Genetic structure of European pine martens (*Martes martes*), and evidence for introgression with *M. americana* in England

C.J. Kyle[1,*], A. Davison[2] & C. Strobeck[1]
[1]*Department of Biological Sciences, University of Alberta, Edmonton, AB, T6G 2E9, Canada;* [2]*Division of Ecology and Evolutionary Biology, Graduate School of Life Sciences, Tohoku University, Aramaki-Aza-Aoba, Aoba-ku, Sendai 980 8578, Japan (*Author for correspondence: current new address: Rabies Research Unit, Ministry of Natural Resources, Trent University, Peterborough, Ontario, Canada; Phone: (705) 755-2293; Fax: (705) 755-2276; E-mail: Christopher.Kyle@mnr.gov.on.ca)*

Abstract

European pine martens (*Martes martes*) were once distributed across much of western Europe. A combination of factors, such as persecution, trapping, and habitat loss have led to sharp declines in the species' numbers and range and, as such, local populations have become more vulnerable to extinction. To evaluate the influence of these factors on both the level of genetic variation and population structure, we genotyped pine martens from across much of their current distribution. Continental *M. martes* populations were found to have a higher level of genetic structure and lower genetic variation than their North American sibling species, *M. americana*, sampled throughout Canada. The differences among mainland populations of these species may lie in greater levels of habitat fragmentation and persecution experienced by European martens, though it is difficult to exclude more ancient processes such as the influence of glaciations. Among island populations of the two species, the Scottish population revealed a similar level of structure and variation to the *M. a. atrata* population of Newfoundland, however Ireland was more differentiated with less genetic variation. Our work using microsatellites also extends previous mtDNA evidence for the presence of *M. americana* haplotypes in England, raising the possibility of hybridization with *M. martes*. These findings may influence current discussions on the status of English martens and the appropriateness of proposed re-introductions by revealing that some indigenous martens persist in England, despite the presence of some potential hybrids in the region.

Introduction

European pine martens (*Martes martes*) are mid-sized mustelids that occur throughout most of western Europe, including Fennoscandia, but excluding parts of the Low Countries. Persecution by dog hunts, poisoning, and trapping, as well as habitat loss and a concomitant increase in predation by foxes, have all contributed to a general decline across much of their distribution (Langley and Yalden 1977; O'Sullivan 1983; Webster 2001). Pine martens are protected in Britain, and in Scotland the population continues to expand following an early 1900s bottleneck attributed to persecution from game-keepers and habitat loss.

In contrast, the species remains rare and difficult to monitor in England and Wales, where its status is disputed (Bright et al. 2000; Messenger and Birks 2000).

The fragmentation of the species' range may have decreased levels of gene flow among regions and resulted in a loss of genetic variation, potentially limiting the evolutionary potential and increasing the risk of localized extinctions for this species (Caro and Laurenson 1994; Lande and Shannon 1996; Mills and Allendorf 1996). For these reasons, it is important to evaluate the levels of genetic variation and gene flow between populations of this species to help identify populations where conservation actions may be appropriate (Crandall et al. 2000).

The phylogeography of *M. martes* has previously been investigated using the control region and a cytochrome *b* fragment of mtDNA (Davison et al. 2001). A general lack of ancient lineages in martens (and polecats *Mustela putorius*) indicated that the present-day animals in central and northern Europe may have colonized from a single European refugium following a recent glaciation. However, genetic structuring was still present, especially involving comparisons with Ireland, Finland or Scotland. Davison et al. (2001) also reported evidence for historic introgression with the sable (*M. zibellina*) in Fennoscandia, along with mtDNA and morphological evidence for introgression with American martens (*M. americana caurina*) in England.

Population genetic studies, using nuclear markers, have also been conducted on other *Martes* species. *M. americana* sampled throughout Canada have a very low level of genetic structure across vast geographic regions, despite the presence of potential barriers to gene flow, including large mountain ranges, as well as an extensive harvest of this species (Kyle et al. 2000; Kyle and Strobeck, submitted). The lack of structure was attributed to high levels of gene flow among regions, high effective population sizes, and relatively continuous habitat. By contrast, a parallel study of fisher populations (*M. pennanti*) sampled from across the Canadian provinces revealed relatively high levels of genetic structuring over short geographic distances (Kyle et al. 2001). The level of structure in fishers could be a reflection of philopatry and the large demographic changes that affected most populations of this species in the early 1900's. Fishers were extirpated from much of their range as a result of anthropogenic influences (logging and fur harvests). Consequently, only a discontinuous distribution of small fisher populations remained across most of its range, not unlike the situation for *M. martes*.

