Molecular Ecology (2001) **10**, 2479–2488

Mitochondrial phylogeography and population history of pine martens *Martes martes* compared with polecats *Mustela putorius*

ANGUS DAVISON,*†§ JOHNNY D. S. BIRKS,† RACHAEL C. BROOKES,*† JOHN E. MESSENGER† and HUW I. GRIFFITHS‡

*Institute of Genetics, Queen's Medical Centre, University of Nottingham, Nottingham, NG7 2UH, UK, †The Vincent Wildlife Trust, 3 & 4 Bronsil Courtyard, Eastnor, Ledbury, Herefordshire, HR8 1EP, UK, ‡Department of Geography, Faculty of Science, University of Hull, Kingston-upon-Hull, HU6 7RX, UK

Abstract

The flora and fauna of Europe are linked by a common biogeographic history, most recently the Pleistocene glaciations that restricted the range of most species to southern refugial populations. Changes in population size and migration, as well as selection, have all left a signature on the genetic differentiation. Thus, three paradigms of postglacial recolonization have been described, inferred from the patterns of DNA differentiation. Yet some species, especially wide-ranging carnivores, exhibit little population structuring between the proposed refugia, although relatively few have been studied due to the difficulty of obtaining samples. Therefore, we investigated mitochondrial variation in pine martens, *Martes martes*, in order to understand the extent to which they were affected by glacial cycles, and compared the results with an analysis of sequences from polecats, *Mustela putorius*. A general lack of ancient lineages, and a mismatch distribution that is consistent with an expanding population, is evidence that the present-day *M. martes* and *Mu. putorius* in central and northern Europe colonized from a single European refugium following a recent glaciation. There has also been interspecific mitochondrial introgression between *M. martes* and the sable *M. zibellina* in Fennoscandia.

Keywords: conservation genetics, control region, introgression, mitochondrial DNA, phylogeography, pine marten

Received 7 February 2001; revision received 14 June 2001; accepted 11 July 2001

Introduction

The flora and fauna of Europe are linked by a common biogeographic history, most recently the Pleistocene glaciations that restricted the range of most, if not all, species to southern refugial populations (Taberlet *et al*. 1998; Hewitt 1999). Changes in population size and migration, as well as selection, all left a signature on the genetic differentiation that is present today. However, the exact nature of the differentiation depends upon the species and their dispersal abilities. Thus some species, in particular those of low mobility, split into separate races and even species, originating from the refugia in which they diverged. Wherever they meet and mate, the races now form hybrid zones with varying degrees of introgression. Many of these zones cluster in the Alps, central Europe, the north Balkans and the Pyrenees, because the postglacial expansion was limited by mountains to the north of the refugium (Taberlet *et al*. 1998; Hewitt 1999). Another major cluster of zones is in central Sweden, probably produced by the final melting of the Scandinavian ice cap, allowing colonists from the east and west to meet (Jaarola *et al*. 1999).

Thus, Iberia (Spain and Portugal), Italy, the Balkans and the Caucasus have been proposed as the main European refugia, and three paradigms of postglacial colonization have been described, inferred from the patterns of DNA differentiation (Hewitt 1999). Common to all three, the Balkans were apparently the source for most populations to the east, and many in the west. In the meadow grasshopper *Chorthippus parallelus* (paradigm no. 1), individuals have a similar genetic background across the whole of Europe,

Correspondence: A. Davison. §Present address: Biological Institute, Graduate School of Science, Tohoku University, Aramaki-Aza-Aoba, Aoba-ku, Sendai 980–8578, Japan. Tel/Fax: 81 22 2177813; E-mail: a.davison@hgmp.mrc.ac.uk

including the Balkans, except in Turkey, Greece, Italy and Spain, which have a large proportion of unique haplotypes (Lunt *et al*. 1998). This implies that an expansion from the Balkans colonized most of Europe, and that the climatic conditions, or the barrier of the mountains, prevented populations in other refugia from migrating. Alternatively, sequences from the west European hedgehog *Erinaceus europeus* (paradigm no. 2) imply colonization from three glacial refugia: a western/Spanish one, a central/Italian one and an eastern/Balkan one (Santucci *et al*. 1998). Finally, the brown bear *Ursus arctos* (paradigm no. 3) appears to have colonized most of Europe from two refugia, in Iberia and the Caucasus/Carpathians (Taberlet *et al*. 1994, 1995).

Yet some species, especially wide-ranging carnivores and birds, exhibit little population structuring across Europe, so that the same mitochondrial haplotype may sometimes be recovered between very distant sites (Vila *et al*. 1999; Walker *et al*. 2001). Perhaps a fourth paradigm is warranted (Davison *et al*. 2000), in taxa where one genetic lineage recently colonized the whole of Europe, presumably because the other lineages went extinct in the other refugia (Vila *et al*. 1999; Cassens *et al*. 2000; Walker *et al*. 2001). Clearly, further examples are required. One problem with carnivores is that it is difficult to obtain samples, especially if they are endangered. Some carnivores are also rare or absent from the main proposed refugia, perhaps because the habitat is not currently suitable. Consequently, with some exceptions (Taberlet *et al*. 1994, 1995; Beltran *et al*. 1996; Vila *et al*. 1999; Waits *et al*. 2000; Walker *et al*. 2001) carnivores have been least studied genetically.

We have therefore undertaken a study on pine martens, *Martes martes*, using mitochondrial control region sequences, and the results are compared with a further analysis of sequences from polecats, *Mustela putorius*, as well as the European mink *Mustela lutreola* (Davison *et al*. 1999, 2000). The ultimate aim is to advise on units for the conservation of martens in Britain and Europe.

M. martes are a circumboreal species, remaining widespread throughout most of western Europe, including Fennoscandia, but excluding parts of the Low Countries (Mitchell-Jones *et al*. 1999). They are present, though uncommon, in Northern Spain and along the border with Portugal. They are rare or absent in much of the rest of southern Europe, with many of the records old and impossible to verify, including Greece (absent), Slovenia (rare), Croatia (rare), Serbia (extremely rare), Bulgaria (extremely rare), Albania (extremely rare), Turkey (may be present on the Black Sea coast, in Anatolia). Finally, many of the island populations were introduced up to 1000 years ago, for their fur, including to the Balearics and possibly Corsica, Sardinia and Ireland (Yalden 1999). In northern and central Europe, *M. martes* are broadly sympatric with beech martens, *M. foina* (Mitchell-Jones *et al*. 1999), though the latter are much more common in the south. *M. martes* and *M. foina* do not hybridize, as far as is known.

