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ORIGINAL ARTICLE

Phylogeography of the weasel (*Mustela nivalis*) in the western-Palaearctic region: combined effects of glacial events and human movements

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The Iberian, Italian or Balkan peninsulas have been considered as refugia for numerous mammalian species in response to Quaternary climatic fluctuations in Europe. In addition to this 'southerly refugial model', northern refugia have also been described notably for generalist and cold-tolerant species. Here, we investigated the phylogeographic pattern of the weasel (*Mustela nivalis*) to assess the impact of Quaternary glaciations on the genetic structure, number and location of refugia as well as to determine the impact of human movements on the colonization of Mediterranean islands. We sequenced 1690 bp from the mitochondrial control region and cytochrome *b* for 88 weasels distributed throughout the western-Palaearctic region, including five Mediterranean islands. Phylogenetic analyses of combined genes produced a clear phylogeographic pattern with two main lineages. The first lineage included all of the western-continental samples (from Spain to Finland) and shows low levels of genetic structure. Demographic analysis highlighted several characteristics of an expanding group, dated approximately at 116 kiloyears (kyr; Riss glaciation). The genetic pattern suggested a northeastern-European origin from which colonization of southwestern Europe took place. The second lineage was divided into five subgroups and indicated a common origin of insular and Moroccan samples from eastern Europe. Eastern-continental weasels did not exhibit signs of sudden expansion, suggesting stable population size during the last ice ages. The time of expansion of Sicilian and Corsican populations was dated around 10 kyr ago, which supports the hypothesis of an early human intervention in the colonization of Mediterranean islands.
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Introduction

Phylogeographic patterns reported for numerous mammalian species in Europe are often interpreted as the consequences of the Quaternary climatic fluctuations, and the southern-European peninsulas (Iberian, Italian or Balkan regions) have been identified as refugia where flora and fauna survived during glacial phases (Taberlet *et al*., 1998; Hewitt, 2000, 2004). If a number of European taxa conformed to this now classical interpretation (see review in Hewitt, 2004; Randi, 2007), several studies indicated that this 'southerly refugial model' (Bhagwat and Willis, 2008) is probably too simplistic and not satisfactory for some taxa. Alternative concepts have been developed involving the existence of northern refugia (Stewart and Lister, 2001; Kotlik *et al*., 2006; Bhagwat and Willis, 2008; Tougard *et al*., 2008), microrefugia (small favourable areas outside the main refugium; Rull, 2009) or nunatak vs lowland refugia (specific to mountain species; Holderegger and Thiel-Egenter, 2009). According to Bhagwat and Willis (2008), populations not originating from southern refugia present common biogeographical traits, such as a small body size, a present-day northerly distribution and they are cold-tolerant animals. Among European mammals, several species of Mustelidae display such characteristics that would have allowed them to persist in northerly areas. However, phylogeographic studies conducted on Mustelidae are few and mostly inconclusive. The study of Davison *et al*. (2001) on *Martes martes* and *Mustela putorius* and the work of Ferrando *et al*. (2004) on *Lutra lutra* showed a lack of structuring and ancient lineages explained by an expansion from a single, but not localized, European refugium. For *M. erminea*, low genetic differentiation and structure were observed among continental Eurasian populations, with only one lineage from Europe (Ireland excepted) to Japan up to Alaska whose origin is not specified (Fleming and Cook, 2002; Kurose *et al*., 2000, 2005; Martinkova *et al*., 2007). Finally, the phylogeographical analysis of *Meles meles* across Eurasia (Marmi *et al*., 2006) evidenced four genetic groups, but only one lineage in Europe possibly resulting from postglacial recolonization from several refugia.

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In this study, phylogeographic variations in the western-Palaearctic region and genetic diversification related to Quaternary climatic changes are investigated for the weasel (*Mustela nivalis*, Linnaeus 1766). This species has a wide distribution covering nearly the entire Holarctic area (Europe, North Africa, northern Asia and North America). It is widespread throughout the entire western-Palaearctic region, with the exception of several Atlantic islands (including Ireland, Iceland and the Canary Islands). The species displays considerable variability across this wide geographic range. Consequently, there is a wealth of literature concerning the identification and description of different weasel lineages in Europe (three or four subspecies are described in Europe and North Africa), mainly based on morphological variations (Frank, 1985; Meia and Mermod, 1992; Zyll De Jong, 1992; Abramov and Baryshnikov, 2000) and chromosomal patterns (Zima and Cenevova, 2002). In contrast, molecular studies are scarce and mainly concern Japanese and Eurasian regions (Kurose *et al*., 1999, 2005; Saarma and Tumanov, 2006). In western Europe, two lineages have been recognized (Lebarbenchon *et al*., 2006), one in the mainland (France, Italy) and one in Corsica. However, these studies are based on short sequences (partial cytochrome *b* (*cytb*) or control region (*ctr*)) and include only a few individuals. This precludes a clear estimation of the number of genetic groups as well as their possible origin and relationships. Finally, the study of Lebarbenchon *et al*. (2006) raised the question of the origin of the two genetic lineages observed for *M. nivalis* in a broader phylogeographic context at the European scale.

The second aim of our study concerns the colonization of Mediterranean islands. Several mammalian species have been studied to identify the genetic link between insular and mainland populations (Santucci *et al*., 1998; Pierpaoli *et al*., 1999; Cosson *et al*., 2005; Dubey *et al*., 2007a, 2008). From these and other studies, it can be concluded that modalities of island colonization are very diverse according to the island and/or species under study, leading to a complex pattern of mammalian composition representing endemic, native or introduced species (Masseti, 1998; Sarà, 1998).

M. nivalis is one of the most common mustelids occurring in the islands of the Palaearctic region, inhabiting all Mediterranean islands larger than 240 km^2 with the exception of Ibiza and Cyprus (Masseti, 1995; De Marinis and Masseti, 2003). As is often reported for small mammals (Blondel and Vigne, 1993; Alder and Levins, 1994; Michaux *et al*., 2002), insular and mainland populations of *M. nivalis* differ morphologically. These variations include larger body size and darker coat colouration in insular populations (Beaucournu and Grulich, 1968; Alcover and Jaume, 1983) and led to the description of potentially one subspecies per island (Beaucournu and Grulich, 1968; De Marinis, 1996). For *M. nivalis*, the prevalent hypothesis is a colonization through human introduction (Masseti, 1995; Bover and Alcover, 2008). Until now, no genetic study has been performed to identify the geographic origin of insular weasel populations or to determine the impact of human movements on the presence of this species in the Mediterranean islands.

We used mitochondrial partial *ctr* and complete *cytb* sequences to investigate the genetic variation and phylogeographic pattern of the weasel in the western-Palaearctic region (from Morocco to Finland) including five Mediterranean islands (Minorca, Corsica, Sardinia, Sicily and Crete). On the basis of this dataset, specific objectives concerning the phylogeography of the weasel were addressed: (1) evaluate the impact of the Quaternary glacial cycles on the genetic diversity of weasels by dating the origin of the phylogenetic groups, (2) infer the locations of potential glacial refugia, (3) assess the demographic history of phylogenetic clades, (4) determine whether colonization of Mediterranean islands occurs through human intervention or natural migration and (5) compare current taxonomic classification with genetic groups.

Materials and methods

Sampling

We sequenced a total of 88 weasels distributed in the western-Palaearctic region (Figure 1). Our samples come from road-killed animals, museums or personal collections (Table 1). We added published sequences from the European Molecular Biology Laboratory Database for weasels of the western-Palaearctic region. Thus, six *ctr* and nine *cytb* (two complete and seven partial sequences; see Table 1) have been added to our dataset. Moreover, five other species of *Mustela* were used as outgroups: the western polecat *M. putorius*, the steppe polecat *M. eversmanni*, the European mink *M. lutreola*, the Japanese weasel *M. itatsi* and the stoat *M. erminea* (see Table 1 for accession numbers and references).