Despite life history traits of European pine martens more closely resembling those of the American marten, the demographic trends of this species seem to parallel the pattern observed for fishers. Therefore, mainland populations of European martens may be similarly structured to fishers where smaller effective population sizes have potentially lead to more genetic drift between populations. In Canadian populations of martens (with the exception of the insular Newfoundland population) the lack of genetic structure may be explained by large effective population sizes and relatively continuous habitat, conditions that are not present for the European species. However, similar to the insular Newfoundland population of martens, populations of European martens on the islands of Britain and Ireland are hypothesized to be distinct from mainland populations.

In this study we attempt to obtain a contemporary view of the levels of gene flow between central and northern populations of *M. martes*, including Britain and Ireland, using microsatellites. These fast evolving markers can potentially reveal barriers to gene flow among regions while not necessarily reflecting genetic patterns strongly influenced by the last ice-age. Further goals of this project were to investigate the levels of genetic variation and structure among European pine marten populations relative to other mustelid species, to determine the origins of individuals from the vulnerable English population, and finally, compare microsatellite data to existing mtDNA data for this species.

Materials and methods

Sampled locations

Samples of *M. martes*, in the form of extracted DNA, were obtained from England, Scotland, Ireland, Italy, Germany, Latvia, Netherlands, Sweden, and Finland (see Figure 1 for map and Davison et al. 2001 for sample collection details). In northern and central Europe, *M. martes* are broadly sympatric with beech martens (*M. foina*), but the species are easily distinguished. In much of southern Europe, *M. martes* is rare or absent. Further east, the data on species distributions are sketchy (Anderson 1970; Bakeyev and Sinitsyn 1994). The sable, *M. zibellina*, is distinguished from *M. martes* by pelt and skull characters, but it 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 where they meet (Grakov 1994). In England and Wales, marten samples were very difficult to collect, with only eight recent records; seven from England and a Welsh marten scat (Davison et al. 2001, 2002). Sample locations for England are detailed in Table 1. For comparative purposes, sixteen *M. americana* samples from the Yukon and Newfoundland in Canada were genotyped at the same microsatellite loci as the *M. martes* samples.

Amplification and visualization of DNA

Eight microsatellite primer sets were used in this study, originally developed by Davis and Strobeck

Table 1. Sources and mtDNA haplotype (Davison et al. 2001) of all known recent English martens, including Vincent Wildlife Trust identification numbers (VWT ID). Haplotypes w and x are from *M. americana*.

English County	Year	MtDNA haplotype	Source	VWT ID	Reference
Lancashire	1994	a	Ellen Davies	139/Mama5	Birks et al. (1997)
Yorkshire	1993	a	Charles Critchley	176/Mama155	Jeffries and Critchley (1994)
Northumberland	1994	a	Colin Simms	99/Mama15	
Northumberland	1995	a	Colin Simms	397/Mama6B	
Northumberland	1990s	w	Colin Simms	Mama6A	
Northumberland	1990	x	Colin Simms	473/Mama16	
Cumbria	1995	x*	Colin Simms	523/Mama117	

*Not reported in Davison et al. (2001).

Table 2. Genetic variation (at 7 loci) of *M. martes* populations and two *M. americana* populations (insular Newfoundland and Yukon), including: the average number of alleles per locus, unbiased expected heterozygosity, and the unbiased probability of identity.

Population	Abbrev.	N	# alleles/ locus	H_E (%)	P_{ID} 1/
Scotland	Sco	59	3.86	42.3	955
England	Eng	7	3.57	66.1	76,300
Ireland	Ire	9	1.86	34.0	156
Germany	Ger	10	3.86	56.2	27,400
Sweden	Swe	16	3.86	57.3	26,500
Finland	Fin	26	4.57	57.2	15,100
Netherland	Net	10	3.57	53.8	8.690
Latvia	Lat	8	3.86	63.8	58,500
Italy	Ita	15	4.57	61.0	53,500
Newfoundland	N F	16	2.43	44.6	554
Yukon	YK	16	3.86	69.0	1,540,000

the Dunn-Sidak experiment-wise error rate. A *G*-test for heterogeneity, summed among loci (Sokal and Rohlf 1997), was then performed for each pair of sampled areas.