Further east, the data on species distributions are sketchy (Anderson 1970; Bakeyev & Sinitsyn 1994). The sable *M. zibellina* is distinguished from *M. martes* by pelt and skull characters (Anderson 1970), but is not present in western Europe (Mitchell-Jones *et al*. 1999). It replaces *M. martes* at some point east of the Ural mountains, and the two species may hybridize when they meet (Grakov 1994). *M. zibellina* is replaced by *M. flavigula* (yellow throated marten) in East Asia and *M. melampus* in Japan (Japanese marten). In North America, two species are present, *M. pennanti* (fisher) and *M. americana* (American pine marten), although the latter may actually be two species that occasionally hybridize, coastal/western *M. a. caurina* and continental/eastern *M. a. americana* (Carr & Hicks 1997; Demboski *et al*. 1999).

The situation is similar with polecats, which also have a wide European distribution. *Mu. putorius* (European polecat) is replaced by the steppe polecat *Mu. eversmannii* in the east. The degree of sequence divergence between the two and North American *Mu. nigripes* (black-footed ferret) is slight (Davison *et al*. 1999). In contrast, European mink *Mu. lutreola* are limited to Russia, France and northern Spain, and Romania (Davison *et al*. 2000). Morphological and molecular analyses strongly suggest that *Mu. lutreola* hybridizes with *Mu. putorius* (Heptner *et al*. 1967; Davison *et al*. 2000).

Materials and methods

Sampling

Samples of 139 *Martes martes* were obtained from 14 European countries (Table 1; approximate location shown in Fig. 1). Since martens are hunted for their fur in some northern European countries, relatively large numbers could be collected. In southern European countries, such as Spain, martens are either protected or very rare, so occasional road traffic accident animals or museum specimens in alcohol were used. Fewer samples were available. We attempted to keep the distance between samples within a single population to a minimum, relative to the distance between populations. The shortest distance between population samples was approximately 300 km (Germany/the Netherlands).

In England and Wales, marten samples were very difficult to collect. Six animals were sampled during the 1990s from England (Jefferies & Critchley 1994; Birks *et al*. 1997), and latterly one from Wales using a faecal sample (Davison *et al*. 2001). In addition, six samples were also taken from captive stock in two British wildlife parks. The stud book suggests that these animals originated from the former Czechoslovakia. The DNA sequences from these latter animals were excluded from the population structure analysis.

Table 1 Source, sample size and haplotype of *Martes martes*

Country	Haplotypes	Collector
Czechoslovakia* (*n* = 6)	g (2), b (4)	Jan Boast, Derek Gow, Jane Howard
England (*n* = 6)	a (4), w, x	JDSB, Colin Simms, Andrew Kitchener
Finland (*n* = 13)	k, b (2), s (2), v (8)	E. Pulliainen, Ahti Karusalmi
France (*n* = 3)	c (2), u	Thierry Lodé, Géraldine Veron
Germany (*n* = 10)	b (4), l, m (3), d (2)	Ruediger Schröpfer
Ireland (*n* = 9)	p (9)	Jim Fox, John Higgins, Andrew Kitchener, Congella McGuire, Paddy Sleeman, Pat Smiddy
Italy (*n* = 3)	f, e, k	Ettore Randi, V Ferbo
Latvia (*n* = 7)	k, d (2), j, t, i (2)	Valdis Pilats
Netherlands (*n* = 10)	l (6), m (2), u (2)	Gerhard Müskens
Scotland (*n* = 52)	a (52)	Andrew Kitchener, Clive Craik, David Balharry, John Dallas, Barry Constantine
Spain (*n* = 1)	i	Xavier Domingo-Roura
Slovenia (*n* = 2)	h, *n*	Boris Krystufek
Sweden (*n* = 16)	b, m (3), o (8), q, s (2), r	Per Larssen
Wales (*n* = 1)	a	George Sanders

*Captive animals from New Forest Nature Quest and Willer's Mill Wild Animal Sanctuary, UK.

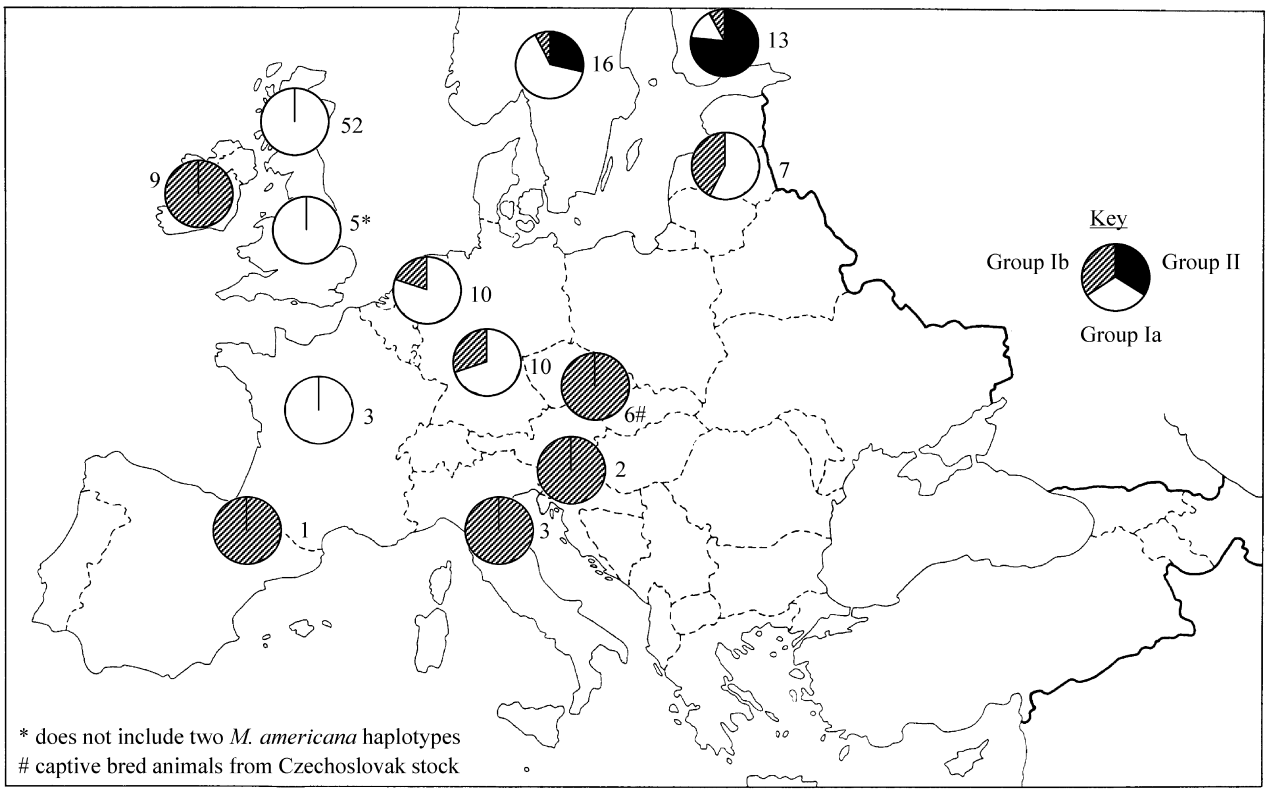

Fig. 1 The distribution of *Martes martes* mitochondrial lineages in Europe. See text for explanation. Sample sizes are also shown.