DNA sequencing and alignment

DNA was extracted from skin samples from the ear (frozen or preserved in ethanol) using the QIAamp DNA Mini Kit (Qiagen, Courtaboeuf, France). The first 550 nucleotides of the 5′ portion of the mitochondrial *ctr* were amplified using the L0Mni 5′-GCC CCR CCA TCA GCA CCC AAA GC-3′ (this study from weasel *ctr* alignment) and MSD (Kurose *et al*., 1999) primers. In case DNA was degraded, we used two internal primers (L1Mni 5′-ACC TCT TCT CGC TCC GGG CCC ATC A-3′; this study from weasel *ctr* alignment and E3 (Arnason *et al*., 1997) to amplify *ctr* in two overlapping fragments of 306 and 363 nucleotides. The mitochondrial DNA *cytb* gene was amplified entirely using L7 and H6 primers (Montgelard *et al*., 2002) or in two overlapping fragments (767 and 742 bp) with the internal primers H2 and L2 (Hassanin *et al*., 1998). All weasels have been sequenced for the *ctr* (65 from this study and 23 from an earlier study by Lebarbenchon *et al*., 2006). For the *cytb*, 68 samples have been sequenced, representing at least one individual from each geographic area and for each *ctr* haplotype. A total of 133 new sequences were obtained and deposited in the European Molecular Biology Laboratory Database with accession numbers given in Table 1.

For both genes, polymerase chain reaction 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 50 °C, 2 min extension at 72 °C and a 10 min final extension at 72 °C. Polymerase chain reaction products were purified with a QIAquick polymerase chain reaction Gel Extraction Kit (Qiagen). Sequencing was carried out with an ABI Prism Big Dye Terminator

Cycle Sequencing Ready Reaction Kit on an ABI Prism 310 Genetic Analyser (Applied Biosystems, Courtaboeuf, France). Electrophoregrams were read and aligned manually using Sequence Navigator software (Applied Biosystems).

The *ctr* and *cytb* sequences were aligned by hand using ED editor of the MUST package (Philippe, 1993). The *cytb* was translated into protein and did not reveal any stop codons, suggesting that functional sequences were obtained. Indels present in the *ctr* alignment were considered as missing characters. The number of variable and informative sites was calculated using DNASP 4.50.2 software (Rozas *et al*., 2003).

Genetic variability and population structure

Genetic variability was estimated for both phylogenetic and geographic groups. The number of different haplotypes (n_H), haplotype diversity (h) and nucleotide diversity (π) as well as the average number of nucleotide differences (k) within groups were estimated using DNASP program. The hypothesis of recent population growth was tested using different statistical tests. Deviation from neutrality of mutations was tested with Fu's Fs (Fu, 1997), Tajima's D statistic (Tajima, 1989) and R_2 test (Ramos-Onsins and Rozas, 2002) implemented in DNASP. The significance of deviation was evaluated with 10 000 boostrap replicates (constant population size for P-values >0.05). Pairwise mismatch distributions of substitution differences were used to test for demographic expansion. Observed and expected values were compared with Arlequin 2.0 (Schneider *et al*., 2000) with 100 bootstrap replicates (demographic expansion rejected for P-values <0.05). Relationships between unique haplotypes were described by a parsimony network constructed with the program TCS 1.21 (Clement *et al*., 2000).

Saturation and phylogenetic analyses

Saturation of nucleotide substitutions was evaluated according to the procedure of Philippe *et al*. (1994). The inferred number of substitutions in maximum likelihood (programs PHYML version 2.4.4; Guindon and Gascuel, 2003 and Treeplot of MUST) was plotted against the pairwise number of observed differences between sequences (programs Comp_mat and Treeplot of MUST). The slope of the linear regression and the coefficient of correlation were then used to evaluate the level of saturation: the slope decreases from one as saturation increases.

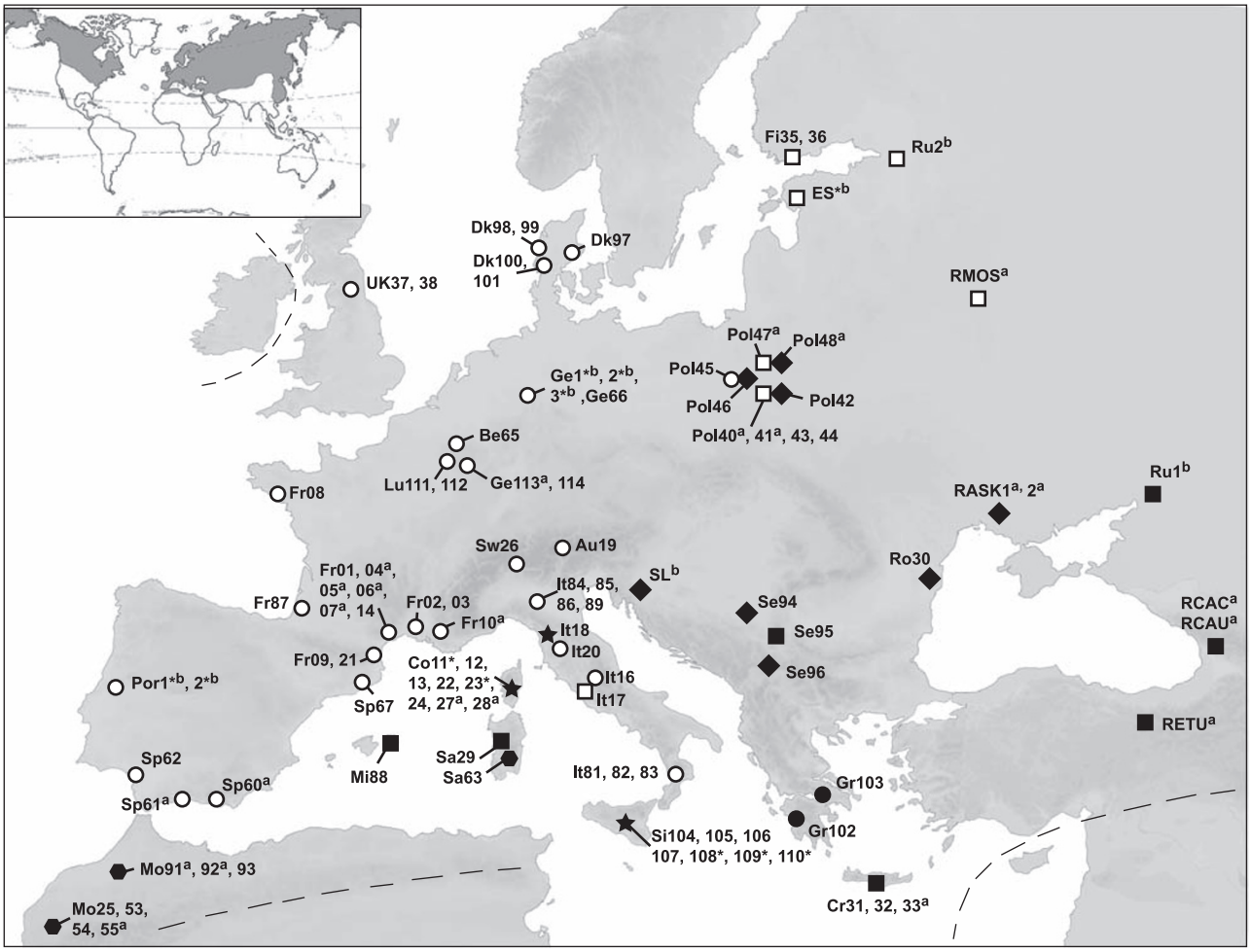

Figure 1 Sample localities of *M. nivalis* (see Table 1 for details). An asterisk (*) denotes samples for which precise locality is unknown. Samples for which only the control region or the cytochrome *b* is available are annotated 'a' and 'b', respectively. White and black symbols correspond to phylogenetic clades identified in Figure 2. The dotted line indicates the approximate distribution of *M. nivalis* and the inset map shows the entire species range.