Genetic variation

The relative genetic variation in each population was assessed using allele frequency data; mean number of alleles, unbiased expected heterozygosity (H_E, Nei and Roychoudhury 1974), and unbiased overall probability of identity (P_{ID}, Paetkau et al. 1998) were calculated. Wilcoxon's signed-ranks test was used to test for significant differences in heterozygosity among populations (Sokal and Rohlf 1997).

Genetic distances and pairwise Fst

Genetic distances between populations were estimated using Nei's standard genetic distance, D_S (Nei 1972) and the genotype likelihood ratio, D_{LR} (Paetkau et al. 1997). Both D_S and D_{LR} were calculated using programs within the website, www.biology.ualberta. ca/jbrzusto/Doh.php, designed by John Brzustowski. GENEPOP 3.1 was used to calculate pairwise F_{ST} estimates (as per Weir and Cockerham 1984).

Assignment test

The assignment test (Paetkau et al. 1995), also found on the above web site by John Brzustowski, was run for all populations. This test determines the probability of a genotype occurring in the region from which it was sampled, and the probability of it occurring in each of the other sampled regions. It then assigns each individual to the population in which that individual's genotype has the highest probability of

(1998) in *M. americana* (MA-1, MA-2, MA-18, and MA-19) and *Gulo gulo* (GG-7, GG-14); Dallas and Piertney (1998) in *Lutra lutra* (L-604); and Walker et al. (2001) in *Gulo gulo* (Ggu454). PCR amplification was performed as in Davis and Strobeck (1998). DNA fragments were visualized using an ABI Prism™ 377 DNA sequencer. The programs GeneScan™ Analysis 2.02 and Genotyper® 2.0 were used to analyze the DNA fragments.

Tests of disequilibrium and heterogeneity

Departure from Hardy-Weinburg equilibrium (H.W.E.) and genotypic disequilibria were assessed, for each of the loci, using GENEPOP 3.1 (Raymond and Rousset 1995). Multiple comparisons were accounted for using

Figure 1. Map of sampled regions of European pine martens, *M. martes*. The abbreviations are explained in Table 2.

occurring (see Waser and Strobeck 1998). We also ran this test making no assumptions about the heterogeneity of the martens from England, but simply added the genotypes from these individuals into a pairwise comparison of Yukon *M. americana* and Scottish and mainland *M. martes* populations, in an attempt to reveal any *M. americana* or *M. americana/M. martes* genotypes among the English samples. The Yukon was used as a representative population for *M. americana* where little genetic structure was found between all mainland Canadian populations sampled (Kyle and Strobeck, submitted).

Isolation by distance

A two-way Mantel test (Mantel 1967), found on Pierre Legendre's webpage: http://www.fas.umontreal.ca/BIOL/legendre/, was used to evaluate the correlation between the genetic and geographic distances of mainland populations. The pairwise geographic and genetic distances of *M. martes* populations were also plotted against *M. americana* pairwise distances (from Kyle et al. 2000) to illustrate the difference between the species. Regressions of each curve were calculated using Excel and were compared with regressions of other mustelid species.

Results

Tests of disequilibrium and heterogeneity

All sampled regions, genotyped using eight loci, conformed to H.W.E. accounting for experiment-wise error, with the exception of Scotland at locus MA-18 and Finland at locus GG-14. Both deviations from H.W.E. were heterozygote deficits, implying that null alleles might exist at these loci in these populations. Inasmuch these deviations only occurred at one locus in each of these populations, all loci were retained for analyses. One deviation from genotypic equilibrium was revealed with locus MA2 and locus Ggu454 in the Scottish population. Similarly, as genotypic disequilibrium was only found in one pair of loci in one population, all eight loci were retained for all analyses. G-tests and assignment tests (randomizing combined gene pools; data not shown) both suggested that all regions sampled differed significantly ($\alpha = 0.05$) in their genotypic frequencies and were treated as distinct populations for all subsequent analyses.

Genetic variation

Levels of genetic variation are summarized in Table 2. Both Scotland and Ireland had significantly lower levels of H_E than continental populations (Wilcoxon's signed-ranks test, $\alpha = 0.05$). All continental populations had relatively homogenous levels of genetic variation, with no significant differences among them. The English population was anomalous, having a relatively high level of genetic diversity compared with Ireland and Scotland.