Molecular analyses

Genomic DNA was extracted from either liver, blood, or skin and hair specimens, alongside an extraction blank, using Qiagen Blood/Tissue purification kits. Nucleon phytopure™ kits were used to extract DNA from the faecal sample, described in detail in Davison *et al*. (2001).

Two separate mitochondrial DNA (mtDNA) fragments, control region and cytochrome *b*, were amplified, resulting in approximately 320 and 364 base pairs of sequence, respectively.

The methods, along with the cytochrome *b* primers, have been described previously in Davison *et al*. (1999). For the control region amplification, the primers L15774 and H16498 were used initially (Shields & Kocher 1991). However, this combination did not always yield a clean polymerase chain reaction (PCR) product, so a slightly smaller product was amplified using either LRCB1 (5′-TGGTCTTGTAAACCAAAAATGG-3′) or LRCB3 (5′-AGACTCAAGGAAGAAGCAAC-3′) and H16498. Samples were screened for sequence variation in the control region using a combination of single-strand conformation polymorphism analysis (SSCP) on PCR amplified material (Sunnucks *et al*. 2000), and DNA sequencing. Where possible, at least three haplotypes were sequenced from each haplotype, and common haplotypes were sequenced more frequently. Nucleotide sequences were determined using either the dRhodamine or Big Dye ABI sequencing kit (Applied Biosystems), and aligned by eye.

Arguably, there has been near complete mitochondrial introgression between *Mustela putorius* and *Mustela lutreola* (Davison *et al*. 2000). Therefore, *Mu. putorius* and *Mu. lutreola* haplotypes were analysed together, because they might represent a single evolutionary unit. Of course, this may not be justified, so variation in the *Mu. putorius* sequences was also analysed separately. There was insufficient sequence variation to do the same with *Mu. lutreola*. A similar problem was encountered with the marten sequences, with probable introgression between *Martes zibellina* and *M. martes*. Thus, the analyses were performed on all sequences together and also a selection of sequences (see Results for further justification).

Descriptive statistics

Nucleotide diversity, π, was estimated using the program DNASP (Rozas & Rozas 1997). The D* and F* tests, first suggested by Fu & Li (1993) and also implemented in DNASP, were used to test the hypothesis that control region variation does not differ from neutral expectations. The tests compare the number of singleton mutations to the total number (D*), and the average number of nucleotide differences between pairs of sequences (F*), both under a neutral model. Statistical significance was determined by comparison of the measured value with the values of Fu & Li (1993).

The relationship between unique haplotypes was described using a minimum spanning network, with the sequences as nodes of a network instead of the terminal tips of a tree. Networks are useful when many of the sequences may be derived from the same ancestral genotype. The algorithm used was MINSPNET, a program within ARLEQUIN 2.0 (Schneider *et al*. 2000).

Phylogenetic analyses

Phylogenetic trees were constructed using the neighbour-joining method with PAUP* version 4 (Swofford 1998). Multiple hits were corrected using the general time-reversible (GTR) model. The rate matrix, base frequencies, proportion of invariant sites and shape parameter (α) of the gamma distribution (based on 16 rate categories) were estimated using likelihood, by iteration from an initial neighbour-joining tree. Parameters estimated from the initial tree were used to make a new neighbour-joining tree. The parameters were then re-estimated, and the process was repeated until there was no further improvement in likelihood. Bootstrap values were then calculated using 1000 replicates. Maximum likelihood methods were also performed on the control region sequences, using DNAML in PHYLIP (Felsenstein 1993). Tree searching for maximum parsimony used a heuristic procedure with tree–bisection–reconnection branch swapping.

Analyses based on coalescent theory

Population bottlenecks and expansions, selective sweeps on the mtDNA, and mutational rate heterogeneity may all result in a Poisson distribution of substitutional differences between pairs of haplotypes (Slatkin & Hudson 1991; Rogers & Harpending 1992). Therefore, the 'mismatch distribution' of differences was calculated in DNASP (Rozas & Rozas 1997), and compared with a fit to the Poisson model.

Analyses of population structure

Population genetic structure was analysed for samples with five or more individuals, with an analogue of F_{ST}, ϕ_{ST}, as implemented in an analysis of molecular variance (AMOVA in ARLEQUIN 2.0; Schneider *et al*. 2000). The method estimates the proportion of genetic variation at different hierarchical levels, using information from the geographical distribution of haplotypes and the pairwise distances between them. The covariance components of variation were calculated among populations and within populations. The significance of estimates was determined by a Markov chain analysis (Schneider *et al*. 2000). All multiple tests of significance were corrected by the 'sequential Bonferroni' method (Rice 1989).

Evidence for an association of geographical distance with genetic distance was tested by plotting a regression of ϕ_{ST} values against ln (geographical distance), with significance determined by a randomization (Mantel) test in the program RT (Mantel 1967; Manly 1997). The geographical distances were estimated using 'Distance Finder' (http://www.indo.com/distance).

