

# Mitochondrial DNA and palaeontological evidence for the origins of endangered European mink, *Mustela lutreola*

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## Abstract

The European mink *Mustela lutreola* is one of Europe's most endangered carnivores, with few vulnerable populations remaining. Surprisingly, a recent phylogeny placed a single mink specimen within the polecat (*M. putorius*, *M. eversmannii*) group, suggesting a recent speciation and/or the effects of hybridization. The analysis has now been extended to a further 51 mink and polecats. As before, phylogenetic methods failed to resolve the relationships between the species. One haplotype (C11) was found in both species, and predominated in European mink from Spain and eastern Europe. The known *M. lutreola* fossils are of very young date, so either mink arose recently, or else the situation is confused by hybridization and a biased fossil recovery. The study highlights the dangers of using a single genetic marker in defining Evolutionarily Significant Units (ESUs). Polecats and European mink are clearly distinct in their morphology and ecology, and should still be considered as separate ESUs, but without further data it is difficult to define Management Units. Following the precautionary principle, we recommend that for the moment European mink in eastern Europe (Belarus, Estonia and Russia) and Spain should be managed separately.

## INTRODUCTION

The European mink *Mustela lutreola* (L., 1761) is one of Europe's most endangered carnivores, since recent population censuses estimate that it now exists in isolated populations covering only one-fifth of its former range (Sidorovich, Savchenko & Budny, 1995; Maran *et al.*, 1998b; Sidorovich, 2000). The territory of the former Soviet Union, specifically the Tver, Pskov (both Russia) and Vitebsk (Belarus) regions was regarded until recently as the remaining stronghold, but surveys have revealed drastic declines and local extinctions. Further vulnerable populations exist in Spain (Palomares, 1991; Ruiz-Olmo & Palazón, 1991), France (Chanudet &

Saint-Girons, 1981) and Romania (Gotea & Kranz, 1999).

In contrast to the European mink, polecats are relatively widespread. They are found throughout western Europe as *M. putorius* (L., 1758; western polecat) and to the steppes of east Asia as *M. eversmannii* (Lesson, 1827; steppe polecat). Although still vulnerable to habitat loss and persecution, in some countries, such as Britain, the western polecat is increasing its range (Birks & Kitchener, 1999). A fourth species, the American mink (*M. vison* Schreber, 1777), was introduced after release from fur farms and has been implicated in the decline of European mink through intra-guild aggression. However, no single satisfactory explanation for the European mink's continuing decline has been discovered to date (Maran & Henttonen, 1995; Maran *et al.*, 1998b).

Although morphological differences between European mink and polecats are well defined (Gromov

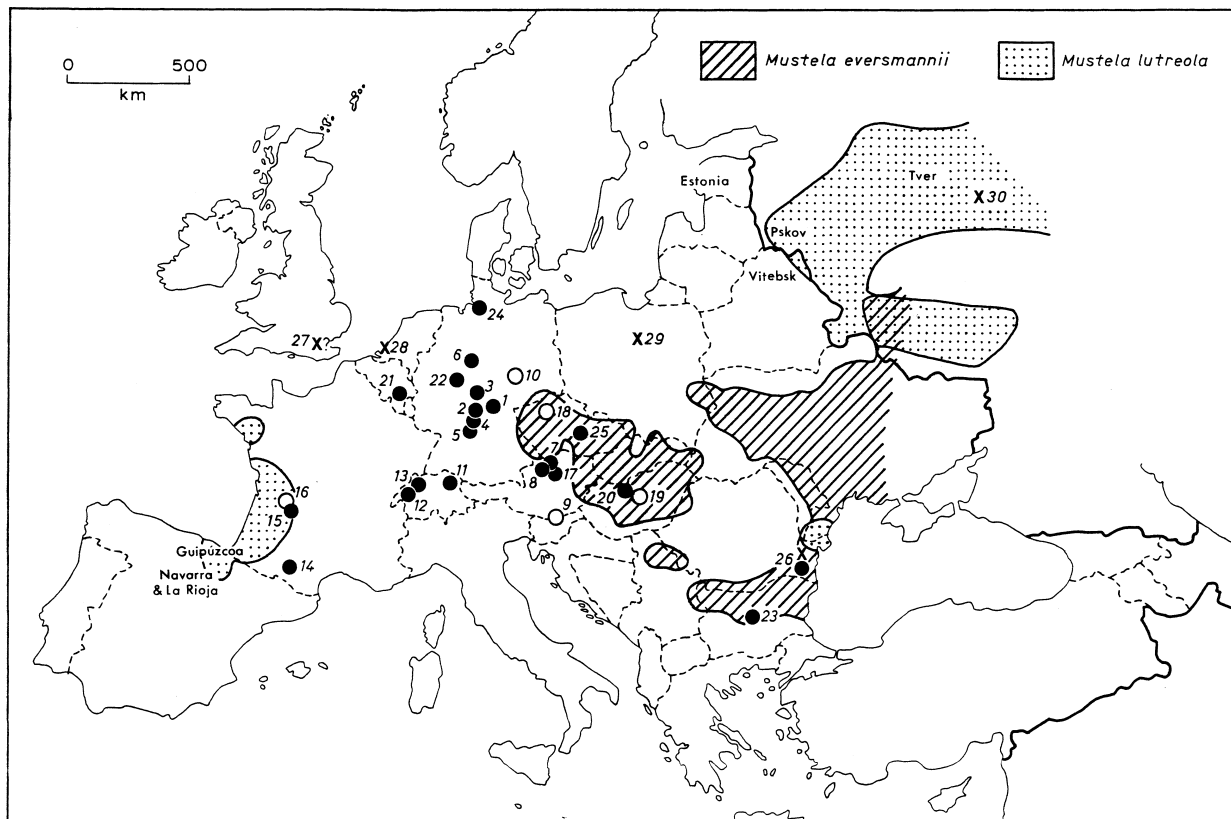
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*et al.*, 1963; Youngman, 1982; Stubbe, 1993), the convergent morphology of European and American mink has meant that both have sometimes been distinguished only as subspecies (Ognev, 1931; Novikov, 1939; Heptner *et al.*, 1967). More recent morphological and molecular phylogenetic analyses place *M. vison* as the most basal member of *Mustela* (Youngman, 1982; Masuda & Yoshida, 1994). However, a mitochondrial phylogeny including both polecat species, black-footed ferrets (*M. nigripes* Audubon & Bachman, 1851), and a single European mink specimen did not resolve the species because the European mink was placed within a polyphyletic polecat group (Davison *et al.*, 1999). This was surprising because European mink have previously been considered to be in the subgenus *Lutreola*, distinct from polecats in the subgenus *Putorius* (Youngman, 1982). The molecular results instead suggest either recent speciation from polecats and/or the effects of hybridization. Both are possible: hybridization between European mink and polecats has been suspected in the