Another comparison was performed using seven microsatellite loci between North American *M. americana* and European *M. martes* populations (locus Ggu454 did not amplify in *M. americana*). The *M. americana* population in the Yukon had the highest level of genetic variation, although not significantly different from continental *M. martes* populations. The insular *M. americana* population on the island of Newfoundland (*M. americana atrata*, listed as endangered by COSEWIC) had a level of genetic variation similar to that of Scottish and Irish *M. martes* populations.

Pairwise genetic distances and Fst

Pairwise estimates of Nei's standard genetic distance, D_S, were found to be highly correlated with both the likelihood ratio genetic distance, D_{LR} ($r = 0.91$, $p = 0.00006$), and pairwise F_{ST} ($r = 0.84$, $p = 0.0003$). D_S was also significantly correlated with geographic distance between mainland populations ($r = 0.55$, $p = 0.007$). Pairwise F_{ST} did not correlate as strongly with geographic distance as did the genetic distance measures ($r = 0.31$, $p = 0.11$).

Both genetic distance values and pairwise F_{ST} suggest that Scottish and Irish martens were differentiated from the continental distribution of pine martens (see Tables 3 and 4). Furthermore, these populations were as differentiated from each other as they are from the continental populations. The level of structure observed in the northern continental populations (Sweden and Finland) was moderately high compared to among the more southerly populations of Germany and Italy. In general, the levels of structure were moderate with an overall F_{ST} value of 0.18 (eight loci).

The pairwise D_S values were plotted against geographic distance between populations (Figure 2). A linear regression of the data revealed a D_S value of 0.140/1000 km (S.E. = 0.018) for the continental *M. martes* populations. When the Scotland and Ireland populations were included the value was 0.198/1000 km (S.E. = 0.05).

Assignment test

The assignment test results, using eight loci, in the absence of *M. americana* populations, also support the suggestion that both Scotland and Ireland are genetically differentiated from the continental populations, with 93 and 100% of the individuals assigning to the population from which they were sampled, respectively (see Table 5). The next most structured populations were in Finland and Netherlands having over 77% of individuals assigned to the population from which they were sampled. Germany, Italy and Latvia shared more cross-assignments (<40% of assignments to the populations from which they were sampled).

The assignment test was also run including the *M. americana* samples (data not shown). There was little effect on the *M. martes* assignments with or without the *M. americana* samples included. Both *M. americana* populations were found to be completely distinct in this test, with all individuals assigned to the populations from which they were sampled and with no cross-assignments to the *M. americana* populations.

The genotype probabilities from the assignment test were also plotted on a graph using individuals from the Yukon (representative of *M. americana*), Scotland (*M. martes*) and England (Figure 3). The same test was also performed using mainland populations in the comparison instead of Scotland (Figure 3). Three individual genotypes from northern England had intermediate probabilities of being from the *M. martes* or *M. americana* populations sampled. Furthermore, in the Scottish/Yukon comparison, the two other Northumbrian martens fell in the centre of the Scottish group, whereas the genotypes from Lancashire and Yorkshire were more distinct, on the outer edge of the Scottish group (Figure 3).

Discussion

Mainland *Martes martes* populations had lower levels of genetic variation and higher levels of genetic structure compared with other *Martes* and mustelid species. We suggest these results may be related to the relative differences in the level and duration of anthropogenic disturbances in Europe and northern North America. These influences may have resulted in smaller, more

Table 3. Genetic distances of *M. martes* populations at 7 loci, including two *M. americana* populations (insular Newfoundland and Yukon). Upper diagonal provides likelihood ratio distance, D_{LR}, lower diagonal provides Nei's standard distance, D_S. Note D_S and D_{LR} are correlated ($r = 0.91$, $p = 0.00006$) by a 2-way Mantel test.

