Mammalian Biology
Zeitschrift für Säugetierkunde
www.elsevier.de/mambio

Short communication

Genetic variation of the weasel (*Mustela nivalis*) in Corsica based on mitochondrial control region sequences

By C. Lebarbenchon, Françoise Poitevin and Claudine Montgelard

Laboratoire de Paléontologie, Phylogénie et Paléobiologie, Institut des Sciences de l'Evolution, Université Montpellier II, Montpellier, France; Laboratoire de Biogéographie et Ecologie des Vertébrés, Université Montpellier II, France; and Laboratoire de Paléontogie des Vertébrés, Université Montpellier II, France

Receipt of Ms. 25.4.2005
Acceptance of Ms. 19.11.2005

Key words: *Mustela nivalis*, France, Italy, insular syndrome

The weasel (*Mustela nivalis*, Linnaeus, 1766) is widespread throughout the entire western Palaearctic region, with the exception of several Atlantic islands (Ireland, Iceland and Canary islands). This species is one of the most common Mustelidae inhabiting islands of the Palaearctic region (Masseti 1995). According to De Marinis and Masseti (2003) *M. nivalis* inhabits all Mediterranean islands larger than 240 km^2. As often reported for small mammals (Blondel 1995) and specially for rodents (Alder and Levins 1994; Michaux et al. 2002), insular and mainland populations often differ by morphological differences. Insular populations of *M. nivalis* present several morphological differences, such as larger body size and darker coat colouration, differences that have led to the description of potentially one subspecies per island (Beaucournu and Grulich 1968; De Marinis 1996).

In Corsica, an important morphological and morphometrical differentiation from the south of France has been observed by Beaucournu and Grulich (1968) and Salotti (1992). However, no data are available concerning genetic variation between Corsican and European mainland populations. The aim of this study is therefore to compare weasel populations between Corsica and the European mainland (south of France and Italy) to estimate the extent of genetic variation. The mitochondrial DNA control region was chosen as a molecular marker because of its high rate of nucleotide change in peripheral regions. We follow Kurose et al. 1999 in sequencing the 5′ portion adjoining the repetitive region of the control region, which is a more informative area for phylogenetic analysis at the population level. The results are discussed in relation to the "insular syndrome" and the "Quaternary glaciation hypotheses".

Our sampling (Table 1) included a total of 23 weasels: 8 from Corsica, 11 from southern France, and 4 from Italy (Fig. 1). Body size and tail length were measured (Table 1) for 21 animals and compared to morphological data previously published. DNA was extracted from ear tissue or dry skin using QIAamp DNA Mini Kit (QIAGEN). We used 2 primers, L0 (5′-CCCAAAGCTGAAATTCTACTTAAACTA-3′; Douzery and Randi 1997) and MSD (5′-GGGGGTGGGTATATGTGTAT-3′; Kurose et al. 1999) to amplify the first 550 nucleotides of the 5′ region. Polymerase chain reaction (PCR) was performed using an initial denaturation of 3 min at 94 °C, followed by 35 cycles of 45 s denaturation at 94 °C, 1 min annealing at

1616-5047/$ - see front matter
doi:10.1016/j.mambio.2005.11.005

Table 1. Geographical origin, sequence accession numbers, head body length (BL) and tail length (TL) of the 23 weasels. Sex is indicated when known.