past (Ognev, 1931; Novikov, 1939; Heptner *et al.*, 1967), and the palaeontological data on *M. putorius*, *M. eversmannii* and *M. lutreola* suggest a recent origin for the latter species (Wolsan, 1993a; EUQUAM database – W. v. Koenigswald, pers. comm.).

However, it is worth questioning whether the palaeontological data are of sufficient depth to reveal any differences between the species. In modern Europe, *M. putorius* is generally distributed throughout (Mitchell-Jones *et al.*, 1999). The species first appeared in the Holsteinian Interglacial of the Middle Pleistocene (c. 500 000 years b.p.), and may have been present in the earlier Elsterian Glacial (Wolsan, 1993a). It is comparatively abundant in European Late Pleistocene sites (Fig. 1), many of which are caves and some records suggest human exploitation (Charles, 2000).

*Mustela eversmannii* has a more restricted modern European distribution than *M. putorius*, and is a considerably less common fossil (Fig. 1). The species' fossil record commences in the Holsteinian Interglacial



**Fig. 1.** Modern and Pleistocene distributions of *M. eversmannii* and *M. lutreola* in the western Palearctic (modern distributions (shaded areas) are based on Wolsan, 1993b; Mitchell-Jones *et al.*, 1999; Tumanov, 1999). Pleistocene distributions of *M. eversmannii* (●, Middle Pleistocene; ○, Late Pleistocene and/or Holocene) and of *M. lutreola* (x). *Mustela eversmannii* records: 1, Koenigswald & Müller-Beck (1975); 2, Koenigswald (1984); 3, Stehlin & Graziosi (1935); 4, Heller (1957); 5, Hahn (1991); 6, 7 and 22, Sickenberg (1968); 8, Zapfe (1966); 9, Pohar (1981); 10, Kurtén (1968); 11, 12, 13 and 15, Koby (1951); 14 and 16, Hugueny (1975); 17, Galik (1997); 18, Horáček & Sánchez Marco (1984); 19 and 20, Jánossy (1986); 21, Gautier (1980); 23, Spassov & Popov cited in Wolsan (1993b); 24, Requate (1956); 25, Hokr (1951); 26, Dumitrescu *et al.* (1963). NB several further early records are given by Remane (1970). Further (unprovenanced) Late Pleistocene and Holocene records from Siberia, the Russian Plain and Crimea, the Caucasus and Central Asia are listed by Vereshchagin & Baryshnikov (1984) and from the Late Last Glacial and Holocene of the former FSR Yugoslavia by Malez (1986). *Mustela lutreola* records: 26, Dumitrescu *et al.* (1963); 27, Pitts & Roberts (1996); 28 and 29, Youngman, (1982); 30, Wolsan (1989, 1993b).

(Wolsan, 1993a), covers parts of western and central Europe, and runs eastwards into western Siberia in the Late Pleistocene (Vereshchagin & Kuz'mina, 1984). Most records are from the European Late Pleistocene and the majority are from the Last (Würm or Weichselian) Glaciation. The small number of earlier records are usually ascribed to the larger-bodied *M. evermannii soergeli* Éhik, 1928.