Results

Phylogenetic analyses

Twenty-five control region sequences from 139 martens fell into three major clades (groups I, II and III) in a

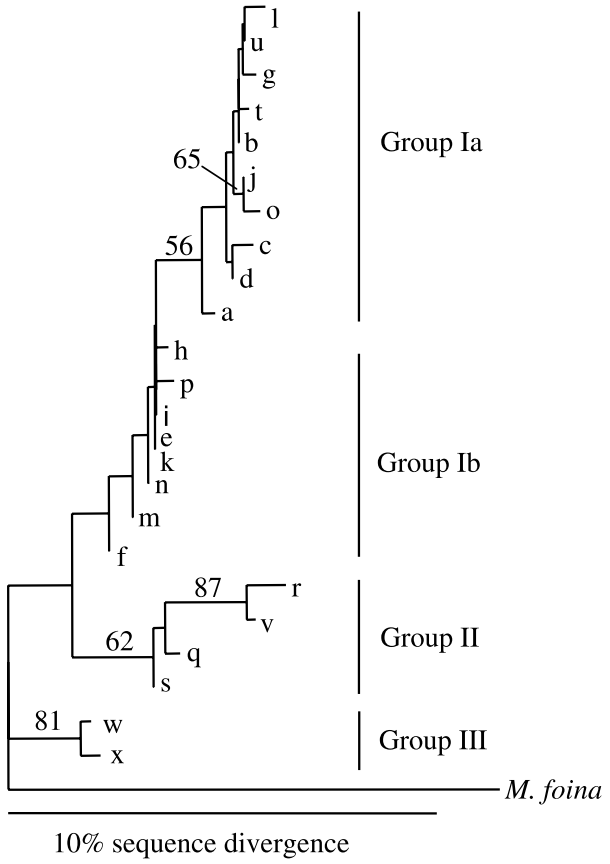

Fig. 2 Neighbour-joining control region tree for martens (gamma shape parameter of $\alpha = 0.16$; transition/transversion ratio = 72). Bootstrap support is shown for nodes found in greater than 50% of 1000 trees. The sequences have GenBank accession numbers AF336949–AF336973.

neighbour-joining tree, with limited bootstrapping support (Fig. 2; GenBank AF336949–73). The same clades were evident using maximum likelihood and maximum parsimony methods. Group I lineages, found throughout Europe (Fig. 1), may further subdivide into a paraphyletic group Ia and outgroup Ib, although again, this is not supported by bootstrapping (Fig. 2). Group II lineages were found in Finland and Sweden only (Fig. 1). Finally, two very divergent haplotypes, group III, were found in two martens from the north of England (Northumberland).

The relationship between different marten species has been investigated by various authors using partial cytochrome *b* sequences (Carr & Hicks 1997; Demboski *et al*. 1999; Kurose *et al*. 1999). Most recently, Hosoda *et al*. (1999, 2000) used the complete cytochrome *b* sequence to show that *Martes martes* and *Martes zibellina* are reciprocally monophyletic sister taxa, and this was supported by bootstrapping. We therefore sequenced a fragment of the cytochrome *b* gene from 19 martens as a comparison: seven from Britain (*w*, *x* and five *a* haplotypes), three from Ireland (all *p*), four from Germany (*l*, *d*, *b* and *m*), two from Sweden (*r* and *q*), and three from Finland (*s* and two *v*).

Five haplotypes were discovered. Over the cytochrome *b* region studied, the single group Ia sequence (*d*, *b*, *l*, *a*) was identical to northwestern Russian and German *M. martes* sequences (GenBank AB051237, AB051251–3), and the single group Ib sequence (*m*, *p*) differed by a single base (the new sequence has GenBank reference AF336975). Cytochrome *b* sequences from group II control region haplotypes were identical to previously reported *M. zibellina* sequences from Khabarovsk in the Russian Far East (Hosoda *et al*. 1999; *s* and *q* identical to AB029420; *r* and *v* identical to AB029421). The simplest explanation is that the group II lineages originated by introgression from *M. zibellina*. Finally, the two English martens from group III had an identical sequence to *M. americana caurina* from Southeast Alaska and the Queen Charlotte Islands, British Colombia (Demboski *et al*. 1999; GenBank AF15468–9). This is consistent with morphological data that suggest that the animals were descendents of American martens that have previously escaped from captivity (Colin Simms, personal communication).

Control region diversity

Within group I, 11 polymorphic sites were identified from 325 aligned sites (3.4%); seven sites (2.6%) were polymorphic within group II (Fig. 3). Within groups (I and II) 15 sites were polymorphic (4.6%), increasing further to 43 sites (13.2%) when group III sequences and the outgroup, *M. foina*, were included (Fig. 3). Two regions of insertion–deletions were difficult to align, and were thus excluded in all further analyses (around positions 116 and 126 in Fig. 3; total aligned sequence = 305 bases). Similar hypervariable regions were found previously in *Mustela putorius* and *Mustela lutreola* (Davison *et al*. 2000).

Differentiation between individual haplotypes within groups was low, with most separated by single base substitutions. The minimum spanning network that describes the relationships between the group I and II haplotypes is shown in Fig. 4. The maximum difference was between haplotypes *r* and *l*, *r* and *g* (both 16 sites), or *r* and *t* (17 sites). Group Ia and Ib differ by three base substitutions in the minimum spanning network (Fig. 4).

The nucleotide diversity (π) of group I haplotypes in *M. martes* (0.013, SD = 0.001) was low, as was the same estimate in *Mu. putorius* (0.009, SD = 0.001). When group I and II haplotypes were considered together the nucleotide diversity was higher, and the same as that found when using introgressed *Mu. putorius* and *Mu. lutreola* haplotypes (0.021, SD = 0.003; 0.021, SD = 0.002, respectively). Values for D* and F* were 0.65 and 0.53, respectively, but −0.20 and −0.26 when group I haplotypes alone were considered. Similar values were obtained for the *Mu. putorius*

```
Haplotype Base position
                    1111111111111111112222222222222223
          4555666678911112334477788889900011123555690
          6278012347425676345978936892645914642189219
a         T~T~TGTCTTGTT~C~TAATATTTATCATCCTATAAGCCCTAA
b         ..................G...C....G..............
c         ........C.............C....G..............
d         ......................C....G..............
o         .............T..CGG...C....G..............
j         ................C.G...C....G..............
t         ..................G...C....G............C..
u         ..C................G...C....G..............
l         ..C................G...C....G..T...........
g         ..................GC..C....G..............
e         .............T....G....CG..G..............
f         ......................C.CG..G..............
h         .............T..C.G....CG..G............C..
i         .................C.G....CG..G..............
k         ..................G....CG..G..............
m         ..................G..C.CG..G..............
n         ..................G....CG..G......G........
p         ................C.GC...CG..G..............
q         ..C...C.....~..CCGC..C.CG.TG...C....A......
s         ..C...C.....~..CC.C..C.CG.TG...C....A......
r         ..C..........T..C.C..C.CGCTGCT.C..G.AT.....
v         ..C..........T..CGC..C.CGCTGCT....G.A......
w         .CC..A....A..T~....C...CG..GC..CG.G.A...C..
x         .CC..A....A..T~....CG..CG..GC..CG...A...C..
M. foina  C.CCCA.T.C.C..~C.....C.CG.TA...C.C.GA.TACGC
```

Group Ia
Group Ib
Group II
Group III

Fig. 3 Variable sites of the mitochondrial DNA control region in martens. Identity with the first sequence is denoted by a dot, and a gap in the alignment by ~.