Sample name	Origin – Locality (French Department or Italian Province)	Sex	BL (mm)	TL (mm)	Accession number
Mni01	French mainland – La Couvertoirade (Aveyron)	♂	247	80	AJ698489
Mni02	French mainland – Saintes-Maries de la Mer (Bouches du Rhône)	♂	190	60	AJ698490
Mni03	French mainland – Montmajour (Bouches du Rhône)	♂	240	75	AJ698491
Mni04	French mainland – Murviel (Hérault)	♂	215	58	AJ698492
Mni05	French mainland – Mireval (Hérault)	♂	235	65	AJ698493
Mni06	French mainland – Le Caroux (Hérault)	♂	210	62	AJ698494
Mni07	French mainland – Mireval (Hérault)		186	54	AJ698495
Mni09	French mainland – Nohédes (Pyrénées Orientales)	♂	190	52	AJ698496
Mni10	French mainland – Vaugines (Vaucluse)		240	85	AJ698497
Mni14	French mainland – St. Martin du Larzac (Aveyron)	♂	210	62	AJ698501
Mni21	French mainland – Banyuls (Pyrénées Orientales)		180	60	AJ698502
Mni11	Corsica – Unknown	♂	230	75	AJ698498
Mni12	Corsica – Querciolo (Haute Corse)	♂	270	100	AJ698499
Mni13	Corsica – Linguezzata (Haute Corse)	♂	270	92	AJ698500
Mni22	Corsica – Portovecchio (Corse du sud)		330	80	AJ698503
Mni23	Corsica – Unknown		340	80	AJ698504
Mni24	Corsica – Muro (Haute Corse)	♂	250	100	AJ698505
Mni27	Corsica – Bonifacio (Corse du sud)	♀	185	60	AJ698506
Mni28	Corsica – Lucciana (Haute Corse)	♂	258	95	AJ698507
Mni16	Italy – Montelibretti (Latium)	♂	252	103	AJ849682
Mni17	Italy – San Polo Dei Cavalieri (Latium)				AJ849683
Mni18	Italy – Pratolino (Tuscany)				AJ849684
Mni20	Italy – Tuscany	♂	160	45	AJ849685

50 °C, 2 min extension at 72 °C, and a 10 min final extension at 72 °C. PCR products were purified with a QIAquick PCR Gel Extraction Kit (QIAGEN). Sequencing was done with ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit, on an ABI Prism 310 Genetic Analyser (Applied Biosystems). Samples were edited using Sequence Navigator software (Applied Biosystems). Sequences have been deposited at the EMBL databank with accession numbers given in Table 1. Two sequences of *M. erminea* (accession number AB006729 and AB006731; Kurose et al. 1999) were used as outgroups. Sequences were aligned by hand using ED editor of the MUST package software (Philippe 1993). We used the HKY85 evolutionary model (Hasegawa et al. 1985), selected by Modeltest 3.0 (Posada and Crandall 1998), for distance and maximum-likelihood (ML) analyses. Phylogenetic trees were reconstructed using neighbour-joining (NJ; Saitou and Nei 1987) and maximum parsimony (MP) methods with PAUP (Swofford 1998). The search for the ML tree was performed with the software PhyML (Guindon and Gascuel 2003). Nucleotide heterogeneity of substitution rates was estimated with a gamma distribution. Nodal support was assessed with 1000 bootstrap replicates generated for each method. A parsimonious network was built using TCS v1.13 software

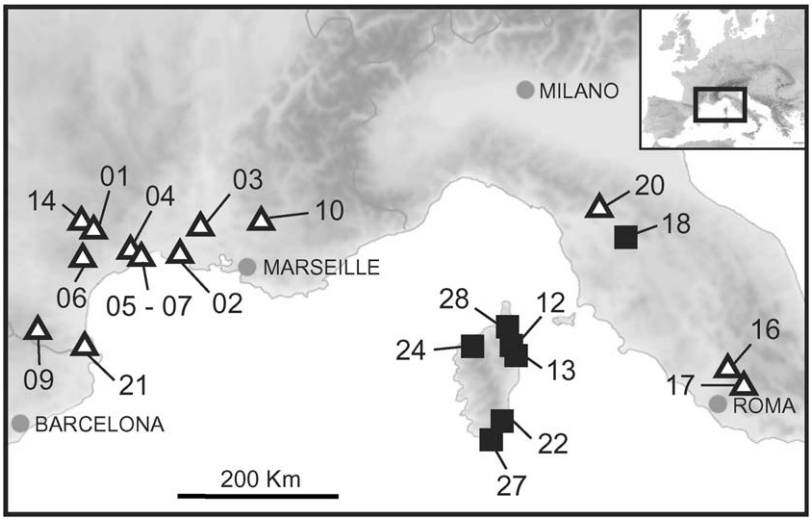

Fig. 1. Geographic location for 21 out of the 23 weasels studied (localities for Mni11 and Mni23 from Corsica are unknown). Numbers are sample names given in Table 1 without the mention Mni. Black squares and white triangles refer to clade I and II of Fig. 2, respectively.