Table 1 Sampling location, sample symbols, tissue origin and sequence accession numbers

Geographical origin of samples		*Symbol*	*Donator or reference*	*Accession number*	
Country	*Locality*			*Control region*	*Cytochrome b*
Austria	Grinzling	Au19	Maria Grazia Filipucci	AM258893[a]	AM258847[a]
Belgium	Blégny Trembleur	Be65	Roland Libois	AM258921[a]	AM258871[a]
Denmark	Glesborg	Dk97	Thomas Secher Jensen	AM258939[a]	AM258887[a]
	Lemvig	Dk98	Thomas Secher Jensen	AM258940[a]	AM258888[a]
	Tyvmose	Dk99	Thomas Secher Jensen	AM258941[a]	AM258889[a]
	Billund, Vandel Dam	Dk100	Thomas Secher Jensen	AM258942[a]	AM258890[a]
	Olgod	Dk101	Thomas Secher Jensen	AM258943[a]	AM258891[a]
Estonia	Unknown	ES	Saarma and Tumanov (unpublished)		AY188793[b]
Finland	Helsinki	Fi35	Janne Sundell	AM258901[a]	AM258859[a]
	Tampere	Fi36	Janne Sundell	AM258902[a]	AM258860[a]
France—Continent	Aveyron, La Couvertoirade	Fr01	Lebarbenchon *et al*. (2006)	AJ698489	
	Bouches du Rhône, Saintes Maries de la Mer	Fr02	Lebarbenchon *et al*. (2006)	AJ698490	
	Bouches du Rhône, Montmajour	Fr03	Lebarbenchon *et al*. (2006)	AJ698491	AM258837[a]
	Hérault, Murviel	Fr04	Lebarbenchon *et al*. (2006)	AJ698492	
	Hérault, Mireval	Fr05	Lebarbenchon *et al*. (2006)	AJ698493	
	Hérault, Le Caroux	Fr06	Lebarbenchon *et al*. (2006)	AJ698494	
	Hérault, Mireval	Fr07	Lebarbenchon *et al*. (2006)	AJ698495	
	Morbihan, Sarzeau	Fr08	François Livet	AM258892[a]	AM258838[a]
	Pyrénées Orientales, Nohedes	Fr09	Lebarbenchon *et al*. (2006)	AJ698496	AM258839[a]
	Vaucluse, Vaugines	Fr10	Lebarbenchon *et al*. (2006)	AJ698497	
	Aveyron, St Martin du Larzac	Fr14	Lebarbenchon *et al*. (2006)	AJ698501	AM258843[a]
	Pyrénées Orientales, Banyuls	Fr21	Lebarbenchon *et al*. (2006)	AJ698502	AM258849[a]
	Landes, Mont de Marsan	Fr87	Elodie Magnanou	AM258930[a]	AM258880[a]
France—Corsica	Unknown	Co11	Lebarbenchon *et al*. (2006)	AJ698498	AM258840[a]
	Haute Corse, Querciolo	Co12	Lebarbenchon *et al*. (2006)	AJ698499	AM258841[a]
	Haute Corse, Linguezzata	Co13	Lebarbenchon *et al*., 2006)	AJ698500	AM258842[a]
	Corse du Sud, Portovecchio	Co22	Lebarbenchon *et al*. (2006)	AJ698503	AM258850[a]
	Unknown	Co23	Lebarbenchon *et al*. (2006)	AJ698504	AM258851[a]
	Haute Corse, Muro	Co24	Lebarbenchon *et al*. (2006)	AJ698505	AM258852[a]
	Corse du sud, Bonifacio	Co27	Lebarbenchon *et al*. (2006)	AJ698506	
	Haute Corse, Lucciana	Co28	Lebarbenchon *et al*. (2006)	AJ698507	
Georgia	Tbilisi District	RCAC	Kurose *et al*. (2005)	AB049770	
	Lagodehi	RCAU	Kurose *et al*. (2005)	AB049764	
Germany	Bielefeld	Ge66	Rainer Hutterer	AM258922[a]	AM258872[a]
	Konz	Ge113	Bettina Schmitt	AM932880[a]	
	Konz	Ge114	Bettina Schmitt	AM932881[a]	AM932885[a]
	Unknown	Ge1	Hosoda *et al*. (2000)		AB051264[b]
	Unknown	Ge2	Hosoda *et al*. (2000)		AB051265[b]
	Unknown	Ge3	Hosoda *et al*. (2000)		AB051266[b]
Greece—Continent	Filiatra—Mesinia	Gr102	Petros Platis	AM749057[a]	AM749048[a]
	Vravrova—Athènes	Gr103	Michel Thévenot	AM749058[a]	AM749049[a]
Greece—Crete	Nida Platau	Cr31	Petros Lymberakis	AM258898[a]	AM258857[a]
	Karteros Iraklion	Cr32	Petros Lymberakis	AM258899[a]	AM258858[a]
	Vrises Hanion	Cr33	Petros Lymberakis	AM258900[a]	
Italy—Continent	Montelibretti	It16	Lebarbenchon *et al*. (2006)	AJ849682	AM258844[a]
	San Polo Dei Cavaliere	It17	Lebarbenchon *et al*. (2006)	AJ849683	AM258845[a]
	Toscana, Pratolino	It18	Lebarbenchon *et al*. (2006)	AJ849684	AM258846[a]
	Toscana	It20	Lebarbenchon *et al*. (2006)	AJ849685	AM258848[a]
	Cosenza, Valle Capra	It81	Licia Colli	AM258924[a]	AM258874[a]
	Cosenza, Rende	It82	Licia Colli	AM258925[a]	AM258875[a]
	Cosenza, Cellara	It83	Licia Colli	AM258926[a]	AM258876[a]
	Parma, Collecchio	It84	Licia Colli	AM258927[a]	AM258877[a]
	Parma, Sala Baganza	It85	Licia Colli	AM258928[a]	AM258878[a]
	Parma, Fugazzolo	It86	Licia Colli	AM258929[a]	AM258879[a]
	Plain of Po	It89	Françoise Poitevin	AM258932[a]	AM258882[a]
Italy—Sardinia	West center	Sa29	Gilles Cheylan	AM258896[a]	AM258855[a]
	Sassari	Sa63	José Cabot	AM258920[a]	AM258870[a]
Italy—Sicily	Castellammare del Golfo—Province de Trapani	Si104	Mario Lovalvo	AM749059[a]	AM749050[a]
	Vicari—Palermo	Si105	Mario Lovalvo	AM749060[a]	AM749051[a]
	St Stefano—Palermo	Si106	Mario Lovalvo	AM749061[a]	AM749052[a]
	Sicile orientale	Si107	Mario Lovalvo	AM749062[a]	AM749053[a]
	Unknown	Si108	Mario Lovalvo	AM749063[a]	AM749054[a]
	Unknown	Si109	Mario Lovalvo	AM749064[a]	AM749055[a]
	Unknown	Si110	Mario Lovalvo	AM749065[a]	AM749056[a]