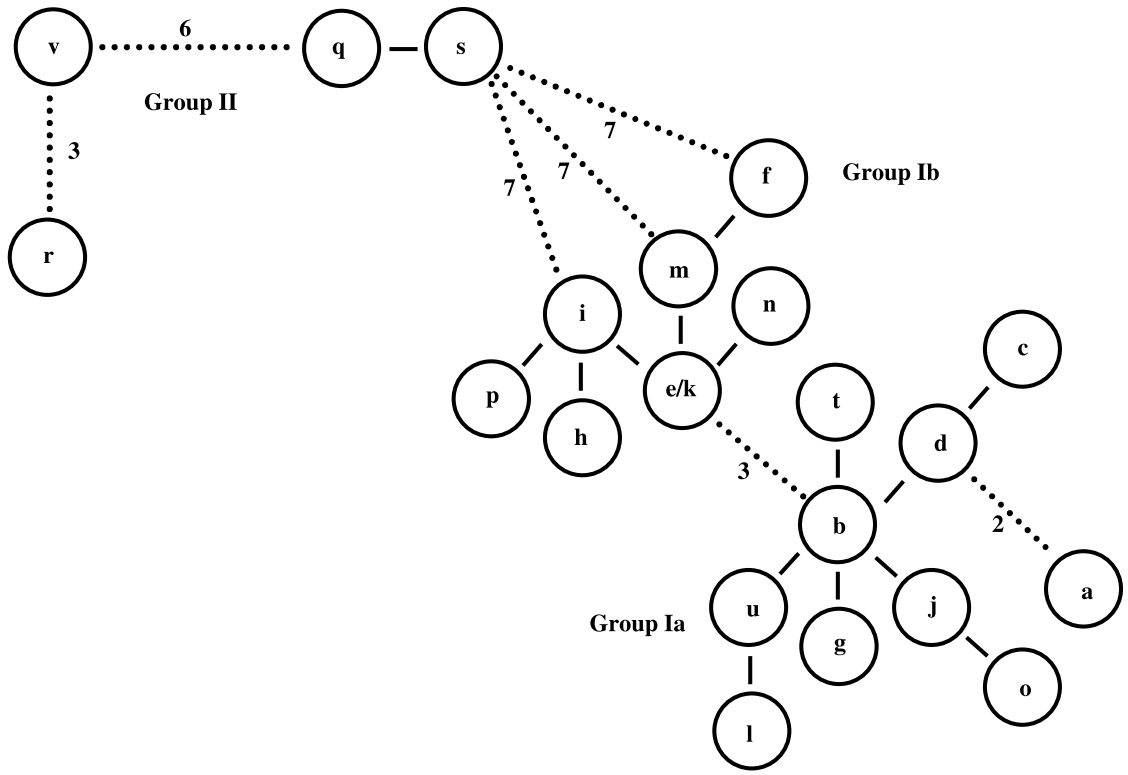

Fig. 4 A minimum spanning network constructed using mitochondrial control region sequences, excluding insertion/deletions. All haplotypes differ by single steps except where shown. The divergent group, haplotypes *s*, *q*, *r* and *v*, were found in Fennoscandia and probably introgressed from *Martes zibellina*. Haplotypes *e* and *k* differ by an insertion/deletion only, so are shown together.

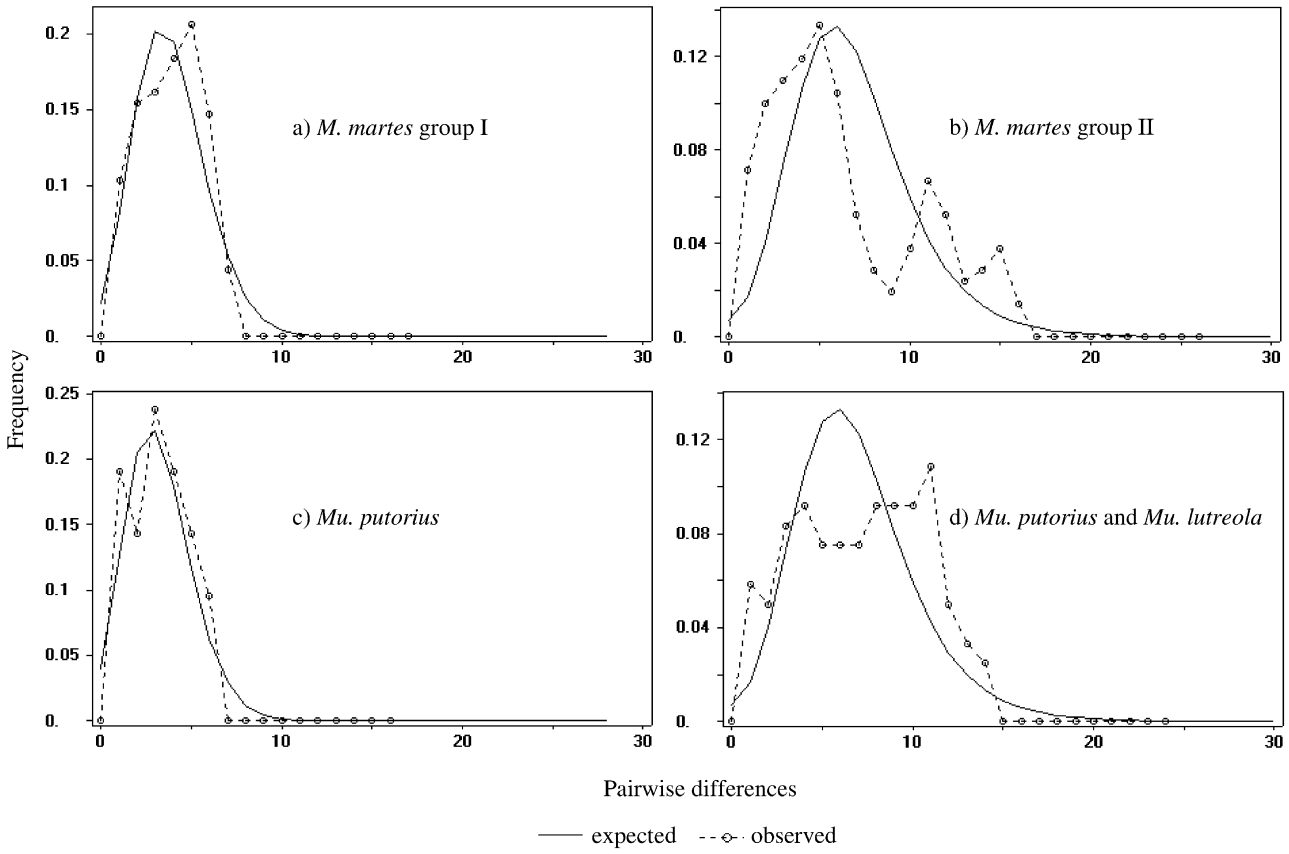

Fig. 5 Distribution of pairwise differences (number of substitutions) between mustelid mitochondrial sequences. The expected frequency is based on a population growth-decline model (Θ initial = 2, Θ final = 200, tau = 5), estimated in DNASP v3.5 (Rozas & Rozas 1997).

and *Mu. lutreola* sequences. However, in all instances values did not deviate significantly from zero.