(Clement et al. 2000) on the diverse haplotypes observed.

On the 23 weasels studied, 16 whole specimens were available to us, giving the possibility to check for the colouration pattern. According to the criteria of Frank (1985), 10 weasels from French mainland have the classical "*vulgaris* pattern" whereas 6 animals from Corsica are more "*nivalis* type" (straight colour demarcation between dorsal and abdominal areas).

From body and tail measurements (Table 1), mean sizes were calculated for Corsican and mainland French populations to allow comparisons with data previously published. Because of the important sexual dimorphism of this species, only males were considered. For southern France weasels ($n = 8$), average body size is 217 ± 22 mm and tail length is 64 ± 9 mm, whereas for Corsican samples ($n = 5$), body and tail lengths are 256 ± 17 mm and 92 ± 10 mm, respectively. These values are statistically different both for body length ($t = 2.77$, $P < 0.01$) and tail length ($t = 5.16$, $P < 0.001$). Saint Girons (1973) mentioned 229 mm ($n = 25$) for French Mediterranean populations whereas Salotti (1992) arrived at 253 mm ($n = 28$; males only) as average body size for Corsican weasels. Both values are congruent with our data. As already mentioned in previous studies, our sampling supports the evidence of an important morphological distinction of body size between Corsican and south France mainland weasels. For Italian samples, Beaucournu and Grulich (1968) indicate a mean body length of 311 mm (273–350 mm) for 60 males, which is a bigger size than in Corsica. However, our 2 samples for which we have measurements are smaller and fall outside this interval with 160 and 252 mm.

Fifteen haplotypes were identified from 23 sequences. Among the 550 sites analysed, 17 were variable (3.09%) and 15 were informative (2.73%). The 17 variable sites included 14 transitions and 3 indels. Genetic distances are low for all populations with $0.25 \pm 0.24\%$ (range 0–0.79%) for Corsica, $0.51 \pm 0.39\%$ (range 0–1.49%) for southern France and 0.79 ± 0.63 (range 0–1.49%) for Italy. Mean genetic distance between populations is higher with $1.54 \pm 0.46\%$ (range 0–2.32%). The ML tree (Fig. 2) includes all Corsican weasels and one Italian sample from Tuscany in a clade (clade I in Fig. 2, see also Fig. 1), moderately well supported by bootstrap values of 82% in NJ, 72% in MP and 73% in ML. These individuals are discriminated