Fossil records of *M. lutreola* are very uncommon, and many reported finds have been based on misidentified material (see Mottl, 1937). For example, Kurtén (1968) and Guérin & Patou-Mathis (1996) both note the presence of *M. lutreola* at Grotte d' Observatoire (Monaco), but this is based on a skull of *M. putorius* (Youngman, 1982). A further, anomalously ancient Cromerian record from the archaeological site at Boxgrove, UK (Pitt & Roberts, 1996) requires further validation. Thus, the only European mink fossils that are known to be valid are an undated, probably Holocene skull from Moscow District, another radiocarbon dated 4300–4100 b.p. from Vlaadingen, the Netherlands (see Youngman, 1982, 1990), material from the Polish Holocene site of Biskupin (Wolsan, 1989), which dates to the Sub-Atlantic (*M.* Wolsan, pers. comm.), and from the Romanian Upper Würmian site of the 'La Adam' Cave (Dumitrescu *et al.*, 1963). All date from within the Last Glaciation or the Holocene, much more recent than the oldest polecat fossils.

Therefore, sampling bias for more recent sites and identification difficulties with fragmentary fossil material may be a real problem. If the fossil record is unreliable, then a genetic analysis could show which species are more ancient, and the evolutionary relationships between them. As mentioned, previously a single European mink specimen was investigated (Davison *et al.*, 1999). It is possible that this animal was actually a recently introgressed hybrid, especially since it was from a region where mink were on the brink of extinction. We have now sequenced a fragment of the mitochondrial cytochrome *b* gene from a further 51 sympatric European mink and polecats from eastern Europe and Spain, as well as a region of the mitochondrial D-loop from representative individuals, in order to understand the relationships within the group, and to assist the conservation of the European mink.

## MATERIALS AND METHODS

### Sampling

European mink samples were collected from Russia (Tver and Pskov regions), Belarus (Vitebsk region), Estonia and Northern Spain (Guipúzcoa, Navarra and La Rioja regions), either from road casualty animals or individuals trapped as part of the European mink captive-breeding programme. Polecats were also collected from the same regions. In addition, polecats (both species) from regions of Europe where European mink are extinct were sampled (see Davison *et al.*, 1999). Details of the numbers sampled from each species and location are

given in Table 1.

A number of suspected western polecat–European mink hybrid animals were also used. Identifications were based on the presence of intermediate pelage characters, judged by two of the authors (T.M. and V.E.S.). Key diagnostic pelage characters for European mink and polecats are dorsal pelage (dark brown and dense in mink, creamy brown and less dense in polecat), mask (no mask in mink, dark mask surrounded by pale fur in polecat), underhair (dark grey in mink, cream/white in polecats), and guardhair (short in mink, long in polecat).

**Table 1.** Cytochrome *b* mitochondrial (Mt) haplotypes of polecats and European mink from eastern Europe and Spain

Population	Species	Mt type (cyt <i>b</i> )	Number
Eastern Europe	<i>M. lutreola</i>	C11	26
		C17	2
		C18	1
		C19	1
		C4	2
	<i>M. putorius</i>	C6	1
		C20	1
		C11	2
	Hybrids	C4	2
		C1	1
		C9	1
	Spain	<i>M. lutreola</i>	C11
C5			1
<i>M. putorius</i>		C11	2
		C5	2
		C21	1

### Mitochondrial DNA sequencing

Genomic DNA was extracted from either liver, blood, or skin and hair specimens, alongside an extraction blank, using Qiagen Blood/Tissue purification kits. Two separate mitochondrial DNA fragments (cytochrome *b* and D-loop) were sequenced, using primers and methods previously described in Davison *et al.* (1999).

### Phylogenetic analysis

Phylogenetic trees were constructed from manually aligned DNA sequences using three methods. A minimum evolution method (neighbour-joining, Kimura 2-parameter) was carried out using PHYLIP (J. Felsenstein, pers. comm.) with a 2:1 transition: transversion ratio. Latterly, a Tamura–Nei distance was used to allow rate variation between sites (calculated using MEGA,  $\alpha$  value set to 0.5; Kumar, Tamura & Nei, 1993). Maximum likelihood methods included five global rearrangements and were also performed using PHYLIP. PAUP v.3.1.1 (Sinauer Associates, MA) was used to identify the most parsimonious tree, using the exhaustive search option where possible; otherwise a heuristic search with the branch swapping option was performed. Trees were bootstrapped 1000 times when the method allowed. The

computer program TREE TOOL was used to help draw trees (Maidak *et al.*, 1994).

Alternatively, the matrix of the number of differences between haplotypes was used to construct a minimum spanning network, with the sequences as nodes of a network instead of the terminal tips of a tree. Minimum spanning networks can be an efficient means of representing relationships between haplotypes when the levels of divergence are low. This was carried out using a program supplied by L. Excoffier (Minspnet: Department of Anthropology, University of Geneva).

## RESULTS

### Morphology

Six animals were identified with pelt characters that were intermediate between European mink and polecats. The characters are diagnostic for the species, so it is likely that these animals were hybrids. To be certain, a more extensive morphological and molecular analysis is necessary. Four of the six hybrids were from Estonia, where

European mink may now be extinct (Maran & Raudsepp, 1994). The remaining two were from the Vitebsk region.

