated our attempt to develop a molecular method that would allow the specific assignment of hare specimens collected from hunting bags. The developed methodology proved to be effective in determining which species prevails on the island, and can in the future be

easily applied to monitor the populations of hares from Corsica.

Although the obtained sampling was not homogeneous (several hare hunters did not participate in the study in Corse-du-Sud; see Fig. 1), in Haute-Corse all the regions with hare hunters known to the hunters’ associations were represented in our screening.

Prevalence of *L. corsicanus* in the hunting bags of hares in Corsica

One of the most striking results obtained in this study was the clear predominance of *L. corsicanus* among all sampled hares. In four communes studied in Corse-du-Sud, as well as in 27 other communes of Haute-Corse, the Italian hare was identified. It is therefore certain that *L. corsicanus* in Corsica is not restricted to the five locations previously described (Riga et al. 2003; Scalera and Angelici 2003; Suchentrunk et al. 2005). Our results show the presence of the Italian hare in at least 32 communes, if we include the

Table 3 Combination of control region haplotypes and transferrin genotypes detected in the 67 hares analyzed from Corsica, and respective sample sizes (see also Fig. 1)

Control region Haplotype	Transferrin		<i>n</i>	Species diagnosis
	Haplotype	Genotype		
CR1	TF1	TF1	37	<i>L. corsicanus</i>
CR2	TF1	TF1	4	<i>L. corsicanus</i>
CR3	TF1	TF1	4	<i>L. corsicanus</i>
CR4	TF1	TF1	1	<i>L. corsicanus</i>
CR5	TF1	TF1	1	<i>L. corsicanus</i>
CR6	TF2	TF2	2	<i>L. europaeus</i>
CR6	TF2	TF3	5	<i>L. europaeus</i>
CR6	TF3	TF3	1	<i>L. europaeus</i>
CR6	TF4	TF4	2	<i>L. europaeus</i>
CR7	TF4	TF5	1	<i>L. europaeus</i>
CR8	TF3	TF3	1	<i>L. europaeus</i>
CR9	TF3	TF4	1	<i>L. europaeus</i>
CR10	TF2	TF4	1	<i>L. europaeus</i>
CR10	TF2	TF6	1	<i>L. europaeus</i>
CR11	TF2	TF5	1	<i>L. europaeus</i>
CR12	TF3	TF3	1	<i>L. europaeus</i>
CR13	TF1	TF1	1	Hybrid <i>L. corsicanus/L. europaeus</i>
CR14	TF2	TF2	1	Hybrid <i>L. europaeus/L. granatensis</i>
CR15	TF1	TF7	1	Hybrid <i>L. corsicanus/L. granatensis</i>
		Total	67	

commune of Sartene identified by Scalera and Angelici (2003). Therefore, we demonstrate the presence of *L. corsicanus* almost all over the island, although, given the less intensive sampling in the South, we cannot account for the presence of *L. corsicanus* in the south-western corner. In the North, where our sampling was more thorough, the Italian hare was shown to be present in 96% of the sampled communes and to represent 95% of the hares harvested. This result has a particular impact for the conservation of *L. corsicanus* in Corsica since data collected about 20 years ago show that 78% of the hunted hares in Corsica came from Haute-Corse (Romi 1988), and that this department contains 73% of the communes known to harbor hares in Corsica (Dubray et al. 1984). This work represents a first estimation of the prevalence of *L. corsicanus* in Corsica, although further studies need to be undertaken to more precisely determine the range and the densities of *L. corsicanus* in the island and to verify the population dynamics of the species.

According to our results, the presence of *L. europaeus* in Corsica is restricted to the central and southern parts of the island (Fig. 1). The identification of *L. europaeus* in Figari and Valle-di-Mezzana (2A in Fig. 1) concerns non-marked hares (i.e., specimens not released by hunters), which means that reproduction *in natura* of this species might have occurred in these two areas. In the commune of Lozzi (2B in Fig. 1), a region where no information about

introductions of brown hares exist since 1980, a young specimen of this species was sampled, suggesting the establishment of a reproducing *L. europaeus* population in this commune (although only *L. corsicanus* seems to occur in the neighboring populations (Fig. 1) and Riga et al. (2003), like Trocchi and Riga (2005), indicate its presence also in Lozzi or nearby).

Historical origin of the hares from Corsica

The existence of several CR sequences in GenBank from hares with known species and geographic location allowed us to speculate about the location of origin of the colonization of Corsica. All haplotypes found in the specimens identified as *L. corsicanus* (Fig. 2) are closely related or similar to the sequences from Central Italy, strongly suggesting that the Italian hare populations from Corsica originated in this region. This Corsica–Central Italy phylogenetic proximity of *L. corsicanus* is in accordance with the inferences made by Pierpaoli and Randi (2005) based in a different set of samples. No records exist that may account for an human-mediated introduction of hares from mainland Italy into Corsica, but this does not preclude earlier introductions.