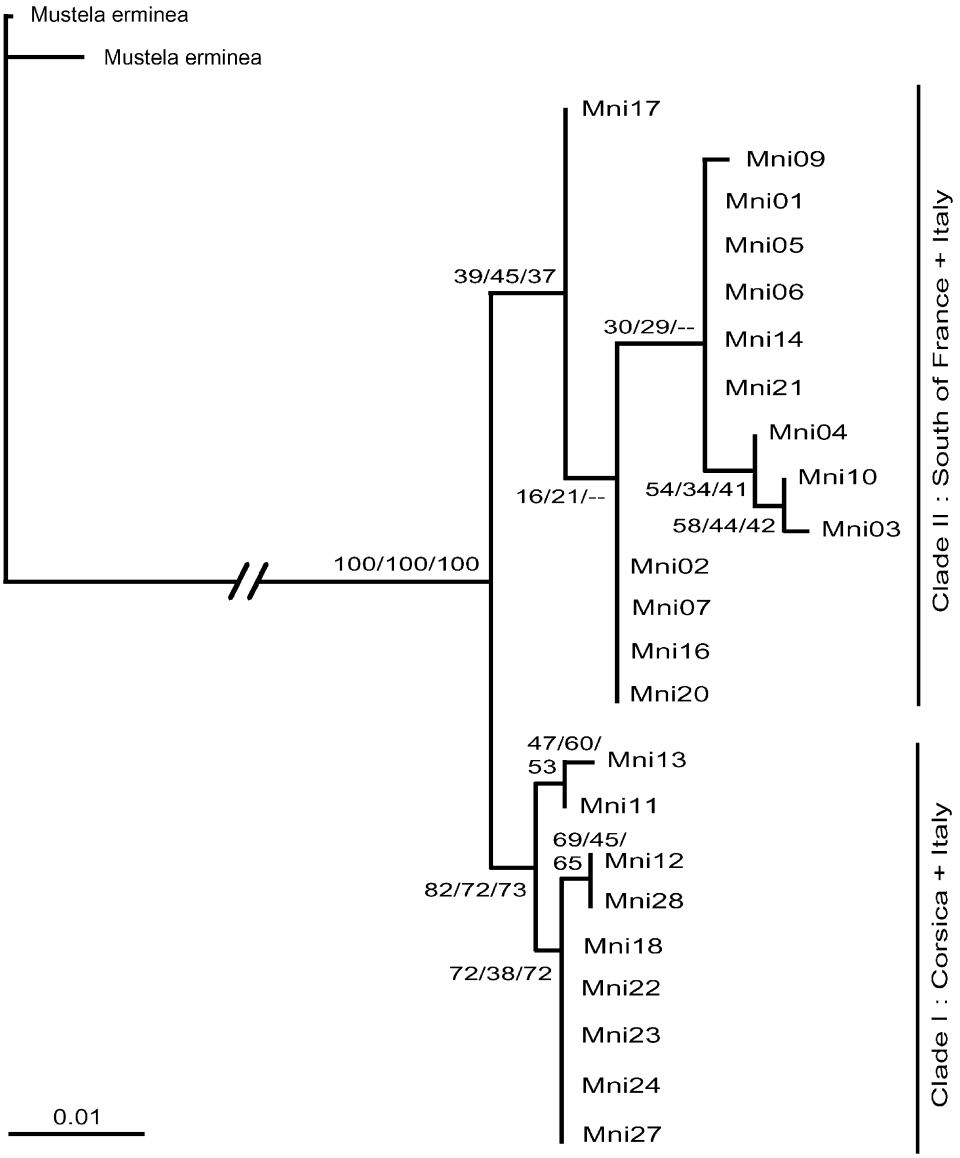

Fig. 2. ML phylogram tree ($-\ln L = 1045.79$) for the mitochondrial control region sequences of 23 *Mustela nivalis*, using the HKY85 $+\Gamma + I$ evolutionary model. Transition–Transversion rate equals 31.66, invariable site proportion is $I = 0.37$, alpha parameter of the gamma distribution is $\alpha = 0.1$ (8 categories), and nucleotide frequencies are $A = 0.25$, $C = 0.25$, $G = 0.17$, $T = 0.33$. Bootstrap proportions calculated after 1000 replications are indicated at nodes in NJ, MP and ML from right to left, respectively. The branch leading to the outgroup has been reduced 5 times. Symbols of samples are given in Table 1.

by 2 out of the 15 informative sites. The remaining animals show little genetic structure (clade II). The haplotypic parsimonious network also exhibited the same 2 groupings, differentiated by 5 missing haplotypes (Fig. 3).

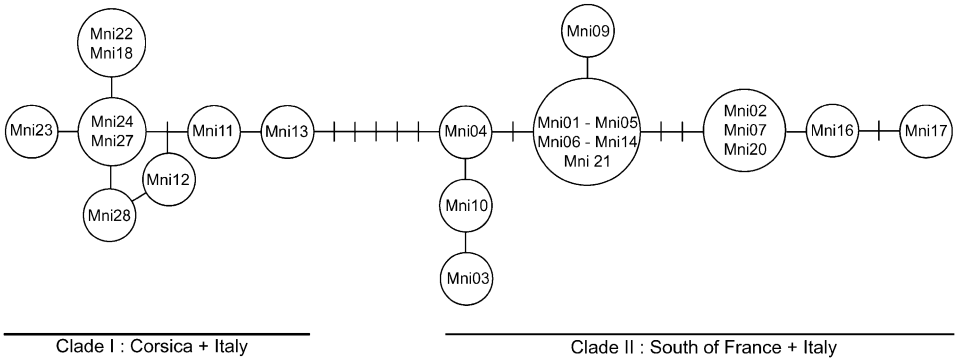

Fig. 3. Parsimonious network between the 15 haplotypes. Slash indicates missing mitotypes. Symbols of samples are given in Table 1.