	Sco	Eng	Ire	Ger	Swe	Fin	Net	Lat	Ita	New	Yuk
Sco	0	3.34	5.31	4.80	3.44	8.75	7.34	6.91	5.33	27.3	20.3
Eng	0.137	0	5.02	2.64	2.73	5.43	5.42	4.59	3.97	20.9	12.9
Ire	0.231	0.255	0	5.95	5.46	8.62	8.12	9.14	7.43	25.3	17.3
Ger	0.230	0.195	0.327	0	0.68	3.10	1.93	1.85	2.21	21.8	14.9
Swe	0.193	0.147	0.263	0.082	0	2.34	3.98	2.97	2.06	22.4	15.5
Fin	0.625	0.376	0.601	0.262	0.251	0	4.26	1.35	2.01	21.5	13.8
Net	0.346	0.320	0.396	0.154	0.252	0.375	0	3.30	3.70	23.1	14.7
Lat	0.473	0.304	0.591	0.219	0.237	0.151	0.317	0	0.41	18.2	12.2
Ita	0.484	0.362	0.347	0.208	0.203	0.289	0.365	0.167	017.1	12.5	
New	2.22	2.58	2.64	2.21	2.08	1.88	2.52	1.45	1.68	0	9.31
Yuk	1.08	0.887	1.13	0.895	0.910	0.664	0.765	0.650	0.904	0.727	0

Table 4. Pairwise Fst values between populations of *M. martes* at 8 microsatellite loci.

	Sco	Eng	Ire	Ger	Swe	Fin	Net	Lat	Ita
Scot	0								
Eng	0.136	0							
Ire	0.223	0.199	0						
Ger	0.207	0.059	0.232	0					
Swe	0.155	0.054	0.186	0.018	0				
Fin	0.316	0.165	0.324	0.129	0.120	0			
Net	0.242	0.118	0.257	0.061	0.112	0.187	0		
Lat	0.280	0.098	0.330	0.072	0.079	0.050	0.135	0	
Ita	0.215	0.082	0.263	0.044	0.044	0.086	0.116	0.016	0

isolated populations in Europe where the effects of genetic drift would lead to more genetic structure. The structure uncovered in this study could also reflect a greater degree of philopatry in this species compared with *M. americana*. However, *M. martes* are believed to be as vagile a species, consistent with evidence for long distance gene flow (Table 5). Another alternative is that more ancient processes still influence the gene frequencies, such as post-glacial founder effects and historical introgression from *M. zibellina* in Fennoscandia. The island populations of Britain and Ireland were genetically distinct from each other and the mainland, a result that can largely be attributed to historical founder effects, but that may have been compounded by anthropogenic pressures in these regions.

The analysis also extends the evidence for the presence of *M. americana* genes in England, and raises the possibility of hybridization with indigenous *M. martes*. This finding may have a significant bearing on current discussions on the status of English martens and the appropriateness of proposed re-introductions.