### Mitochondrial analysis

A 337 base-pair (bp) fragment of the mitochondrial cytochrome *b* gene was sequenced from 36 European mink, nine polecats (all *M. putorius*) and six putative European mink/polecat hybrids from Eastern Europe and Spain. The same region was previously sequenced from approximately 100 polecats (including 10 *M. eversmannii* from Mongolia, Serbia and Poland: Davison *et al.*, 1999) and a single European mink. Only five new haplotypes were discovered (C17 to C21). In total, 31 variable sites were present when compared against the *M. itatsi* and *M. sibirica* outgroups (Masuda & Yoshida, 1994), giving rise to 16 different haplotypes between the polecats, European mink and black-footed ferrets. These changes are detailed in Table 2. (Note: haplotypes C12 to C16 are not included since they were assigned to more distant taxa in Davison *et al.*, 1999.) The mean sequence variation between all pairs of haplotypes was 1.2%

**Table 2.** Alignment of the variable positions of the cytochrome *b* haplotypes

Haplotypes	14	17	23	25	29	43	47	49	118	133	166	169	179	187	196	202
C1	T	A	T	A	G	T	G	A	C	A	C	C	G	T	T	A
C2	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.
C3	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.
C4	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.
C5	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.
C6	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.
C7	.	.	C	.	A	.	.	.	.	.	.	.	.	.	.	.
C8	.	.	C	.	.	.	.	.	.	.	T	.	.	.	.	.
C9	.	.	C	.	.	.	.	.	.	.	T	.	.	.	.	.
C10	.	.	C	.	.	.	.	.	.	.	T	.	.	C	.	.
C11	C	.	C	.	.	.	.	.	.	.	T	.	.	.	.	.
C17	C	.	C	.	.	.	.	.	.	.	T	.	.	.	.	G
C18	C	.	C	.	A	.	.	.	.	.	T	.	.	.	.	.
C19	.	.	C	.	.	.	.	.	.	.	T	.	.	.	.	.
C20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C21	.	.	C	.	.	.	A	.	.	.	.	.	.	.	.	.
<i>M. itatsi</i>	.	.	.	.	.	.	.	G	T	G	T	.	.	.	C	.
<i>M. sibirica</i>	.	G	C	T	.	C	.	.	.	.	T	T	.	.	C	.

Haplotypes	205	211	223	235	250	256	259	262	265	274	280	285	310	315	322
C1	C	C	T	T	G	A	A	G	T	T	T	T	C	T	G
C2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C5	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
C6	.	.	.	.	.	.	G	.	.	.	.	.	.	C	.
C7	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
C8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C9	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.
C10	.	.	.	C	A	G	G	T	C	.	.	C	.	.	.
C11	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
C17	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
C18	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
C19	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
C20	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.
C21	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
<i>M. itatsi</i>	T	T	C	.	A	.	G	A	C	C	C	.	.	.	A
<i>M. sibirica</i>	.	.	.	C	A	.	G	A	.	C	.	.	.	.	.

A dot indicates identity with the first sequence. Haplotypes C1 to C11 were first reported in Davison *et al.* (1999); C10 is *Mustela nigripes*. The *M. itatsi* and *M. sibirica* sequences are from Masuda & Yoshida (1994).

(range 0.3–3.6%, mean 0.9% excluding black-footed ferret). The majority of European mink possessed haplotype C11 (32 out of 37 individuals; see Table 1), which was also identified in the Spanish *M. putorius*.

Phylogenetic trees were constructed from the mink and polecat cytochrome *b* sequences using *M. sibirica* and *M. itatsi* sequences as an outgroup. The neighbour-joining tree using Tamura–Nei  $\gamma$  corrected distances is shown in Fig. 2(a). All of the haplotypes identified in European mink were either shared with polecats or fell within the polecat group (i.e. including *M. putorius* and *M. evermannii* specimens). The low sequence divergence is reflected in the lack of bootstrapping support for the branches (Fig. 2(a)).

Support for the cytochrome *b* tree was tested by sequencing a D-loop fragment from at least one representative of each species from the cytochrome *b* haplotypes. This resulted in 10 new haplotypes (D15 to D24), in addition to the 14 reported in Davison *et al.* (1999). The variable positions are shown in Table 3. The hyper-variable C<sub>n</sub>T<sub>n</sub> array was not used in the phylogenetic analysis because it was not possible to unambiguously align all individuals, although some pairs were obviously similar (e.g. D7 and D8, which differed by only a single C insertion). Sixteen variable D-loop haplotypes remained after removal of the C<sub>n</sub>T<sub>n</sub> array. The neighbour-joining tree constructed using Tamura–Nei  $\gamma$  corrected distances is shown in Fig. 2(b). Three reasonably supported clades are present, one of which corresponds to the main European mink group in Fig. 2(a) (C19, C18, C17, C11). However, the monophyly of each species may be illusory because of the limited and biased sampling procedure. For instance the D-loop of the European mink with haplotype C5 was unfortunately not sequenced. Maximum likelihood and parsimony trees resulted in trees of similar, although not identical, topology.