Our molecular study, suggests a low level of genetic variation for weasel populations (both Corsican and mainland) which is nevertheless in agreement with values observed for other Mustelidae: *M. putorius* (Michaux et al. 2004) shows 2.4% ($n = 10$) intraspecific divergence for mitochondrial control region sequences for samples from Russia to Spain. The same authors found 0–1.5% ($n = 43$) divergence for *M. lutreola* among western European populations.

Mean genetic distance between mainland and island weasel populations shows somewhat higher differentiation (1.54%) than seen within populations (0.25–0.79%). Phylogenetic analyses (Fig. 2) suggest a well-supported clade that includes all the Corsican weasels along with 1 of the 4 Italian samples (Mni18 from near Firenze). This differentiation is also evidenced in the network (Fig. 3) showing a gap of 5 missing haplotypes between this clade and the remaining French and Italian samples. Our data thus suggest both a morphological and genetic differentiation of *M. nivalis* between Corsica and southern France. Two hypotheses can be put forward to explain the origin of this differentiation: either the genome of the insular population has been affected after the settlement in Corsica (insular syndrome), or weasels introduced on Corsica originated from a population already differentiated.

Under the island syndrome hypothesis, 3 factors are usually invoked to explain the genetic divergence of immigrant populations (see for example, Blondel 1995): (1) founder effect: the genome sampling included only a part of the genetic diversity of the source population, (2) genetic drift due to low initial population size, (3) divergence resulting from selective pressures in a new environment. All these factors could contribute to explain the lower genetic variability for the Corsican population (0.25% as compared to 0.51% and 0.79% for the French and Italian mainland populations, respectively). However, finding 1 haplotype shared by Italian and Corsican weasels as well as the recent introduction of *M. nivalis* in the last millenniums (Blondel and Vigne 1993; Masseti 1998) do not argue in favour of an insular differentiation. Humans introduced domestic species to Corsica (sheep, goats, pigs, cattle and dogs) as early as 7000 BP (Blondel and Vigne 1993). Most mammals, including *M. nivalis*, are most likely to have immigrated and founded new populations after this date. For some genera (*Apodemus*, *Glis* or *Erinaceus*), fossils have been found as early as 4000 BP. However, no fossils of *M. nivalis* were recorded in Corsica during the Pleistocene and the Neolithic (Blondel and Vigne 1993), preventing our knowing exactly when this species entered Corsica.

The second hypothesis, that weasels were already genetically differentiated before arrival on the island, can be tested using the 2 mainland populations (south of France and Italy). Missing haplotypes between Corsica and French mainland populations (Fig. 3)

and the high bootstrap values retrieved for the Italo-Corsican clade (Fig. 2) strongly suggest that Corsican weasels did not originate from southern France. On the other hand, the inclusion of 1 sample from Italy associated with the Corsican individuals could indicate colonization from the Italian peninsula. This result agrees with other studies showing an Italian origin for different Corsican mammals, such as the woodmouse *Apodemus sylvaticus* (Michaux et al. 1998), the Italian hare *Lepus corsicamus* (Pierpaoli et al. 1999) and the hedgehog *Erinaceus europaeus* (Santucci et al. 1998).

The morphological differentiation of weasels is more difficult to interpret in the hypothesis of colonization from Italy. Measurements published by Beaucournu and Grulich (1968) indicate a bigger size for Italian weasels that would corroborate this assumption. On the other hand, De Marinis (1996) stated that 13 out of 15 skull measurements are not different in size between weasels from 6 Mediterranean Islands and their mainland relatives (Iberian and Italian peninsula). Concerning our 2 Italian samples, we cannot exclude the fact that Mni20 might be a juvenile or a subadult because of its very small size, the other one (Mni16) being in the range observed for Corsican weasels (256 ± 17 mm for BL). Because morphology can undergo rapid change in response to adaptive environmental pressures (Fleming and Cook 2002), more careful studies taking into account variability between sex and age classes, as well as local geographical diversity would be necessary to understand morphological evolution.