The distribution of the pairwise number of differences in the control region did not differ significantly from the null Poisson model in all comparisons (Fig. 5a–d), although the best fit was obtained using *M. martes* group I haplotypes, or *Mu. putorius* sequences alone (Fig. 5a,c). The mean number of differences was very similar in martens compared with *Mu. putorius*/*Mu. lutreola* (Fig. 5a, mean = 3.9 and Fig. 5c, mean = 3.2; Fig. 5b, mean = 6.4 and Fig. 5d, mean = 7.2).

Population structure

Within-population nucleotide diversity was highly variable, because some populations were monomorphic, others had both group I and II lineages and also, due to sampling bias (Table 1; Fig. 1). Consequently, the most diverse populations were those in Fennoscandia. There was some evidence for structuring between group Ia and Ib haplotypes, because the former were the predominant type in Sweden, Scotland, France, Germany, Latvia and the Netherlands (Fig. 1). However, small sample sizes mean that this result should be interpreted with caution.

The AMOVA on control region variation indicated significant differentiation between certain sites, mostly involving comparisons with Ireland, Finland, or Scotland. Among-population differentiation explained 64.8% of the covariance component; within-population differentiation explained 35.2% (ϕ_{ST} = 0.65, P = 0, based on 4970 permutations). No evidence for an isolation-by-distance was found, following the regression of log (distance between populations) against ϕ_{ST}.

Discussion

The phylogeography of Martes martes *and* Mustela putorius *in Europe*

The absence of ancient lineages in *Martes martes* and *Mustela putorius* (with the exception of the introgressed *Martes zibellina* haplotypes, see below), as well as a mismatch distribution that is consistent with an expanding population (Fig. 5), is evidence that the present-day *M. martes* and *Mu. putorius* in central and northern Europe colonized from a single European refugium following a recent glaciation. The refugium was not necessarily the

same for each species, nor does it rule out unsampled divergent lineages in southern Europe. These lineages may exist but they have probably not contributed to the present day diversity in the rest of Europe. Low levels of mitochondrial variability have been reported in other mustelids, such as wolverines (Walker *et al*. 2001) and otters (Cassens *et al*. 2000), suggesting that a similar process may be responsible. This could be because they are predominantly northern species. The south Mediterranean is near their range limits, except during glaciations, so the relicts of the refugial populations have periodically become extinct. An alternative explanation is that they have a low effective population size, so diversity is rapidly lost. It suggests that there may be a spectrum of biogeographic patterns across Europe, instead of three paradigms (Hewitt 1999).

Nevertheless, there are two minor mitochondrial lineages in *M. martes* (Fig. 2), and *Mu. putorius* are similarly differentiated (Davison *et al*. 1999, 2000). These types may represent postglacial recolonization from different refugia, with subsequent intermixing, but it is impossible to confirm without further sampling. As mentioned, sampling of *M. martes* in southern Europe was especially difficult, so it is possible that some undiscovered lineages remain (Fig. 1).

In wolves (*Canis lupus*) statistical parsimony, AMOVA analysis and Mantel tests all showed a lack of genetic differentiation by distance, and it has been argued that this is due to the multiple expansions and contractions into refugia, combined with their extreme mobility, dispersal over 1000 km has been recorded, and they have crossed the Bering land bridge a number of times (Vila *et al*. 1999). For mustelids, it is possible that the distribution of alleles is patchy as a consequence of a leptokurtic dispersal following recolonization (Ibrahim *et al*. 1996), but other more 'current' explanations are possible, such as population fragmentation and isolation.

Mitochondrial introgression in mustelids

Mustelid species often hybridize when sympatric (Griffiths 2000). Hybridization has previously been detected by altered pelt and cranial features, between *Mu. putorius* and *Mu. lutreola*, and between *M. martes* and *M. zibellina*. It is now clear that the history of hybridization has had an impact upon the mitochondrial diversity as well. In *Mu. putorius* and *Mu. lutreola* at least one haplotype is shared between the species (Davison *et al*. 2000). The remainder of the haplotypes are so similar that the mitochondrial phylogeny does not resolve the species, although this could be because of the recent speciation of *Mu. lutreola*, rather than hybridization (Davison *et al*. 2000). Similarly, the most likely explanation for the divergent group II lineage in Fennoscandian *M. martes* is that it originated by introgression from *M. zibellina*, because the same type was found in *M. zibellina* from the Russian Far East, over 6000 km away. Presumably, *M. zibellina* further west also have this sequence but they have not been sampled. Alternative explanations, such as the diffusion of *M. martes* mitochondria to the Far East or the retention of an ancestral polymorphism are less parsimonious.

In addition, introgression has been reported in other Fennoscandian taxa, such as field voles (Jaarola *et al*. 1999). This is because Fennoscandia was completely covered with ice at the height of the last glaciation, *c.* 18000 years BP (Jaarola *et al*. 1999). As the ice melted with the onset of the Holocene, immigrants arrived both via a southern land bridge and via a northeast route. Secondary contact between the southern and northeastern colonizers was established around 9000 years BP, so there is now a cluster of hybrid zones in central Sweden (Jaarola *et al*. 1999). In the field vole, *Microtus agrestis*, the southern mitochondrial lineages probably derive from a Balkan refugium, whereas the northeast lineage arrived via Russia. Similar phylogeographic patterns have also been observed in bank voles *Clethrionomys glareolus*, the common shrew *Sorex araneus* and the brown bear *Ursus arctos* (Taberlet *et al*. 1995; Jaarola *et al*. 1999). Furthermore, bank voles in northern Sweden and Finland have mtDNA originating from a different species, the red-backed vole *Clethrionomys rutilus*.