Genetic variation and structure relative to other mustelid species

M. martes populations had an average H_E of 53%, excluding England, and 58%, further excluding the island populations of Scotland and Ireland. Although it is difficult to make a direct comparison with other studies, since different loci were used, *M. martes* had a lower level of genetic variation than Canadian *M. americana*, $H_E = 63\%$ (Kyle and Strobeck, submitted); Canadian *M. pennanti*, $H_E = 62\%$ (Kyle et al. 2001); and North American wolverines, $H_E = 63\%$ (Kyle and
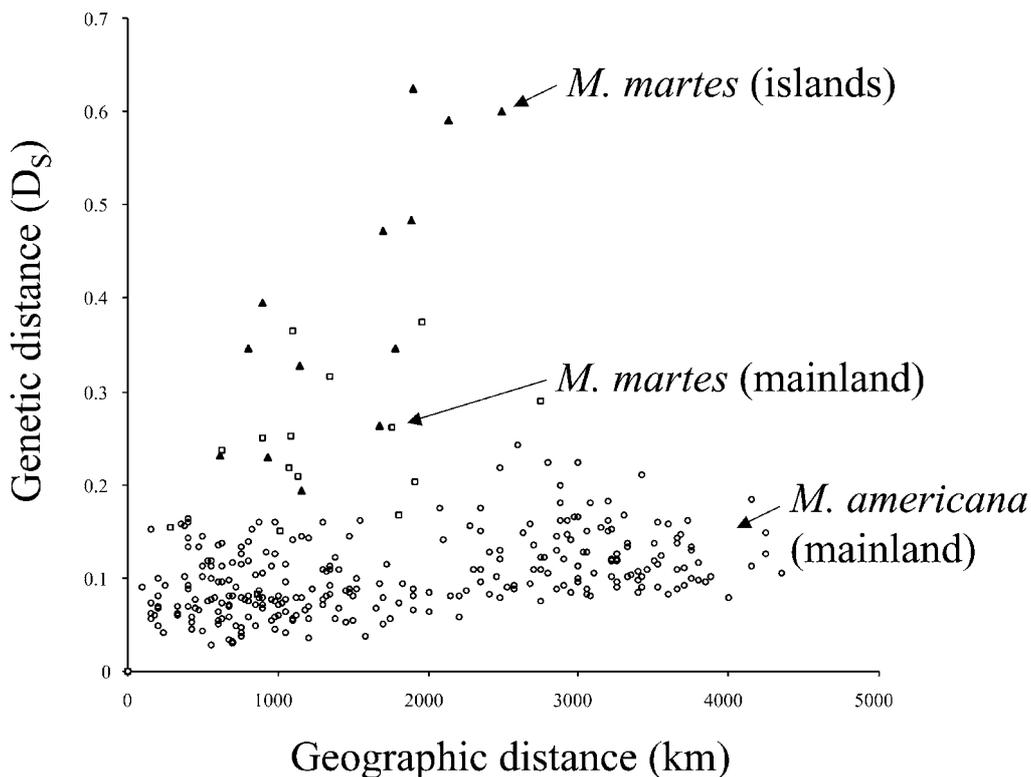
Figure 2. Relationships of genetic distance, D_S, to geographic distance for *M. martes* and *M. americana* populations including, 1) the island populations of Scotland and Ireland (excluding English samples), 2) mainland *M. martes* populations, and 3) mainland *M. americana* populations from Canada.
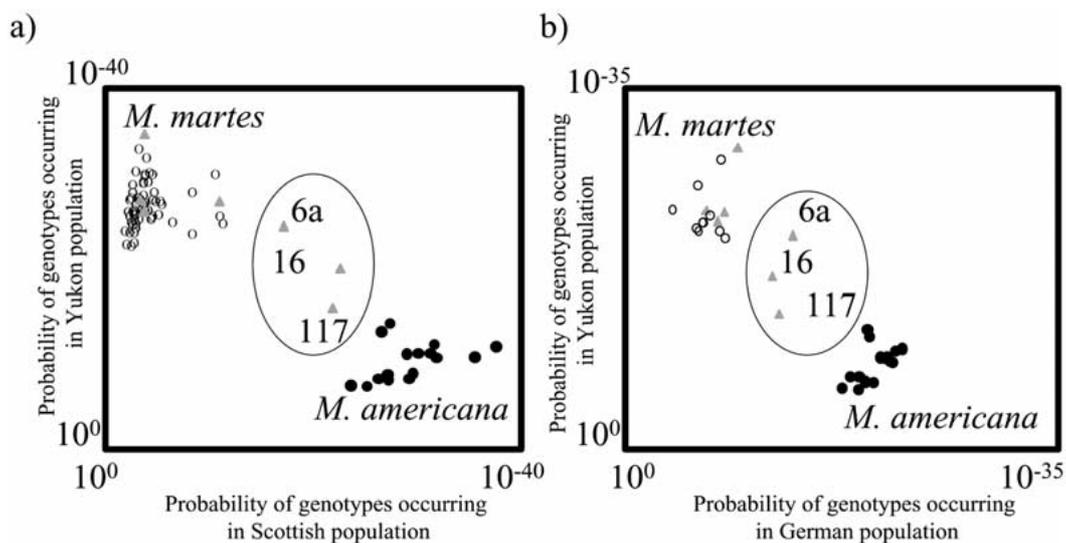

Figure 3. (a) Genotype assignments between North American *M. americana* (closed circles), Scottish *M. martes* (open circles), and martens from England (grey triangles). Some of the genotypes obtained from England (identified as 6a, 16, and 117), have intermediate probabilities of coming from either the Scottish or *M. americana* population, the same animals identified with *M. americana*-like mtDNA haplotypes by Davison et al. (2001). The remainder of the English samples grouped with the Scottish samples. (b) Genotype assignments between Yukon *M. americana* (closed circles) and mainland European *M. martes* (Germany; open circles), again shows same individuals captured in England (grey triangles) have genotypes with intermediate probabilities of occurring in either species.

Table 5. Summary of genotype assignment test for *M. martes* populations (8 loci, without *M. americana* populations). Left column represents where individuals were sampled from and top row represents where individuals were assigned to. Example: of the 59 individuals sampled from Scotland, 55 assigned to Scotland, 3 to Sweden, 1 to Finland, and none to the other populations included in the test.