Figure 3(a) shows the distribution of cytochrome *b* haplotypes by geographical region in the minimum spanning network. Some region-specific haplotypes are present, but other haplotypes are clearly widespread across Europe: in Spain and eastern Europe, or shared between Spain/eastern Europe and another country. Figure 3(b) shows the distribution of haplotypes by species. Again, some haplotypes were shared between species, even discounting C1, C4 and C9 which were shared polecat/hybrid haplotypes. Other haplotypes were found only in one species. The D-loop network showed a similar pattern: haplotypes do not fully separate by country, although they may separate by species. Again, this may be a sampling artifact.

## DISCUSSION

### The evolution and biogeography of polecats and European mink

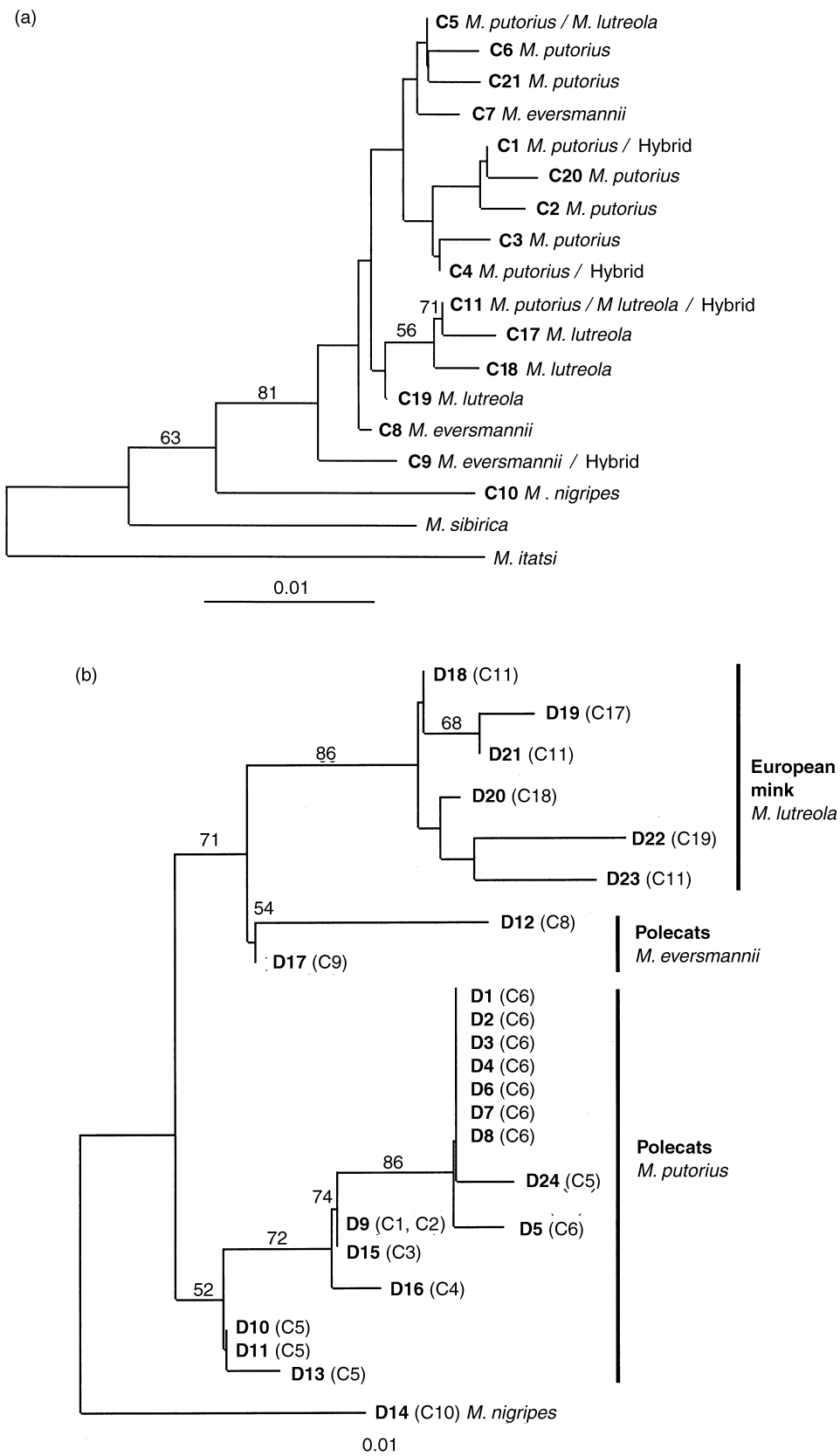
European mink have previously been considered to be members of the subgenus *Lutreola* within the genus *Mustela* (with *M. sibirica*, *M. itatsi* and *M. nudipes*), dis-

tinct from polecats in the subgenus *Putorius* (Youngman, 1982). We carried out a genetic analysis of inter- and intra-specific mitochondrial variation in eastern European/Spanish *M. lutreola* and *M. putorius*. The study confirms the earlier finding (Davison *et al.*, 1999) that polecats are the most closely related congener to European mink, perhaps through reticulate evolution (hybridization). Phylogenetic methods failed to resolve the relationships between species (Fig. 2) because each species was not reciprocally monophyletic. Some haplotypes were shared between species (Fig. 3(a), Table 1), and others between countries (Fig. 3(b), Table 1). Only the analysis of D-loop sequences showed some degree of monophyly for European mink (Fig. 2(b)). However, the degree to which the results may have been affected by sampling bias is uncertain, particularly since it was not possible to obtain specimens from threatened populations in France and Romania. In addition, sampling of steppe polecats was poor. European mink are sympatric with western polecats over most of their range, but interactions with steppe polecats may be particularly frequent in central Europe.