Table 1 Continued

Geographical origin of samples		*Symbol*	*Donator or reference*	*Accession number*	
Country	*Locality*			*Control region*	*Cytochrome b*
Luxembourg	Berbourg	Lu111	Bettina Schmitt	AM932878[a]	AM932883[a]
	Berbourg	Lu112	Bettina Schmitt	AM932879[a]	AM932884[a]
Morocco	Marrakech region	Mo25	Fabrice Cuzin	AM258894[a]	AM258853[a]
	Marrakech region	Mo53	Fabrice Cuzin	AM258914[a]	AM258867[a]
	Marrakech region	Mo54	Fabrice Cuzin	AM258915[a]	AM258868[a]
	Marrakech region	Mo55	Fabrice Cuzin	AM258916[a]	
	Casablanca region	Mo91	Michel Thévenot	AM258933[a]	
	Casablanca region	Mo92	Michel Thévenot	AM258934[a]	
	Casablanca region	Mo93	Michel Thévenot	AM258935[a]	AM258883[a]
Poland	Bialowieza	Po40	Karol Zub	AM258905[a]	
	Bialowieza	Po41	Karol Zub	AM258906[a]	
	Bialowieza	Po42	Karol Zub	AM258907[a]	AM258863[a]
	Bialowieza	Po43	Karol Zub	AM258908[a]	AM258864[a]
	Bialowieza	Po44	Karol Zub	AM258909[a]	AM258865[a]
	Kampinos	Po45	Karol Zub	AM258910[a]	AM932882[a]
	Kampinos	Po46	Karol Zub	AM258911[a]	AM258866[a]
	Biebrza	Po47	Karol Zub	AM258912[a]	
	Biebrza	Po48	Karol Zub	AM258913[a]	
Portugal	Unknown	Port1	Fernandez *et al*. (2007)		EF689080
	Unknown	Port2	Fernandez *et al*. (2007)		EF689081
Romania	Delta's Danube	Ro30	Johan Michaux	AM258897[a]	AM258856[a]
Russia	Rostov	RU1	Hosoda *et al*. (2000)		AB051267[b]
	Leningrad	RU2	Saarma and Tumanov (unpublished)		AY188794[b]
	Moscow Province	RMOS1	Kurose *et al*. (2005)	AB049766	
Serbia	Dobanovci	Se94	Dusko Cirovic	AM258936[a]	AM258884[a]
	Backo Dobro Polje	Se95	Dusko Cirovic	AM258937[a]	AM258885[a]
	Svilajnac, Sedlare	Se96	Dusko Cirovic	AM258938[a]	AM258886[a]
Slovenia	East Slovenia	SL	Davison *et al*. (1999)		AF068545[b]
Spain—Continent	Granada	Sp60	José Cabot	AM258917[a]	
	Malaga	Sp61	José Cabot	AM258918[a]	
	Huelva	Sp62	José Cabot	AM258919[a]	AM258869[a]
	Barcelona, Natural Park of Montseny	Sp67	Alexis Ribas	AM258923[a]	AM258873[a]
Spain—Baleares	Minorca	Mi88	Evarist Coll	AM258931[a]	AM258881[a]
Switzerland	Tessin, Cadagno lake	Sw26	François Catzeflis	AM258895[a]	AM258854[a]
Turkey	Kars Province	RETU	Kurose *et al*. (2005)	AB049776	
Ukraine	Askania—Nova	RASK1	Kurose *et al*. (2005)	AB049765	
	Askania—Nova	RASK2	Kurose *et al*. (2005)	AB049768	
United Kingdom	Kielder	UK37	Xavier Lambin	AM258903[a]	AM258861[a]
	Kielder	UK38	Xavier Lambin	AM258904[a]	AM258862[a]
OUTGROUP					
Mustela putorius			Kurose *et al*. (1999); Kurose *et al*. (2000)	AB010379	AB026107
Mustela eversmanni			Michaux *et al*. (2004); Kurose *et al*. (2000)	AJ548476	AB026102
Mustela lutreola			Michaux *et al*. (2005); Kurose *et al*. (2000)	AJ548803	AB026105
Mustela itatsi			Kurose *et al*. (2005); Kurose *et al*. (2000)	AB007327	AB026104
Mustela erminea 1			Kurose *et al*. (1999); Kurose *et al*. (2000)	AB006729	AB026101
Mustela erminea 2			Kurose *et al*. (1999); Fleming and Cook (2002)	AB006731	AF271061

[a]Submitted for this article.
[b]Sequences incomplete for the cytochrome *b* (about 350 bp).

Phylogenetic trees were reconstructed in maximum parsimony with PAUP (version 4.0b10; Swofford, 2002), in maximum likelihood using PHYML and with the Bayesian inference using MRBAYES (version 3.1.2; Ronquist and Huelsenbeck, 2003). The search for the maximum parsimony trees was performed with the option stepwise addition with simple addition sequence and stability of nodes was assessed with 1000 boostrap replications. For the two probabilistic methods, the optimal model of sequence evolution was determined with MODELGENERATOR (version 0.82; Keane *et al*., 2006), using the majority model indicated by the four tests (LRT, AIC1, AIC2 and BIC). As PHYML does not allow for data partition, the model of sequence evolution was inferred by MODELGENERATOR on whole genes (separately or in combination) and stability of nodes was tested with 1000 bootstrap replications. With MRBAYES, a mixed-model analysis was performed on partitioned genes using different models (selected by MODELGENERATOR) and parameters for four partitions (*ctr* and each codon position for the *cytb*). MRBAYES was run using four chains of four millions of generations sampled for each 100 generations, with the first 10 000 trees discarded as burn-in after checking stationary of log-likelihood values.

Dating analysis

Divergence dates were estimated by Bayesian coalescent analysis with the program BEAST (version 1.4.6;

Drummond and Rambaut, 2007) using the complete combined dataset (68 weasels sequenced for both genes and 6 outgroups). We used the HKY model of nucleotide substitution with a proportion of invariable site (I) and a Gamma distribution (G). Three coalescent priors (constant size, exponential growth and expansion growth) as well as three molecular clock models (strict, relaxed exponential and lognormal) were evaluated and tested using the Bayes factor (BF) as implemented in the program TRACER (version 1.4; Rambaut and Drummond, 2007). BF significance was determined from the values of 2LnBF as described in Brandley *et al*. (2005). Chains were run twice independently for 30-million generations each, sampled every 1000 iterations with the first 10% of trees discarded as burn-in. The program TRACER was used to check for convergence of MCMC chains to stationarity (effective sample size >600). The results of the two independent analyses were combined using the program Logcombiner to calculate the time of divergence from the most recent common ancestor (TMRCA) and 95% highest posterior density (HPD) intervals.

Molecular dating was derived using as calibration point the age of the first occurrence of *M. nivalis*, which is dated at 300 kiloyear (kyr) in North America and 100 kyr in Eurasia (The PaleoBiology Database, http://paleodb.org). A lognormal distribution suitable for modelling fossil data (Ho, 2007) was used as prior with parameter values of 100 kyr as the minimum age (lower bound parameter), 300 kyr as the mean and the standard deviation of the distribution was chosen to 2, so that 95% of the HPD for the time of divergence of the *M. nivalis* clade lies in the interval 100–500 kyr (late-middle Pleistocene; Sheffield and King, 1994).

The expansion time was estimated from the mismatch distribution as $t=\tau/(2\mu)$, where t is the time since expansion in generation, τ is the mode of the mismatch distribution and μ is the mutation rate per nucleotide multiplied by the sequence length (Rogers, 1995). We assumed one generation per year, and for the substitution rate, we used the mean and 95% HPD estimations obtained from BEAST analyses to calculate the expansion time with 95% confidence interval (CI).

Results

Phylogenetic analyses

Ninety-four *ctr* weasel sequences have been analysed for 550 nucleotides (including 7 indels), which yielded 49 haplotypes showing 43 (7.8%) variable and 33 (6%) informative sites. Intraspecific sequence variation between haplotypes varies from 0.18 to 3.85%. The slope of the regression and correlation coefficient of the saturation analysis were 0.77 and 0.83, respectively. These values indicate that the *ctr* was little affected by saturation, although values were slightly lower than for the *cytb* (see below). The complete *ctr* dataset includes 100 animals (94 weasels and 6 outgroups) and HKY + I + G was selected as the best-fitting model of sequence evolution. On the whole, the *ctr* did not reveal strong phylogeographic structure with the exception of a clade including samples from western Europe (clade I in Supplementary Appendix S1a).

Thirty-six different haplotypes have been identified for 70 weasel complete *cytb* sequences (Table 1), which showed 82 (7.2%) variable sites among which 42 (3.7%) were informative. Intraspecific sequence variation between haplotypes varied from 0.088 to 2.28%. The slope of the regression and correlation coefficient of the saturation analysis was 0.85 and 0.98, respectively, indicating that the *cytb* was not affected by saturation.

Phylogenetic analyses were performed on 83 *cytb* when partial sequences and outgroups were included. The best model of sequence evolution was TrNef + I, HKY and TrN, for the first, second and third position, respectively, whereas the HKY + G model was selected for the whole *cytb*. In Bayesian analysis, we used the GTR model instead of TrNef and TrN, which are not available in MRBAYES. The resulting trees revealed two moderately supported clades (I and II in Appendix S1b). Two groups can be identified in clade I. Subclade Ia contained all weasels from the western part of Europe from Spain to Finland among which a uniform phylogeographic pattern was observed. Subclade Ib was weakly supported and included seven samples (two individuals from Poland, two from Finland and one from central Italy, Estonia and Russia each). In contrast, group II was clearly structured in five rather well-supported subclades including individuals from (i) Greece; (ii) Crete, Minorca, Sardinia and Serbia; (iii) Poland, Romania, Slovenia and Serbia; (iv) Corsica, north Italy and Sicily and (v) Morocco and Sardinia.