Based on our molecular data, we attempt to estimate the divergence of the Italo-Corsican group (clade I), using the estimation of 21.1×10^{-9} substitutions/site/year given by Pesole et al. 1999 for the 5′ portion of the control region of Carnivora. When applying this estimation to our average genetic distance, the divergence time estimated for the clade I is around 0.08 ± 0.01 Myr. This tentative dating falls during the Pleistocene glaciations, suggesting that the phylogeographic subdivision observed could be the consequence of the Quaternary glaciations, as reported for numerous mammalian species in Europe (Hewitt 2000). For *M. nivalis*, the geographic differentiation observed seems to be different from the general pattern detected. In particular, the Alps do not seem to act as a barrier as often described (Seddon et al. 2001; Michaux et al. 2003). Of the 4 Italian weasels only 1 clustered in the Corsican clade whereas the 3 others from Tuscany and Roma showed more affinities with the French samples (1 haplotype in common). Hence, the existence of 2 different lineages in Italy remains to be explained in a broader geographic context.

In conclusion, our data lead us to recognize 2 weasel lineages, one on the mainland (France and Italy), and one on Corsica. Biogeographical processes, such as glacial refuge and post-glacial recolonisation, may have affected weasel evolution before its introduction in Corsica. This interpretation better explains the genetic divergence observed between the 2 lineages than the hypothesis of an island syndrome. Answering the questions on the origin of genetic structure of *M. nivalis* on mainland and islands imply to extend sampling to cover the whole Mediterranean basin.

Acknowledgements

We thank Gilles and Marc Cheylan, Maria-Grazia Filippucci, Roger Fons, Robin Letscher, Arnaud Lyet, Johan Michaux and Philippe Perret for supplying precious weasel tissues and skins. This research was financially supported by the PPF-EPHE "Populations Insulaires et Fractionnées". This is Contribution No. 2005-094 of the Institut des Sciences de l'Evolution de Montpellier (UMR 5554 CNRS).

References

Alder, G. H.; Levins, R. (1994): The island syndrome in rodent populations. Q. Rev. Biol. **69,** 473–490.

Beaucournu, J. C.; Grulich, I. (1968): A propos de la belette de Corse. Mammalia **32,** 341–371.

Blondel, J. (1995): Biogéographie, approche écologique et évolutive. Paris: Masson.

Blondel, J.; Vigne, J. D. (1993). Space, time, and man as determinants of diversity of birds and mammals in the Mediterranean region. In: Species Diversity in Ecological Communities—Historical and Geographical Perspectives. Ed. by R.E. Ricklefs and D. Schulter. Chicago: The University of Chicago Press. Pp. 135–146.

Clement, M.; Posada, D.; Crandall, K. A. (2000): TCS: a computer program to estimate gene genealogies. Mol. Ecol. **9,** 1657–1659.

De Marinis, A. M. (1996): The weasel (*Mustela nivalis*) on the Mediterranean region: a morphometric approach. Vie Milieu **46,** 376.

De Marinis, A. M.; Masseti, M. (2003): The weasel (*Mustela nivalis*) on the Mediterranean islands. Mamm. biol. **68,** 181–186.

Douzery, E. J. P.; Randi, E. (1997): The mitochondrial Control Region of Cervidae: Evolutionary patterns and phylogenetic content. Mol. Biol. Evol. **14,** 154–166.

Fleming, M. A.; Cook, J. A. (2002): Phylogeography of endemic ermine (*Mustela erminea*) in southeast Alaska. Mol. Ecol. **11,** 795–807.

Frank, F. (1985): Zur evolution und systematik der kleinen wiesel. Zeitsh Säugetierkd. **50,** 208–225.

Guindon, S.; Gascuel, O. (2003): A simple, fast, and accurate algorithm to estimate large phylogenies by Maximum Likelihood. Syst. Biol. **52,** 696–704.