The failure to resolve the species relationships is strongly suggestive of either a recent origin of one or all of the species, or else a result of introgressive hybridization, as has occurred between Scandinavian mountain and brown hares (Thulin, Jaarola & Tegelstrom, 1997). Conceivably, both processes are implicated. It is unlikely that the explanation lies solely in the slow evolution of the mustelid cytochrome *b* gene, because it has proved useful in resolving relationships within other closely related mustelid species groups, such as otters, martens and weasels (Carr & Hicks, 1997; Koepfli & Wayne, 1998; Davison *et al.*, 1999). In contrast to the polecat/European mink situation, in other European mammals distinct lineages *within* species have frequently been found, presumed to have arisen through isolation in glacial refugia (Taberlet & Bouvet, 1994; Hewitt, 1999). Often, the degree of differentiation, both molecular and non-molecular, is sufficiently great to warrant distinct subspecies or species status (Beltran, Rice & Honeycutt, 1996; Santucci, Emerson & Hewitt, 1998).

The polecat and mink fossil record is poorly known, probably due to the limited use of mustelids in biostratigraphy, and their overall paucity in the Quaternary (see Introduction, above). Consequently, it is difficult to establish when each species arose. A direct comparison of the appearance of fossil *M. lutreola* and the time to coalescence of the mitochondrial lineages is impractical, because of large errors in the estimation of both, primarily due to stochastic effects and other problems with calibrating the molecular clock (Hillis, Moritz & Mable, 1996; Avise, 2000). The situation has probably been further confused by hybridization. However, since a single haplotype (C11) predominated in the sampled European mink from Spain and eastern Europe, the simplest interpretation is that the present-day animals have a relatively recent common origin. This does not preclude an ancient origin for European mink.

We have now sampled polecats (both species) or



**Fig. 2.** Neighbour-joining trees of haplotypes based upon Tamura–Nei distances. Bootstrap values (>50% only) are shown below each node. Haplotypes C1 to C11 and D1 to D14 are from Davison *et al.* (1999). C17 to C21 have GenBank accession numbers AF207712 to AF207716 and D15 to D24 have GenBank accession numbers AF207717 to AF207726. (a) Tree constructed using cytochrome *b* sequences, with the haplotype and species at each tip. ‘Hybrids’ are putative polecat–European mink animals, identified by their morphology. The *M. sibirica* and *M. itatsi* sequences are from Masuda & Yoshida (1994). (b) Tree constructed using D-loop sequences with the haplotype (and corresponding cytochrome *b* haplotype) at each tip.

**Table 3.** Alignment of the unique D-loop haplotypes, including the variable sequence positions and all bases of the C<sub>n</sub>T<sub>n</sub> repeat region (bases 147–174)

Haplotype	1	29	58	98	104	111	115	116	121	129	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164		
D1	T	T	T	T	T	T	A	T	T	G	T	C	T	T	T	T	T	-	-	-	-	-	-	C	T	T	T	T		
D2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
D3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
D4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
D5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
D6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
D7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
D8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
D9	.	A	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	
D10	.	A	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	C	.	.	-	-	-	
D11	.	A	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	T	.	.	.	.	.	C	.	.	-	-	-	
D12	.	A	C	.	.	.	C	.	.	C	.	.	.	-	-	-	C	C	C	C	T	C	C	.	.	-	C	-		
D13	.	A	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	C	.	.	-	-	-	
D14	.	A	.	.	.	.	C	C	.	.	.	.	.	-	-	-	T	.	.	.	.	.	.	.	.	-	-	-	.	
D15	.	A	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	-	-	-	.	
D16	.	A	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	-	-	-	.	
D17	.	A	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	-	-	-	.	
D18	C	A	.	C	.	.	C	.	.	.	.	.	.	-	-	-	T	T	T	T	.	.	.	.	-	-	-	-	.	
D19	C	A	.	C	.	.	G	C	.	A	.	.	.	.	.	.	T	T	T	T	.	.	.	.	-	-	-	-	.	
D20	C	A	.	C	.	.	C	.	.	.	.	.	.	.	.	.	T	T	T	T	.	.	.	.	-	-	-	-	.	
D21	C	A	.	C	.	.	C	.	.	A	.	.	.	.	.	.	T	T	T	T	.	.	.	.	-	-	-	-	.	
D22	C	A	.	C	C	C	.	C	.	.	.	.	.	.	.	.	T	T	T	T	.	.	.	.	-	-	-	-	.	
D23	C	A	.	C	.	.	C	.	.	.	.	.	.	.	.	.	T	T	T	T	.	.	.	.	-	-	-	-	.	
D24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	.