The combined analysis included 74 samples (68 weasels and 6 outgroups) for 1690 nucleotides, and HKY + I + G was selected as the best model of sequence evolution. The phylogenetic tree (Figure 2a) strengthened the arrangement in two main clades (I and II) observed with the *cytb* alone (Appendix S1b), and the same five subclades were strongly supported in group II. This indicates that while less information was brought by the *ctr*, there was little conflict with the structure given by the *cytb*. The phylogenetic analysis of the western-Palaearctic weasels thus reveals an opposition between the clade I unstructured and including only one strong group (subclade Ia) and the clade II split into five well-supported subgroups.

The minimum spanning network (Figure 2b) performed on the whole combined dataset (68 weasels) showed the same general pattern with clades I and II separated by 18 mutational steps. The absence of structure inside subclade Ia was also apparent, along with a star-like topology indicative of a demographic expansion (stronger evidence with *cytb* alone, data not shown).

Genetic structure

The mean divergence, estimated from combined markers by the uncorrected *p* distance (percentage of divergence), was low within each clade ($0.49 \pm 0.3\%$ for clade I and $0.6 \pm 0.31\%$ for clade II), whereas the differentiation between the two groups was $<2\%$ ($1.8 \pm 0.18\%$). These estimates are not uncommon for carnivores and, for example, are in agreement with the mean percentage divergence observed for stoat (*M. erminea*; Fleming and Cook, 2002; Martinkova *et al*., 2007).

Genetic diversity was calculated for phylogenetic (Figure 2a) and geographic (each Mediterranean island, Morocco and eastern-continental individuals) groups. Haplotype diversity was high ($h > 0.80$) for all clades, whereas nucleotide diversity was heterogeneous between different groups (Table 2), with lower values recorded for groups including insular samples. This analysis, combined with the average number of

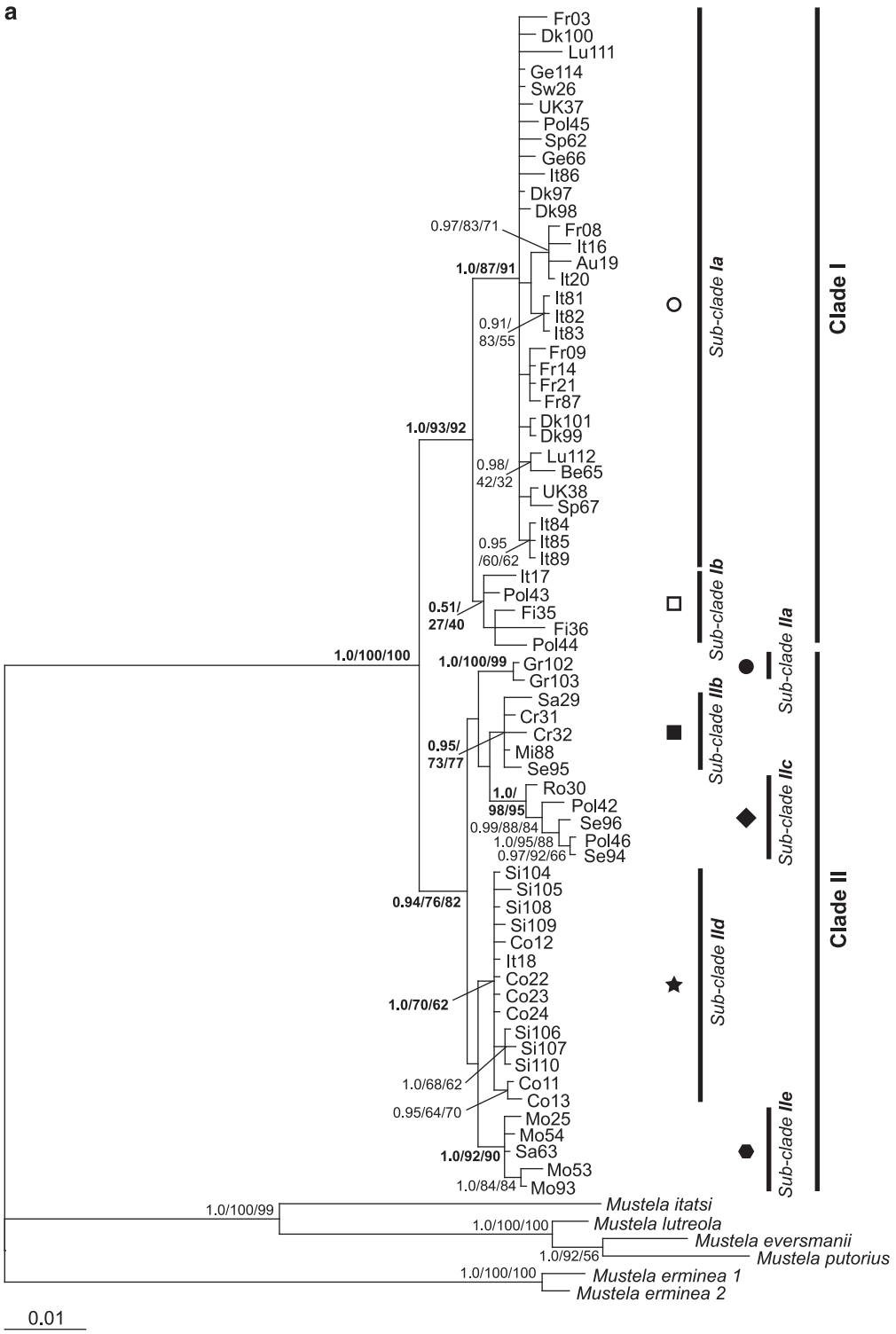

Figure 2 (**a**) Bayesian phylogram derived from the combined control region and cytochrome *b* for 68 *M. nivalis* (see Table 1 for sample names). Bayesian posterior probabilities and bootstrap proportions in maximum likelihood and maximum parsimony are shown at nodes from left to right, respectively. With the exception of clade Ib, only nodes supported by at least 90% support for at least one method are indicated. Supports for main clades and subclades are indicated in bold. (**b**) Minimum spanning network for the same 68 sequences. Black dots represent missing haplotypes. Unless specified, the number of substitution between haplotypes is one.