At present, it is impossible to know exactly how the introgression occurred between martens in Fennoscandia, nor whether the original *M. martes* colonization was bi-directional. However, as *M. zibellina* is an eastern species that is better cold adapted than *M. martes*, it may have been the first to colonize the northeast of Fennoscandia. As the climate ameliorated, *M. martes* could have replaced *M. zibellina*, with mitochondrial introgression as the dwindling *M. zibellina* population mated with *M. martes*.

An alternative scenario is suggested by reports from hunters' bags. *M. zibellina* is generally limited by the Ural mountains to the west, but occasionally penetrates deep into Europe. From the fifteenth to the seventeenth centuries, *M. zibellina* reached into northern Finland and Sweden at 50° N latitude (Heptner *et al*. 1967; Bakeyev & Sinitsyn 1994). They also moved southwest into Byelorussia, Lithuania and eastern Poland. This range extension coincided with a marked downturn in temperatures, between 1550 and 1850, which is known as the 'Little Ice Age'. Similar cold effects were noted in the forests, as northern spruce extended its range up to 600 km southward. As the temperatures increased again, the range of *M. zibellina* was restricted once again to the Urals. During this period there will have been ample opportunities for contact between *M. martes* and *M. zibellina*, since records from the levying of taxes on pelts show that the species were sympatric for a number generations (Bakeyev & Sinitsyn 1994).

A significant problem with most of the studies that have reported bi-directional colonization in Fennoscandia is that nuclear markers have rarely been used. Results from

microsatellites or nuclear gene sequences do not always corroborate mitochondrial studies, though a distinction should be made between intra- and interspecific studies (Tegelström *et al*. 1988; Hare & Avise 1998; Avise 2000; Waits *et al*. 2000). In part, the explanation may be due to the smaller effective population size of mtDNA. However, a lack of correlation between mitochondrial sequences and microsatellites in a recent study on brown bears was explained by differences in the levels of male and female gene flow (Waits *et al*. 2000). Female bears are strongly philopatric. Between species, the patterns of introgression between two species will tend to be shaped by the type and extent of reproductive barriers.

Units for conservation in M. martes

In normal circumstances, group I and group II mitochondrial types might define an Evolutionary Significant Unit (ESU; Moritz 1994). However, defining ESUs based on a single molecular genetic marker is not ideal: combining ecological and genetic data (mitochondrial and nuclear; see Waits *et al*. 2000) is preferred (Crandall *et al*. 2000). The full description of ESUs in *M. martes* must await further samples from southern Europe and studies using nuclear markers.

In Scotland, the *M. martes* population continues to expand following their near extinction, whereas it has been argued by some authors that *M. martes* is now functionally extinct in England and Wales (Strachan *et al*. 1996; Bright *et al*. 2000), though this is disputed by others (Messenger & Birks 2000; Davison *et al*. 2001). For five specimens in this study, the mtDNA type was the same as that for animals from Scotland, and different from captive bred animals. Further work using microsatellites could determine whether the English and Welsh *M. martes* are relicts from the original population, recent covert introductions from Scotland, or the results of introgression between the two. More worrying is the discovery of *M. americana caurina* mitochondrial haplotypes in northern England. Although *M. martes* and *M. americana* represent opposite ends of a Holarctic species group, it is likely that they may still hybridize given the opportunity. However, at present it is unknown whether there is even an extant population of *M. americana* in Northumberland, nor whether they were accidental escapes (perhaps from fur farms) or deliberate releases.

Acknowledgements

We are especially grateful to The Vincent Wildlife Trust for funding this project, and to the many people (listed in Table 1) who donated marten samples. Thanks also to the staff at Nottingham for their help with this project, including Bryan Clarke, David Parkin, Linda Parkin and Chris Wade. Helpful comments from Jon Bridle and two anonymous referees greatly improved the manuscript.

References

Anderson E (1970) Quaternary evolution of the genus *Martes* (Carnivora, Mustelidae). *Acta Zoologica Fennica*, **130**, 1–132.

Avise JC (2000) *Phylogeography*. Harvard University Press, Cambridge, MA.

Bakeyev N, Sinitsyn AA (1994) Status and conservation of sables in the Commonwealth of Independent States. In: *Martens, Sables and Fishers: Biology and Conservation* (eds Buskirk SW, Harestad AS, Raphael MG, Powell RA), pp. 246–261. Cornell University Press, Ithaca.

Beltran JF, Rice JE, Honeycutt RL (1996) Taxonomy of the Iberian Lynx. *Nature*, **379**, 407–408.

Birks JDS, Messenger JE, Davison A (1997) A 1994 pine marten *Martes martes* (L.) record for Lancashire, including a preliminary genetic analysis. *Naturalist*, **122**, 13–18.

Bright P, Halliwell E, Mitchell-Jones T (2000) *Return of the pine marten to England: proposed recovery programme for one of Britain's rarest mammals*. Public consultation document. The People's Trust for Endangered Species and English Nature, London.

Carr SM, Hicks SA (1997) Are there two species of marten in North America? Genetic and evolutionary relationships within *Martes*. In: *Martes: Taxonomy, Ecology, Techniques, and Management* (eds Proulx G, Bryant HN, Woodard PM), pp. 15–28. Provincial Museum of Alberta, Edmonton.

Cassens I, Tiedemann R, Suchentrunk F, Hartl GB (2000) Mitochondrial DNA variation in the European otter (*Lutra lutra*) and the use of spatial autocorrelation analysis in conservation. *Journal of Heredity*, **91**, 31–35.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution*, **15**, 290–295.

Davison A, Birks JDS, Griffiths HI, Kitchener AC, Biggins D, Butlin RK (1999) Hybridization and the phylogenetic relationship between polecats and domestic ferrets in Britain. *Biological Conservation*, **87**, 155–161.

Davison A, Griffiths HI, Brookes RC *et al*. (2000) Mitochondrial DNA and palaeontological evidence for the origins of endangered European mink, *Mustela lutreola*. *Animal Conservation*, **3**, 345–355.

Davison A, Birks JDS, Brookes RC, Braithwaite TC, Messenger JE, (2001) On the origin of faeces: morphological versus molecular methods for surveying rare carnivores from their scats. *Journal of Zoology (London)*, in press.

Demboski JR, Stone KD, Cook JA (1999) Further perspectives on the Haida Gwaii glacial refugium. *Evolution*, **53**, 2008–2012.

Felsenstein J (1993) PHYLIP *(Phylogeny Inference Package), Version 3.5c*. Distributed by the Author. Department of Genetics, University of Washington, Seattle.