Haplotype	165	166	167	168	169	170	171	172	173	174	178	224	225	228	229	233	254	255	259	264	268	270	301	316	342	355	360	372	
D1	T	T	T	T	-	C	C	C	C	C	A	C	A	T	T	A	C	T	T	C	G	G	C	A	A	T	C	T	
D2	.	.	-	-	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D3	-	-	-	-	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D4	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.
D6	.	.	.	.	T	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D7	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D8	.	.	.	.	T	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D9	-	-	-	-	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D10	-	-	-	-	.	-	-	-	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	C	.
D11	-	-	-	-	.	-	-	-	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	C	.
D12	-	-	-	-	.	-	-	-	.	T	.	.	.	.	.	G	T	.	C	.	.	.	.	.	.	.	C	T	C
D13	-	-	-	-	.	-	-	-	.	.	.	G	.	.	.	G	.	.	C	.	.	.	.	.	.	.	C	.	C
D14	.	.	.	.	T	-	.	.	.	.	.	.	.	.	.	G	.	.	.	T	A	A	T	G	.	C	.	C	
D15	-	-	-	-	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D16	-	-	-	-	.	-	-	-	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D17	-	-	-	-	.	-	-	-	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	C	.	C
D18	-	-	-	-	.	-	-	-	.	.	T	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	C	.	C
D19	-	-	-	-	.	-	-	-	.	.	T	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	C	.	C
D20	-	-	-	-	.	-	-	-	.	.	T	.	.	.	.	G	T	.	.	T	.	.	.	.	.	.	C	.	C
D21	-	-	-	-	.	-	-	-	.	.	T	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	C	.	C
D22	-	-	-	-	.	-	-	-	.	.	T	.	.	.	.	G	T	C	.	T	.	.	.	.	.	C	.	C	
D23	-	-	-	-	.	-	-	-	.	.	T	.	.	.	.	G	T	C	.	.	.	.	.	.	.	.	C	.	C
D24	-	-	-	-	T	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	.	C

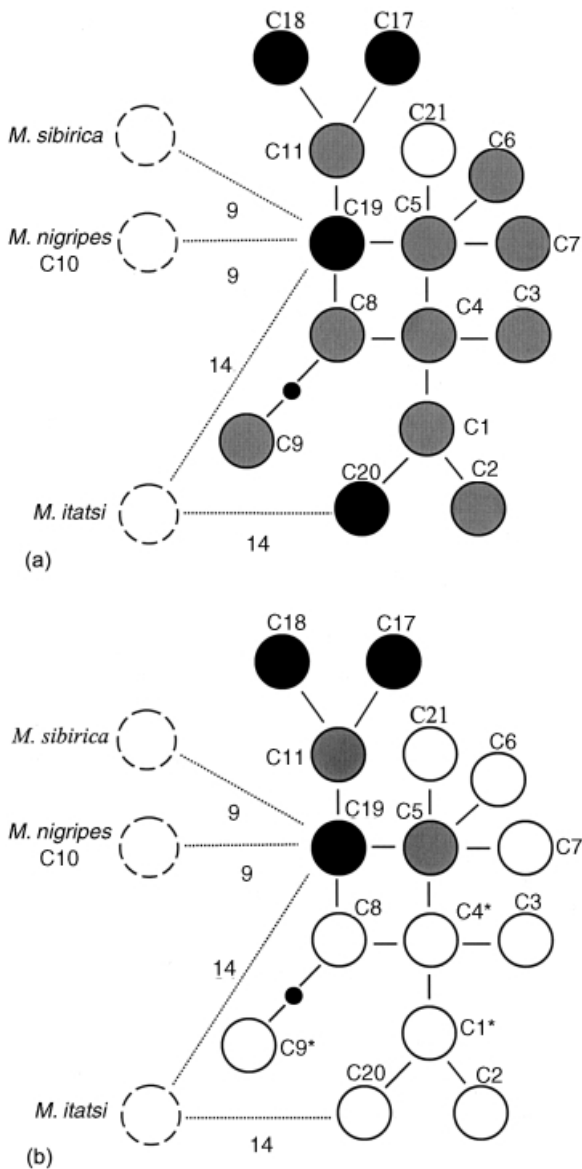
A dot indicates identity with the first sequence; a hyphen indicates a deletion. Haplotypes D1 to D14 were first reported in Davison *et al.* (1999).

European mink from Belarus, Estonia, Germany, Mongolia, Poland, Russia, Serbia, Slovenia, Spain, Turkey and the UK (the USA as well, if the black-footed ferret is included), yet have found very little sequence divergence. There is no evidence that distinct lineages have survived in southern European refugia over many glaciations, yet, in other species one of three broad genetic patterns has usually been found, a result of the post-glacial recolonization of Europe (Hewitt, 1999). The examples of the ‘grasshopper’, ‘hedgehog’ and ‘bear’ have been described recently as paradigms (Hewitt, 1999). Perhaps, with further investigation, the situation in polecats and European mink will be similar to that of the wolf, where both species (grey wolf and

coyote) demonstrate little differentiation of haplotypes within species, even over continents (Vila *et al.*, 1999). This is presumably because they disperse over great distances in search of territories or mates. Conceivably, this kind of differentiation (or lack thereof) should be considered a fourth paradigm, after Hewitt (1999).