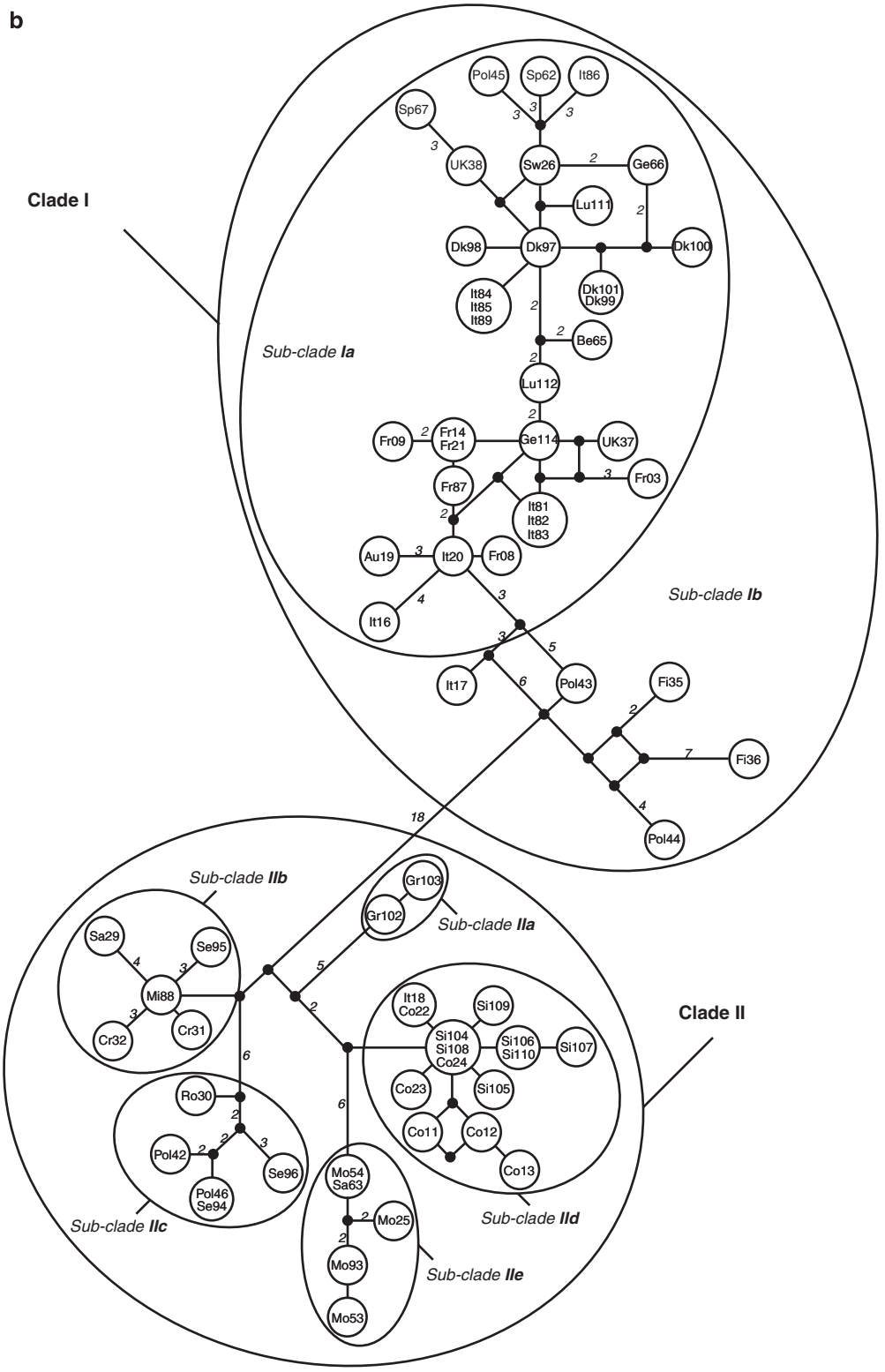

Figure 2 Continued.

nucleotide differences (*k* in Table 2), highlights that subclades IIa and IId, and especially samples from Corsica and Sicily, present the lowest genetic diversities.

Three statistical tests (Fu's Fs, Tajima's *D* statistic, R_2 test; Table 2) as well as mismatch distributions were performed to infer demographic histories (stability or expansion of population). The Tajima's *D* statistic was the most restrictive test with 10 out of 13 groups tested showing a probability above 0.05 (thus rejecting the hypothesis of expansion), whereas the two other tests reject this hypothesis ($P>0.05$) for seven groups. In contrast, mismatch distributions accepted the hypothesis

Table 2 Genetic polymorphism and neutrality tests

Groups	n	n_H	h *(s.d.)*	π *(s.d.)*	k	*Fs*	D	R_2	τ
Phylogroups[a]									
All clades	68	53	0.99 (<0.001)	0.0092 (<0.001)	14.96	−27.87***	−0.99	0.07	31.93
Clade I	37	31	0.99 (<0.001)	0.0043 (<0.001)	7.03	−22.64***	−1.73*	0.06**	3.55
Subclade Ia	32	26	0.98 (<0.001)	0.0033 (<0.001)	5.49	−19.19***	−1.39	0.07*	5.08
Subclade Ib	5	5	1 (0.13)	0.0058 (<0.005)	9.8	−0.37	−0.83	0.09*	10.8
Clade II	31	22	0.96 (<0.001)	0.0049 (<0.001)	8.09	−7.62*	−1.41	0.07*	13.23
Subclade IIa	2	2	1 (0.50)	0.0006 (<0.001)	1.00	NA	NA	NA	NA
Subclade IIb	5	5	1 (0.13)	0.0024 (<0.001)	4.00	−1.72	−1.19*	0.14*	4.78
Subclade IIc	5	4	0.90 (0.16)	0.0027 (<0.001)	4.60	0.36	−0.30	0.16	6.65***
Subclade IId	14	8	0.82 (0.09	0.0009 (<0.001)	1.48	−4.54***	−1.55*	0.09**	3.02
Subclade IIe	5	4	0.90 (0.16)	0.0019 (<0.001)	3.20	−0.23	−0.33	0.20	2.15
Geographic groups									
Eastern−continental	9	8	0.97 (0.06)	0.0064 (<0.001)	10.80	−0.88	0.06	0.15	17.74
Sicily	7	5	0.91 (0.10)	0.0010 (<0.001)	1.71	−1.89*	−0.79	0.16	NA
Corsica	6	4	0.80 (0.17)	0.0007 (<0.001)	1.20	−1.45	−0.45	0.18	3.66
Morocco	4	4	1 (0.18)	0.0026 (<0.001)	4.33	−0.72	−0.07	0.16	6.02

Abbreviations: *D*, Tajima's *D*; Fs, Fu's Fs; *h*, haplotype diversity; *k*, the average number of nucleotide differences; *n*, number of sequences; NA, not available (low sample size or computational limits of the program); n_H, number of haplotypes; R_2, R_2 test; s.d., standard deviation; τ, time since expansion (in units of mutational time) estimated from mismatch distributions; π, nucleotide diversity.
Probability of the test, *** <0.001, ** <0.01, * <0.05, no star: non-significant.
[a]Defined from the phylogenetic tree obtained on combined sequences (1690 bp) (Figure 2a).

of recent population growth for all populations except one (subclade IIc). Discrepancies between tests can be explained by differences in their statistical power or in the sequence information used (Ramos-Onsins and Rozas, 2002). Therefore, we considered a population in expansion only if results from at least three tests were significant. According to these constraints, five phylogenetic clades (clade I, subclade Ia, clades II, IIb and IId) were considered as expanding groups.

Molecular dating

The difference in likelihood between the strict (−5038.26), relaxed lognormal (−5025.55) and relaxed exponential (−5015.01) molecular clock reveals that the exponential model was significantly more adapted to our dataset (2LnBF>10). Similarly, the test of three demography priors indicated that the constant size (−5015.28) hypothesis cannot be rejected (2LnBF<2) as compared with the exponential (−5014.84) and expansion (−5015.99) growth coalescents. Consequently, the relaxed exponential clock and constant population size prior were used to estimate the age of the TMRCA for different phylogenetic and geographic groups (Table 3; and Figure 4).

Three periods of diversification can be recognized for weasels: (i) 116 kyr for the TMRCA of the whole western-Palaearctic lineage (clade I), (ii) 50–60 kyr for the timing of the eastern-Palaearctic continental and western (subclade Ia) populations and (iii) 18–28 kyr for the TMRCA of the Moroccan, Sicilian and Corsican populations. For the outgroups, the split between *M. lutreola* and *M. eversmanni*+*M. putorius* was 133 kyr, which is the same timing as the divergence of clade I. The divergence of *M. itatsi* from other mustelids was dated at 389 kyr.

The mean mutation rate was evaluated at 112 substitutions per site per kyr (95% HPD: 8.9–233 kyr) by BEAST analyses. This value and 95% HPD interval were used to infer time of expansion from mismatch analysis for different clades (Table 3). Values were rather different, notably for clades I and Ia.

Table 3 Estimated time (in kiloyears) to the most recent common ancestor (TMRCA) and expansion time for different groups of interest using a Bayesian coalescent analysis (BEAST program) and the mismatch distributions

Group	*BEAST*		*Mismatch distribution*	
	TMRCA	*95% HPD*	*Expansion time*	*95% CI*
Clade I	116	126–287	9	5–117
Subclade Ia	62	7–158	13	7–167
Corsica	18	1.2–48	10	5–120
Sicily	18	1.4–49	NA	NA
Morocco	28	1.3–78	16	8–198
Clade II-continent	50	5–130	35	23–583
All weasels	220	100–520	84	41–1049

Abbreviations: CI, confidence interval; HPD, highest posterior density; NA, not available; TMRCA, time of divergence from the most recent common ancestor.

Discussion

Differentiation in northern refugia?