Regarding *L. europaeus*, haplotypes from the four major known mtDNA haplogroups (as determined by Stamatis et al. 2009) were included in our phylogeny (Fig. 2). We

observed that four of the detected haplotypes in Corsica (CR6, CR7, CR9, and CR13) are included in a clade together with the sequences from the European-type haplogroup, subgroup A (Fig. 2a). This haplogroup can be detected all over Central Europe and is also present in Italy (Stamatis et al. 2009). Also, other haplotype groups, with sequences from the European-type haplogroup, but from subgroup B, which are present only in the Balkans from where some *L. europaeus* may then have been brought to Corsica. Then, one haplotype group with those from southeastern European-type haplogroup, also present in the Balkans, but also present in Italy. Finally, haplotypes CR11 and CR12 cluster with sequences from the Anatolian/Middle Eastern haplogroup which is present in the Middle East and Anatolia, but also in the Balkans. This suggests that *L. europaeus* may have arrived to Corsica from several origins, covering a wide geographical range of its distribution, likely with independent introductions. This hypothesis had been previously put forth by Pietri (2007) who suggested that the introduced *L. europaeus* during the last 30 years came from several regions as Central and Eastern Europe and France.

Hybridization

Many instances of hybridization among different species of hares have so far been reported, with different levels of introgression and involving different species (e.g., Thulin et al. 1997, 2006; Alves et al. 2003; Melo-Ferreira et al. 2005, 2007, 2009; Suchentrunk et al. 2005), a phenomenon that may be even more extensive than presently detected (Alves et al. 2008b). The tendency for interspecific hybridization among species of hares and the suspicion that many different species have been introduced in Corsica made us wonder whether we could detect any signs of hybridization in Corsica. In fact, we did detect three situations where the species assignment was not concordant between the mtDNA and the nuclear marker. Curiously, two of these three specimens possess the mtDNA lineage of *L. granatensis*, a species known to have been introduced in Corsica in the past, although no presumably pure specimen of this species was detected here. The analysis of the nuclear marker suggests that part of the ancestry of one of these individuals is *L. europaeus* and that of the other is *L. corsicanus*. We cannot exclude the possibility that the first individual was introduced from northern Spain where hybridization between *L. granatensis* and *L. europaeus* occurs (Freitas 2006; Freitas et al. 2006), but the second one, detected in Calcatoggio, must have resulted from *in loco* hybridization since these species have natural allopatric distributions. Finally, the third detected specimen possesses genetic material ascribed to *L. corsicanus* and *L. europaeus* a cross that, although can theoretically occur in natural conditions in Italy where the species contact, was previously discarded (Pierpaoli et al.

1999; Riga et al. 2001; Randi et al. 2007a, b) and may therefore have occurred in Corsica.

The discovery of hybrids involving crosses between three species in the hares of the island brings forth the question of the maintenance of the genetic integrity of the *L. corsicanus* population from Corsica. Indeed, although only three specimens with hybrid origin were detected here, this number may be underestimated since our assignment is based solely in the analysis of two molecular markers. A more thorough screening of the genetic variability of the hares from Corsica is needed in order to further assess the dimension of this phenomenon.

Conclusions and conservation implications

This work has contributed to a better understanding of the status quo on the Corsican population of hares. Our results demonstrated that: (1) *L. corsicanus* prevails among the species of hares present in Corsica, (2) apart from the Italian hare, two other hare species exist or may have existed in the island (*L. europaeus* and *L. granatensis*), and (3) these species are hybridizing in Corsica. These settings make this island on one hand a unique natural laboratory for the study of hybridization and speciation, and on the other hand, call up to attention a tendency for the deterioration of the genetic integrity of *L. corsicanus*, a currently threatened hare species (VU, Vulnerable) with a restricted and declining native range in continental Italy, that should be actively preserved (Temple and Terry 2007). Thus, we consider that the hybridization between *L. corsicanus* and others hare species in Corsica must be further monitored in future studies. Finally, we hope that this study can act as an opportunity to launch the discussion on the most adequate management policies for the hare populations in Corsica.

Acknowledgements Funding for this research was provided by Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO), Fundação para a Ciência e a Tecnologia (PPCDT/BIA-BDE/58817/2004, PTDC/BIA-EVF/111931/2009, and PTDC/BIA-EVF/115069/2009 research projects, and SFRH/BPD/43264/2008 post-doc grant to José Melo-Ferreira), the Fédération des Chasseurs de Haute-Corse, and the Fédération des Chasseurs de Corse-du-Sud. DNA sequencing was performed on the platform of Centro de Testagem Molecular (CTM) of CIBIO. We thank Nuno Ferrand for his comments and suggestions on this work. We are indebted to the people who were directly involved in collecting the biological material used, particularly all hare hunters and Roger Maupertuis, Paul Etori, Christian Olivesi, and Victor Acquaviva. We also thank Nils Meunier and two anonymous reviewers for their helpful comments on the manuscript.