Hasegawa, M.; Kishino, H.; Yano, T. A. (1985): Dating of the human–ape by molecular clock of mitochondrial DNA. J. Mol. Evol. **22,** 160–174.

Hewitt, G. (2000): The genetic legacy of the quaternary ice ages. Nature **405,** 907–913.

Kurose, N.; Masuda, R.; Yoshida, C. (1999): Phylogeographic variation in two Mustelines, the least weasel *Mustela nivalis* and the ermine *M. erminea* of Japan, based on mitochondrial DNA control region sequences. Zool. Sci. **14,** 971–977.

Masseti, M. (1995): Quaternary biogeography of the Mustelidae family on the mediterranean islands. Hystrix **7,** 17–34.

Masseti, M. (1998): Holocene endemic and anthropochorous wild mammals of the Mediterranean islands. Anthropozoologica **28,** 3–20.

Michaux, J. R.; Sara, M.; Libois, R.; Matagne, R. (1998): Is the woodmouse (*Apodemus sylvaticus*) of Sicily a distinct species? Belg. J. Zool. **128,** 211–214.

Michaux, J. R.; Goüy De Bellock, J.; Sarà, M.; Morand, S. (2002): Body size increase in insular rodent populations: a role for predators? Global Ecol. Biogeogr. **11,** 427–436.

Michaux, J. R.; Libois, R.; Davison, A.; Chevret, P.; Rosoux, R. (2004): Is the western population of the European mink (*Mustela lutreola*) a distinct management unit for conservation? Biol. Conserv. **115,** 357–367.

Michaux, J. R.; Magnanou, E.; Paradis, E.; Nieberding, C.; Libois, R. (2003): Mitochondrial phylogeography of the woodmouse (*Apodemus sylvaticus*) in the western paleartic region. Mol. Ecol. **12,** 685–697.

Pesole, G.; Gissi, C.; De Chirico, A.; Saccone, C. (1999): Nucleotide substitution rate of mammalian mitochondrial genomes. J. Mol. Evol. **48,** 427–434.

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.

Philippe, H. (1993): MUST: a computer package of management utilities for sequences and trees. Nucleic Acids Res. **21,** 5264–5272.

Posada, D.; Crandall, K. A. (1998): Modeltest: testing the model of DNA substitution. Bioinformatics **14,** 817–818.

Saint Girons, M. C. (1973): Les Mammifères de France et du Benelux (faune marine exceptée). Paris: Doin.

Saitou, N.; Nei, M. (1987): The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4,** 406–425.

Salotti, M. (1992). Carnivores sauvages actuels de Corse. Encyclopédie des carnivores de France. Nort s/Erdre: Société Française pour l'étude et la protection des mammifères.

Santucci, F.; Emerson, B. C.; Hewitt, G. M. (1998): Mitochondrial DNA phylogeography of European hedgehogs. Mol. Ecol. **7,** 1163–1172.

Seddon, J. M.; Santucci, F.; Reeve, N. J.; Hewitt, G. M. (2001): DNA footprints of European hedgehogs, *Erinaceus europaeus* and *E. concolor*: Pleistocene refugia, postglacial expansion and colonization routes. Mol. Ecol. **10,** 2187–2198.

Swofford, D. L. (1998): PAUP*—Phylogenetic Analysis Using Parcimony (*and other methods). Ver. 4. [Computer Software and Manual]. Sunderland, MA: Sinauer.

Authors' addresses:
Camille Lebarbenchon, Laboratoire de Paléontologie, Paléobiologie et Phylogénie, Institut des Sciences de l'Evolution (UMR5554 CNRS), CC64 Université

Montpellier II, 34095 Montpellier Cedex 05, France
Françoise Poitevin, Laboratoire de Biogéographie et Ecologie des Vertébrés, (EPHE), CC94 Université Montpellier II, 34095 Montpellier Cedex 05, France
Claudine Montgelard, Laboratoire de Paléontologie des Vertébrés, (EPHE), Institut des Sciences de l'Evolution (UMR5554 CNRS), CC64. Université Montpellier II, 34095 Montpellier Cedex 05, France
(e-mail: montgela@isem.univ-montp2.fr)