On the basis of extensive sampling in the western-Palaearctic region and the use of two mitochondrial genes (1690 bp), we have shown for the first time a clear phylogeographic pattern for the weasel, revealing a split into two major groups (clades I and II in Figure 2). Clade I includes all individuals sampled in the western-Palaearctic region, from Spain to Finland and including the United Kingdom (white symbols in Figure 1), and clade II encompasses samples from eastern Europe to the

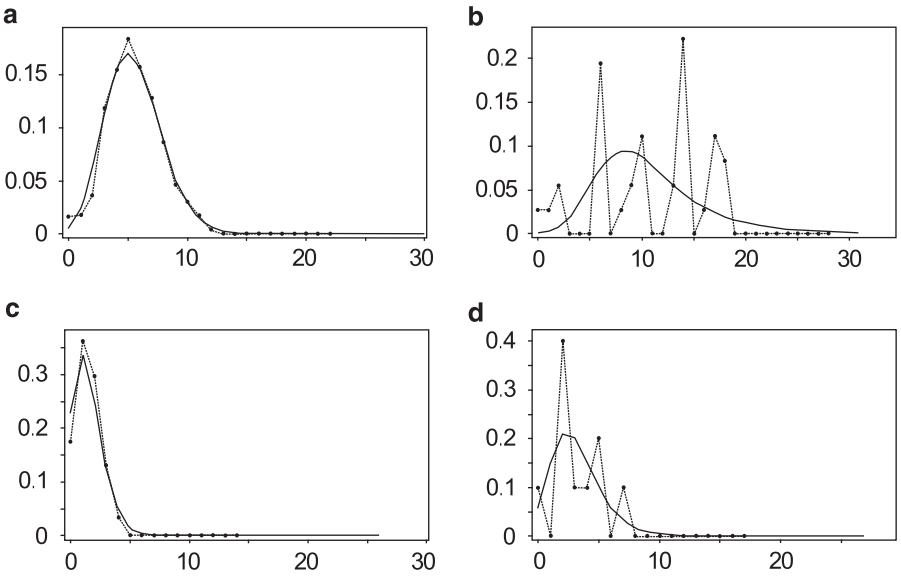

Figure 3 Observed (dot line) and expected (solid line) mismatch distributions under a population growth-decline model for different *M. nivalis* clades (see Figure 2a) of interest: (**a**) clade Ia, (**b**) clade II east-continental samples (after exclusion of insular and Moroccan samples), (**c**) clade IId and (**d**) clade IIe.

Black Sea as well as all Moroccan and insular weasels (black symbols in Figure 1).

Group I contained a strongly supported clade (Ia) including all western-European weasels (except It18) and a weakly supported clade (Ib) including five individuals located at the edge of the distribution area of clade I (Figure 1). As these haplotypes are the oldest among clade I and are mostly located in Finland and Poland, it can be inferred that clade I probably originated in northeastern Europe. Several explanations can be put forward to account for these individuals. First, they could carry ancestral polymorphisms because of incomplete lineage sorting (Maddison and Knowles, 2006). There is, however, little support for this hypothesis because samples are geographically localized, which is not consistent with the retention of ancestral variation. Second, these individuals could result from admixture with another genetic group not identified in our study because it is located farther east. To check for the existence of another group in the eastern part of Eurasia, we added all complete weasel *cytb* sequences available in European Molecular Biology Laboratory. Only two sequences from Japan and one from Taiwan were found. The resulting tree (not shown) indicates that these sequences also clustered at the base of clade I, thus suggesting that clade I might effectively include other lineages expanding to the eastern part of the Palaearctic region. Third, a replacement event can be proposed by which these intermediate samples would represent a residual clade I (formerly covering the whole western-Palaearctic region) displaced by range expansion of subclade Ia. This hypothesis is supported by the observation that subclade Ia is nestled within clade I, and, therefore, group Ia likely originated from group I. Such a replacement process has been used to explain the different lineages observed in Scotland and England/Wales for the water vole (*Arvicola terrestris*, Piertney *et al*., 2005) or in Ireland and Britain for the stoat (Martinkova *et al*. 2007).

The subclade Ia showed no phylogeographic structure and has characteristics consistent with sudden expansion: a bell-shaped curve of the mismatch distribution (Figure 3a), rejection of the hypothesis of neutrality (Table 2) and a star-like network (particularly for the *cytb*). These features suggest some contraction of population size in the past, likely during the last glacial cycles as indicated by the time of expansion (13 kyr; 95% CI: 7–167 kyr). For this clade, our data do not support the hypothesis of refugia located in the Mediterranean peninsulas because observation of the network (Figure 2b) reveals that individuals from Spain or Italy are mostly located at the periphery of the network, whereas central nodes are occupied by individuals from Denmark (Dk97), Switzerland (Sw26) or Germany (Ge114). These results thus lead to the assumption that clade Ia originated from clade I in northeastern Europe and then spread to southwestern regions. The interpretation of northern-European refugium would also be consistent with the ecology of weasels, which are presently found from the southern and northern extremes of Europe and in mountains up to 3000 m. These characteristics suggest that, as cold-tolerant animals, weasels would have been able to survive at different latitudes (to the boundary of the ice sheet) during successive glaciations. The presence of weasel subfossils recorded during the pleniglacial age (15–75 kyr) of central Europe (Germany, Poland; Sommer and Benecke, 2004) is also consistent with this hypothesis. Of course, more samples from northern countries (Sweden, Norway) to the Extreme Orient would be needed to support this scenario.

Clade II showed a topology with five well-supported subgroups (Figure 2). When samples from clade II are restricted to continental individuals (nine weasels from Greece, Serbia, Romania, Italy, Poland), there is no indication of recent population growth: the mismatch distribution curve is multimodal (Figure 3b) and close to the rejection threshold ($P = 0.08$; see also subclade IIc).

Moreover, the failure to reject the neutrality hypothesis (Table 2) rather suggests constant population size. In contrast to western-European weasels, eastern-continental populations seem to have been more stable during the ice ages and would not have undergone reduction in population size. Although based on a small sampling, our data do not support the hypothesis of a refugium located in the Balkan area. This pattern of more stable populations in the Balkans has already been described for the bi-coloured shrew (*Crocidura leucodon*; Dubey *et al*., 2007b) and the yellow-necked fieldmouse (*Apodemus flavicollis*; Michaux *et al*., 2005). This might be explained by milder climatic conditions prevailing in places farther from the ice sheet. A more comprehensive sampling from northern and middle-eastern regions (Carpathian, Balkan, Black Sea until Caspian areas) would help to delineate the geographic distribution of clade II and to better identify refugia potentially located in more northern (Carpathian) or eastern (Caucasus) areas (see Jaarola and Searle, 2002; Brunhoff *et al*., 2003).

Molecular dating indicates that the divergence of subclade Ia and clade II (continental samples) occurred 62 and 50 kyr ago, respectively (95% HPD: 5–158 kyr; see Table 3; Figure 4). The proximity of these dates suggests that the two clades originated from the same event and fall during the last glacial period, namely the Würm glaciation (10–80 kyr). Thus, we can reasonably assume that the last glacial period was responsible for the emergence of these two distinct genetic groups, but with quite different consequences. Whereas the western-Palaearctic group has likely undergone all consequences of a distribution restricted to glacial refugia, the eastern-continental group does not express any characteristic of a refugium zone. The molecular dating obtained for the clade I (extended western Palaearctic) is dated approximately 116 kyr ago (95% CI: 126–287 kyr), which corresponds to the end of the Riss glaciation.

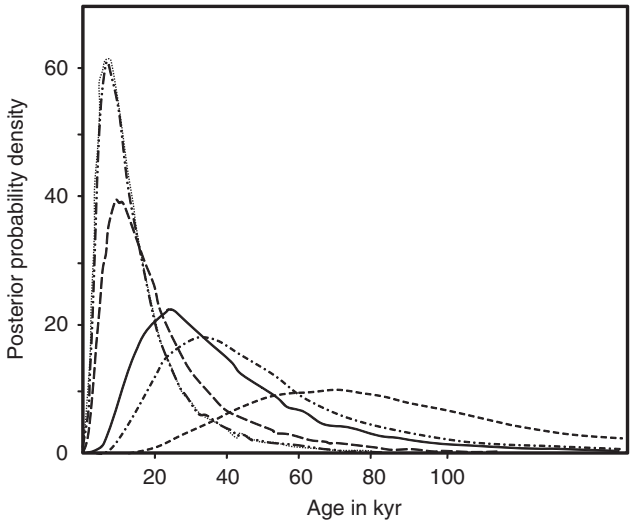

Figure 4 Bayesian posterior density plot showing the distribution of the age of the most recent common ancestor for clade I (- - - -), clade Ia (—) and Corsican (—··—), Sicilian (··· ···), Moroccan (— — —) and east-continental (— · — ·) populations of *M. nivalis* (see Table 3 for estimated ages).