**Implications for the conservation of European mink**

The results show that the European mink may have speciated relatively recently, or else there has been frequent hybridization. If the former is true, then reciprocal monophyly would not be expected because insufficient



**Fig. 3.** Minimum spanning networks constructed using cytochrome *b* sequences. All haplotypes differ by single steps, except between the species *M. sibirica*, *M. itatsi* and *M. nigripes* and the rest, where the number of steps is indicated next to the dotted line. The small filled circle between C8 and C9 represents a hypothesized haplotype. (a) By region. Open circles are haplotypes found in Spain only; black circles are haplotypes found in eastern Europe only; grey circles are haplotypes shared between Spain and eastern Europe or one of these and other sites in Europe. (b) By species. Open circles are haplotypes found in polecats only; black circles are haplotypes found in European mink only; grey circles are haplotypes shared between mink and polecats. Haplotypes C1, C4 and C9 (indicated by an \*) were shared between polecats and putative European mink–polecat hybrids.

generations have elapsed since speciation. For the latter, monophyly may have been obscured by introgression.

The study highlights the problem in defining Evolutionarily Significant Units (ESUs) for conservation (Moritz, 1994), based on a single molecular genetic

marker, especially since mitochondrial DNA may easily introgress between taxa. Combining ecological and genetic data is preferred (Crandall *et al.*, 2000). In this case, polecats and European mink are easy to distinguish from their morphology (Gromov *et al.*, 1963; Youngman, 1982; Stubbe, 1993), and have distinct ecologies and behavioural preferences (Wolsan, 1993*b,c*; Maran *et al.*, 1998*a,b*; Sidorovich *et al.*, 1998; Sidorovich, Kruuk & Macdonald, 1999). Both polecat species and European mink should be considered as separate ESUs.

A mitochondrial study such as this could fail to identify ‘proper’ ESUs when investigating recently evolved, or hybridizing, cryptic species. The use of further markers, such as rapidly evolving microsatellites, on both the polecats/mink or any hypothetical cryptic species, would enable identification of ESUs. It could also help understand whether European mink have a recent origin or, alternatively, an ancient origin with partial genomic introgression (including the mitochondria). For the latter, we would expect at least a proportion of markers to be quite divergent. A caveat, however, is that in hybridizing sympatric species, even if hybridization is relatively rare, introgression of neutral alleles may be so great that only those loci under selection remain differentiated between the species (Goodman *et al.*, 1999). Finally, many of the legal issues arising from hybridization have been discussed with respect to the origins of the red wolf (*Canis lupus*; Brownlow, 1996).

More problematical is whether European mink from eastern Europe (Belarus, Estonia and Russia) and Spain should be considered a single Management Unit (*sensu* Moritz, 1994), depending upon whether they meet the defined criteria of differences in haplotype frequencies. As in a recent study of the red squirrel (Barratt *et al.*, 1999), the problem of identifying differentiation when sampling rare alleles in a rare species becomes clear. We found that a single cytochrome *b* haplotype was shared between most European mink individuals, yet some population specific alleles were also present. The chances of finding further population specific alleles could only increase if we had sequenced the D-loop fragment across all individuals. Whether any haplotypes are indeed population specific could only be addressed with further extensive sampling. If this was augmented by an analysis of nuclear markers and fitness related phenotypic differences (Hedrick, 1999), then it would be possible to strictly define Management Units.

In the meantime, it is likely that European mink from Estonia are already completely extinct. The management of the captive bred populations can not wait for further molecular analyses. Following the precautionary principle, mink from the two regions for the moment should be managed separately, since differentiation in haplotype frequencies is likely. It is also possible that the animals are locally adapted, so that outbreeding depression (Lynch, 1991) could result from some mating combinations, especially once the animals were released into the wild. A priority for future studies must be to investigate *M. lutreola* from the remaining isolated populations in



Romania and France, as well as any animals to be used for reintroductions.

At an individual level, six possible hybrid animals were identified by their morphology in this study, and hybridization between European mink and polecats has frequently been suspected in the past (Ognev, 1931; Novikov, 1939; Heptner *et al.*, 1967). Whether interactions of European mink with polecats, by hybridization or through better adaptation to an intensively farmed landscape (Maran *et al.*, 1998b; Sidorovich, 2000), are directly implicated in the decline of the former is debatable. It is unknown whether hybrids are maladapted to either or both ecological niches, or whether outbreeding depression operates (Lynch, 1991). Perhaps incorrectly, extinction by hybridization is not usually cited as a major cause of species loss (Rhymer & Simberloff, 1996). The key factors that may threaten European mink survival, such as interactions with American mink and habitat degradation, are increasing and could act synergistically with polecat hybridization. Therefore, whilst polecats are almost certainly not the key factor in the catastrophic decline of the European mink, introgressive hybridization may be detrimental to attempts to save small, local populations, living at low density.

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