References

- Alves PC, Ferrand N, Suchentrunk F, Harris DJ (2003) Ancient introgression of *Lepus timidus* mtDNA into *L. granatensis* and *L. europaeus* in the Iberian Peninsula. *Mol Phylogenet Evol* 27:70–80

- Alves PC, Melo-Ferreira J, Branco M, Suchentrunk F, Ferrand N, Harris DJ (2008a) Evidence for genetic similarity of two allopatric European hares (*Lepus corsicanus* and *L. castroviejoi*) inferred from nuclear DNA sequences. *Mol Phylogenet Evol* 46:1191–1197
- Alves PC, Melo-Ferreira J, Freitas H, Boursot P (2008b) The ubiquitous mountain hare mitochondria: multiple introgressive hybridization in hares, genus *Lepus*. *Philos Trans R Soc Lond B Biol Sci* 363:2831–2839
- Angelici FM, Luiselli L (2001) Distribution and status of the Apennine hare *Lepus corsicanus* in continental Italy and Sicily. *Oryx* 35:245–249
- Aragon T (2008) EpiTools: epidemiology tools. R package version 0.5–7. <http://www.medepi.com>
- Bandelt HJ, Forster P, Rohlf A (1999) Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* 16:37–48
- Dubray D, Roux D, Gardes ONC (1984) Répartition des lagomorphes (lièvre commun et lapin de garenne) en Corse. *Bull Mens Off Natl Chasse* 85:36–38
- Ellerman JR, Morrison-Scott TCS (1951) Checklist of palaeartic and indian mammals 1758 to 1946. Trustees of the British Museum (Natural History), London
- Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evol Bioinform Online* 1:47–50
- Freitas H (2006) Natural hybridization between the Iberian hare (*Lepus granatensis*) and the brown hare (*L. europaeus*) in northern Iberian Peninsula. MSc thesis, Faculdade de Ciências do Porto, Porto
- Freitas H, Melo-Ferreira J, Alves PC (2006) Hibridação natural entre lebre-europeia e lebre-ibérica na província de Álava: evidência de novos marcadores genéticos. *Accazadores* 11:46–48
- Gissi C, Gullberg A, Arnason U (1998) The complete mitochondrial DNA sequence of the rabbit, *Oryctolagus cuniculus*. *Genomics* 50:161–169
- Kasapidis P, Suchentrunk F, Magoulas A, Kotoulas G (2005) The shaping of mitochondrial DNA phylogeographic patterns of the brown hare (*Lepus europaeus*) under the combined influence of Late Pleistocene climatic fluctuations and anthropogenic translocations. *Mol Phylogenet Evol* 34:55–66
- Melo-Ferreira J, Boursot P, Suchentrunk F, Ferrand N, Alves PC (2005) Invasion from the cold past: extensive introgression of mountain hare (*Lepus timidus*) mitochondrial DNA into three other hare species in northern Iberia. *Mol Ecol* 14:2459–2464
- Melo-Ferreira J, Boursot P, Randi E, Kryukov A, Suchentrunk F, Ferrand N, Alves PC (2007) The rise and fall of the mountain hare (*Lepus timidus*) during Pleistocene glaciations: expansion and retreat with hybridization in the Iberian Peninsula. *Mol Ecol* 16:605–618
- Melo-Ferreira J, Alves PC, Freitas H, Ferrand N, Boursot P (2009) The genomic legacy from the extinct *Lepus timidus* to the three hare species of Iberia: contrast between mtDNA, sex chromosomes and autosomes. *Mol Ecol* 18:2643–2658
- Miller GS (1912) Catalogue of the mammals of Western Europe (Europe exclusive of Russia) in the collection of the British Museum. British Museum (Natural History), London
- Palacios F (1996) Systematics of the indigenous hares of Italy traditionally identified as *Lepus europaeus* Pallas, 1778 (Mammalia: Leporidae). *Bonn Zool Beitr* 5:59–91
- Pierpaoli M, Randi E (2005) Evoluzione del genere *Lepus* in Europa. In: Trocchi V, Riga F (eds) I Lagomorfi in Italia. Linee guida per la conservazione e gestione. Min. Politiche Agricole e Forestali - INFS, Documenti Tecnici 25, pp 8–11
- Pierpaoli M, Riga F, Trocchi V, Randi E (1999) Species distinction and evolutionary relationships of the Italian hare (*Lepus corsicanus*) as described by mitochondrial DNA sequencing. *Mol Ecol* 8:1805–1817
- Pietri C (2007) Caccia e protezione delle popolazioni di lepore (*Lepus* sp.) in Corsica. In: De Filippo G, De Riso L, Riga F, Trocchi V, Troisi SR (eds) Conservazione di *Lepus corsicanus* De Winton, 1898 e stato delle conoscenze. IGF Publication, Napoli, pp 52–62