Colonization of Mediterranean islands

The question of how (natural or introduced populations) and when (during the Pleistocene or at human scale) the current mammalian species colonized the different Mediterranean islands is still open to debate (Vigne, 1999; Lister, 2004; Marra, 2005). During the Holocene, the replacement of endemic mammalian species by a poorly diversified introduced fauna occurred on all of the larger Mediterranean islands (Blondel and Vigne, 1993). For *M. nivalis*, there is no clear explanation concerning the modality of island introduction. However, the lack of fossil records (Vigne and Alcover, 1985; Masseti, 1995; Vigne, 1999) suggests the absence of *M. nivalis* not only on most islands, but also in the western part of North Africa (Morocco, Algeria and Tunisia) during the Pleistocene and early Holocene ages. Hence, colonization through human intervention (voluntary or not) seems to be the prevailing hypothesis (Vigne, 1999; Bover and Alcover, 2008).

According to our results, it is noticeable that, with the exception of Crete, all Mediterranean islands do not belong to the same genetic group as the nearest continent. Corsica, Sardinia, Sicily and Minorca are included in the genetic clade II, whereas their corresponding closest mainland regions (France, Italy and Spain) are in the genetic group I (Figures 1 and 2). It can thus be inferred that islands have not been colonized from the nearest continent, as already shown from a smaller sampling (Lebarbenchon *et al*., 2006). Rather, the clustering of insular samples with weasels from Serbia, Greece and Romania suggests that islands were likely colonized by individuals originating from an eastern-European group. Owing to this common origin, our genetic data argue in favour of a human introduction, which is also congruent with the early trade routes that have been established in eastern civilizations since the Bronze age (De Marinis and Masseti, 2003; Costa, 2004). Moreover, a common eastern origin through natural colonization across land masses seems to be unlikely because of the high bathymetry of the Mediterranean Sea: large islands (Corsica, Sardinia and Crete) are surrounded by deep marine pits and only coastal islands were connected to the continents during glacial ages (Shackleton *et al*., 1984).

In our analysis, Sicilian and Corsican samples are clustered in the same subclade (IId; Figure 2) suggesting that both islands were colonized from the same eastern genetic group. Moreover, the low nucleotide diversity and high haplotype variability (Table 2) observed for this grouping as well as the unimodal mismatch distribution (Figure 3c) are indicative of population growth from a small number of individuals (founder effect). These results thus support the hypothesis of a recent introduction on both islands, which is compatible with the hypothesis of colonization through human activity. Similar to the ferret (*Mustela putorius furo*), which has been used for centuries for hunting purposes in western Europe, the weasel may be considered as an 'anthropophilous' species (Vigne, 1999 and references herein). As a matter of fact, it was used as a house animal (to preserve food from small rodents; Masseti, 1995) as well as for food, fur and even traditional medicine as is still the case in Morocco. The weasel was thus part of human activities and as such was involved in the Holocene immigration wave that colonized Mediterranean islands

with man. It is also possible that in Sicily, introduced weasels replaced some autochthonous lineage because the remains from *M. nivalis* are documented in the Terminal Pleistocene (Masseti, 1995).

As supported by Mesolithic sites in Sardinia and Corsica, the early human settlements on large Mediterranean islands are dated approximately 11 000 before present (Vigne, 1999). However, the main wave of island colonization started with the Neolithic age, that is from the seventh millennium, while the permanent colonization of Corsica was realized during the sixth millennium. These dates are more recent than the 18 kyr (95% HPD: 1.2–48 kyr) we obtained from analyses of individuals from Corsica and Sicily (Table 3). In spite of a large CI, dating obtained for the time of expansion (10 kyr, 95% HPD: 5–120 kyr) remains more compatible with an early human intervention than by a colonization initiated by the Phoenician or Greek civilizations that started about 3 kyr ago (De Marinis and Masseti, 2003; Costa, 2004) in the whole Mediterranean region.

Colonization of North Africa

Weasels from Morocco are included in clade II (subclade IIe; Figure 2a) with insular and eastern-European samples and thus do not belong to the same group as western-European (Iberian, French or Italian) weasels. This result suggests that, like insular individuals, those in Morocco originated from the eastern part of Europe. Different routes have been proposed to explain colonization of North Africa (Dobson and Wright, 2000). The absence of genetic similarity between Iberian and Moroccan samples strongly suggests that North African weasels do not come from western Europe (route 1 in Dobson and Wright, 2000). Colonization through this route is, however, advocated for other mammals, including the woodmouse (*Apodemus sylvaticus*), which colonized Morocco from the Iberian peninsula (Michaux *et al*., 2003).

Neutrality tests (Table 2; Figure 3d) performed on Moroccan samples are not indicative of recent population expansion, and nucleotide diversity is at least twice that seen in insular samples (Table 2). Although the number of analysed samples is low, these results suggest that North African populations did not undergo a recent bottleneck. With dating analyses, we obtained 28 kyr (95% HPD: 1.3–78 kyr) for the time of divergence of the Moroccan population, which is slightly older than estimations obtained for Sicilian and Corsican populations (18 kyr, 95% HPD: 1.4–49 kyr). However, considering the large CI, it is difficult to determine whether colonization occurred through human movements or naturally from the middle-eastern area along the northern coast of Africa (route 2 in Dobson and Wright, 2000). To corroborate the hypothesis of an eastern route of colonization, it would be very interesting to include weasel samples from Egypt (from the Nile delta). These weasels comprise the most eastern African population and are described as a different subspecies (*M. n. subpalmata*; Reig, 1997) or even a distinct species (*M. subpalmata*; Zyll De Jong, 1992), representing a glacial relict (Dayan and Tchernov, 1988).

Taxonomic considerations

The extreme variability of weasel morphological characteristics (body size, pattern of colouration, but also sexual, seasonal and geographic variation) led to the description of numerous taxonomic forms (and thus subspecies). Most recent studies on weasel taxonomy recognize 3–4 subspecies in the western-Palaearctic region (Frank, 1985; Zyll De Jong, 1992; Abramov and Baryshnikov, 2000; Zima and Cenevova, 2002), but there is no consensus about the geographic range or the evolutionary history of these taxa. Instead of arbitrarily adopting one particular taxonomic classification, we preferred to revert to the type locality (Sheffield and King, 1994) in which the different subspecies were originally described: (i) *M. nivalis nivalis:* 1776 'Province of Vesterbotten, Sweden'; (ii) *M. nivalis vulgaris*: 1777 'near Leipzig, Germany'; (iii) *M. nivalis boccamela*: 1800 '*Sardinia*' and (iv) *M. nivalis numidica*: 1855 '*Tangier, Morocco*'. Our mitochondrial data lead to the recognition of two main phylogenetic groups in the western-Palaearctic region (I and II on Figure 2) and the attempt to compare them with assumed subspecies constitutes only a preliminary approach to the revision of weasel taxonomic classification. As individuals from Germany fall within group I, we might assign this clade to the *vulgaris* group, which would thus include the whole western Europe. There is no genetic evidence for the occurrence of two subspecies in Morocco (*numidica*) and Sardinia (*boccamela*) because these samples belong to the same genetic clade (II). Finally, the identification of the *nivalis* subspecies would necessitate analysing weasels from Scandinavia, and in particular Sweden. In any case, linking morphotypes to genetic lineages would require concurrent analyses of the same individuals for both morphological and molecular characteristics (including nuclear markers). Such studies remain unfortunately rare in the literature, especially for carnivores, because of the inherent logistical complexities therein.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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