Fu YX, Li WH (1993) Statistical tests of neutrality of mutations. *Genetics*, **133**, 693–709.

Grakov NN (1994) Kidus — a hybrid of the sable and the pine marten. *Lutreola*, **1**, 1–4.

Griffiths HI (2000) *Mustelids in a Modern World: Management and Conservation Aspects of Small Carnivore: Human Interactions*. Backhuys, Leiden.

Hare MP, Avise JC (1998) Population structure in the American oyster as inferred by nuclear gene genealogies. *Molecular Biology and Evolution*, **15**, 119–128.

Heptner VG, Naumov NP, Yurgenson PB, Sludsky AA, Chirkova AF, Bannikov AG (1967) *Mammals of the USSR*, Part 2, Vol. 1 [in Russian]. Akademia Nauk USSR, Moscow.

Hewitt GM (1999) Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*, **68**, 87–112.

Hosoda T, Suzuki H, Iwasa MA *et al.* (1999) Genetic relationships within and between the Japanese marten *Martes melampus* and the sable *M. zibellina*, based on variation of mitochondrial DNA and nuclear ribosomal DNA. *Mammal Study*, **24**, 25–33.

Hosoda T, Suzuki H, Harada M *et al.* (2000) Evolutionary trends of the mitochondrial lineage differentiation in species of genera *Martes* and *Mustela*. *Genes and Genetic Systems*, **75**, 259–267.

Ibrahim KM, Nichols RA, Hewitt GM (1996) Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity*, **77**, 282–291.

Jaarola M, Tegelstrom H, Fredga K (1999) Colonization history in Fennoscandian rodents. *Biological Journal of the Linnean Society*, **68**, 113–127.

Jefferies DJ, Critchley CH (1994) A new pine marten *Martes martes* (l) record for the North Yorkshire Moors: skull dimensions and confirmation of species. *Naturalist*, **119**, 145–150.

Kurose N, Masuda R, Siriaroonrat B, Yoshida MC (1999) Intraspecific variation of mitochondrial cytochrome *b* gene sequences of the Japanese marten *Martes melampus* and the sable *Martes zibellina* (Mustelidae, Carnivora, Mammalia) in Japan. *Zoological Science*, **16**, 693–700.

Lunt DH, Ibrahim KM, Hewitt, GM (1998) MtDNA phylogeography and post-glacial patterns of subdivision in the meadow grasshopper, *Chorthippus parallelus*. *Heredity*, **80**, 630–641.

Manly BJF (1997) *Randomization, Bootstrap, and Monte Carlo Methods in Biology*, 2nd Edn. Chapman and Hall, London.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.

Messenger JE, Birks JDS (2000) Monitoring the very rare: pine marten populations in England and Wales. In: *Mustelids in a Modern World Management and Conservation Aspects of Small Carnivore: Human Interactions* (ed. Griffiths HI), pp. 217–230. Backhuys, Leiden.

Mitchell-Jones AJ, Amori G, Bogdanowicz W *et al.* (1999) *The Atlas of European Mammals*. T and AD Poyser Ltd, London.

Moritz C (1994) Defining evolutionarily significant units for conservation. *Trends in Ecology and Evolution*, **9**, 373–375.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552–569.

Rozas J, Rozas R (1997) DNASP, version 2 0: a novel software package for extensive molecular population genetic analysis. *Computer Applications in the Biosciences*, **13**, 307–311.

Santucci F, Emerson BC, Hewitt GM (1998) Mitochondrial DNA phylogeography of European hedgehogs. *Molecular Ecology*, **7**, 1163–1172.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN, Version 2.000: a Software for Population Genetic Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Geneva.

Shields GF, Kocher, TD (1991) Phylogenetic relationships of North-American ursids based on analysis of mitochondrial DNA *Evolution*, **45**, 218–221.

Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, **129**, 555–562.

Strachan R, Jefferies DJ, Chanin PRF (1996) *Pine Marten Survey of England and Wales 1987–88*. Joint Nature Conservation Committee, Peterborough, UK.

Swofford DL (1998) *PAUP* Phylogenetic Analysis Using Parsimony and Other Methods*. Sinauer Associates, Sunderland, MA.

Sunnucks P, Wilson ACC, Beheregaray LB, Zenger K, French J, Taylor AC (2000) SSCP is not so difficult: the application and utility of single-stranded conformation polymorphism in evolutionary biology and molecular ecology. *Molecular Ecology*, **9**, 1699–1710.

Taberlet P, Bouvet J (1994) Mitochondrial DNA polymorphism, phylogeography, and conservation genetics of the brown bear *Ursus arctos* in Europe. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **255**, 195–200.

Taberlet P, Fumagalli L, Wust-Saucy AG, Cossons JF (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453–464.

Taberlet P, Swenson JE, Sandegren F, Bjarvall A (1995) Localization of a contact zone between highly divergent mitochondrial lineages of the brown bear *Ursus arctos* in Scandinavia. *Conservation Biology*, **9**, 1255–1261.

Tegelström H, Wyöni P-I, Gelter H, Jaarola M (1988) Concordant divergence in proteins and mitochondrial DNA between two vole species in the genus *Cleithrionomys*. *Biochemical Genetics*, **26**, 223–237.

Vila C, Amorim IR, Leonard JA *et al.* (1999) Mitochondrial DNA phylogeography and population history of the grey wolf *Canis lupus*. *Molecular Ecology*, **8**, 2089–2103.

Waits L, Taberlet P, Swenson JE, Sandegren F, Franzen R (2000) Nuclear DNA microsatellite analysis of genetic diversity and gene flow in the Scandinavian brown bear (*Ursus arctos*). *Molecular Ecology*, **9**, 421–431.

Walker CW, Vilà C, Landa A, Lindén M, Ellegren H (2001) Genetic variation and population structure in Scandinavian wolverine (*Gulo gulo*) populations. *Molecular Ecology*, **10**, 53–63.

Yalden DW (1999) *The History of British Mammals*. T & AD Poyser, London.

Angus Davison completed this work at Nottingham, where he worked on the genetics of land snails (funded by NERC) and various mustelid species (funded by The Vincent Wildlife Trust). He is now in Japan on a JSPS/Royal Society 2 + 2 fellowship, investigating speciation in land snails. Rachael Brookes was a VWT research assistant working on martens, and is now researching a PhD on the genetics of slugs. Johnny Birks and John Messenger have diverse interests, centred around the conservation of British mammals, and are also employed by The VWT. Huw Griffiths is a biogeographer, with a special interest in the distribution and origins of European mammals.