- Randi E, Mengoni C, Mucci N (2007a) Genetic distinction and assessment of interspecific hybridisation among three of hares (*Lepus*) in Italy. V European Congress of Mammalogy, Siena, Italy, 21–26 September 2007. *Hystrix* (N.S.) II, Supp., 362
- Randi E, Mengoni C, Mucci N (2007b) Genetica di *Lepus corsicanus*: evoluzione, speciazione ed assenza di ibridazione interspecifica. In: De Filippo G, De Riso L, Riga F, Trocchi V, Troisi SR (eds) Conservazione di *Lepus corsicanus* De Winton, 1898 e stato delle conoscenze. IGF Publication, Napoli, pp 37–43
- R Development Core Team (2008) R: a language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria. <http://www.R-project.org>
- Riga F, Trocchi V, Toso S (2001) Morphometric differentiation between the Italian hare (*Lepus corsicanus* De Winton, 1898) and European brown hare (*Lepus europaeus* Pallas, 1778). *J Zool* 253:241–252
- Riga F, Trocchi V, Angelici FM, Randi E, Pierpaoli M (2003) *Lepus corsicanus* De Winton, 1898—Apenninenhase. In: Krapp F (ed) Handbuch der Säugetiere Europas Hasentiere. Aula-Verlag, Wiebelsheim, pp 117–135
- Romi R (1988) La chasse en Corse. In: Couret A, Ogé F (eds) Droit et Animal. IEP, Toulouse, pp 185–208
- Sambrook E, Fritsch F, Maniatis T (1989) Molecular cloning. Cold Spring Harbour Press, Cold Spring
- Scalera R, Angelici FM (2003) Rediscovery of the Apennine Hare *Lepus corsicanus* in Corsica. *Bolletino del Museo Regionale Scienze Naturali Torino* 20:161–166
- Scherrer B (1984) Biostatistique. Gaëtan Morin, Boucherville
- Stamatis C, Suchentrunk F, Moutou KA, Giacometti M, Haerer G, Djan M, Vapa L, Vukovic M, Tvrtkovic N, Sert H, Alves PC, Mamuris Z (2009) Phylogeography of the brown hare (*Lepus europaeus*) in Europe: a legacy of south-eastern Mediterranean refugia? *J Biogeogr* 36:515–528
- Stephens M, Scheet P (2005) Accounting for decay of linkage disequilibrium in haplotype inference and missing data imputation. *Am J Hum Genet* 76:449–462
- Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 68:978–989
- Suchentrunk F, Mamuris Z, Stamatis C, Ben Slimen H, Hackländer K, Haerer G, Giacometti M (2005) Introgressive hybridization in wild living mountain hares (*L. timidus varronis*) and brown hares (*L. europaeus*) and morphological consequences. *Mamm Biol* 70 (suppl):39–40
- Suchentrunk F, Ben Slimen H, Stamatis C, Sert H, Scandura M, Apollonio M, Mamuris Z (2006) Molecular approaches revealing prehistoric, historic, or recent translocations and introductions of hares (genus *Lepus*) by humans. *Hum Evol* 21:151–165
- Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 10:512–526
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599
- Temple HJ, Terry A (2007) The status and distribution of European mammals. Office for Official Publications of the European Communities, Luxembourg

- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680
- Thulin CG, Jaarola M, Tegelstrom H (1997) The occurrence of mountain hare mitochondrial DNA in wild brown hares. *Mol Ecol* 6:463–467
- Thulin CG, Fang M, Averianov AO (2006) Introgression from *Lepus europaeus* to *L. timidus* in Russia revealed by mitochondrial single nucleotide polymorphisms and nuclear microsatellites. *Hereditas* 143:68–76
- Toschi A (1965) Fauna D'italia: Mammalia. Lagomorpha–Rodentia–Carnivora–Artiodactyla–Cetacea. Calderini, Bologna
- Trocchi V, Riga F (2005) I Lagomorfi in Italia. Linee guida per la conservazione e gestione. Ist. Naz. Fauna. Selvatica, Documenti Tecnici 25:1–128
- Vigne JD (1999) The large “true” Mediterranean islands as a model for the Holocene human impact on the European vertebrate fauna? Recent data and new reflections. In: Benecke N (ed) The Holocene history of European vertebrate fauna. Modern aspects of research. Deutsches Archäologisches Institut Eurasien-Abteilung, Verlag Marie Leidorf GmbH Rahden/Westf, pp 295–322