	N	Sco	Eng	Ire	Ger	Swe	Fin	Net	Lat	Ita
Sco	59	55	0	0	0	3*	1*	0	0	0
Eng	7	3*	3	0	0	0	0	0	0	1
Ire	9	0	0	9	0	0	0	0	0	0
Ger	10	0	0	0	4	3	0	1	2	0
Swe	16	0	0	1	3	9	1	0	1	1
Fin	26	0	0	0	0	2	20	0	4*	0
Net	10	0	0	1*	1	0	0	8	0	0
Lat	8	0	0	0	1	1	2	0	3	1
Ita	15	0	0	0	2	1	2	0	5*	5

*Significant at $\alpha = 0.01$ by randomization of individual gene pools, assuming HWE.

Strobeck 2001, 2002); but higher than Scandinavian wolverines, $H_E = 37\%$ (Walker et al. 2001).

The island populations of Scotland and Ireland were found to have significantly lower levels of genetic variation than mainland populations of this species. This result is not unexpected given their isolation. These results are consistent with findings for *M. a. atrata* found on Newfoundland island (Table 2), and other insular carnivore populations (e.g. brown bears on Kodiak Island, Alaska, Paetkau et al. 1998; wolves on Banks Island, Northwest Territories, Carmichael et al. 2001). The unexpected finding among the English samples with $H_E = 66\%$ may be partially explained by introgression with *M. americana*, but also by errors in sampling due to a low sample size.

The pairwise genetic distances and F_{ST} found in *M. martes* were higher than in other mustelid species. Here, a moderately high overall F_{ST} value of 0.18 at eight loci was obtained for *M. martes* relative to that found for: *M. americana*, $F_{ST} = 0.020$, *M. pennanti*, $F_{ST} = 0.14$, North American wolverines, $F_{ST} = 0.043$, and Scandinavian wolverines, $F_{ST} = 0.045$. The results obtained from the assignment test for *M. martes* were more ambiguous. Both Scotland and Ireland were found to be relatively isolated, but for the continental populations, only the Netherlands and Finland had a high percentage of individuals assigned to the population from which they were sampled. Germany, Italy, Latvia, and to a lesser degree Sweden had many cross-assignments to other sampled regions. These results should be interpreted with some caution, however, given relatively low sample size in several of the populations.

To illustrate the differences in genetic structuring among the mustelid studies, a linear regression of the D_S and geographic distances was performed for all mainland populations. *M. martes* had the highest level of structure per unit distance, 0.140/1000 km (S.E. = 0.02; Figure 2) using continental populations alone, followed by *M. pennanti* 0.092/1000 km (S.E. = 0.008), *M. americana* 0.057/1000 km (S.E = 0.009), and then North American *Gulo gulo* 0.018/1000 km (S.E. = 0.005). These results may suggest that European martens are more philopatric that other mustelids, but the level of genetic structuring observed among *M. martes* populations may also be a result of population fragmentation and bottlenecks leading to genetic drift.

Origins of M. martes in England

In England and Wales controversy exists as to the origins and status of pine martens. Despite evidence for populations persisting through the 20th century (Strachan et al. 1996), some authors have suggested that no viable populations remain and reintroduction to England has been proposed (Bright et al. 2000). Others have argued that while martens remain, we should try to understand their failure to expand (Messenger and Birks 2000). We have eight recent records (seven English martens and one Welsh marten scat; Davison et al. 2001, 2002), in addition to a sightings survey (Messenger and Birks 2000). Based on previous morphological and mtDNA evidence for the presence of *M. americana* individuals and genes in Britain, we screened all *M. martes* for *M. americana*-specific nuclear alleles. For most loci the allele ranges overlapped, so instead a pairwise comparison of genotypic probabilities was used between *M. martes* and *M. americana* populations to determine the probability of the genotypes of the English samples being from either species (Figure 3). Three of the seven individuals had genotypes with intermediate probabilities of being from the *M. martes* populations and the *M. americana* population from the Yukon, Canada (used as a representative population of *M. americana*). These three samples were also the three individuals that were found to have *M. americana*-like haplotypes by Davison et al. (2001; see Table 1). In the Scotland/Yukon pairwise comparison, the other four samples from England fell with the Scottish samples, though two were on the edge of this group.

The combined results from the mtDNA and nuclear markers suggest that some remaining English animals may be indigenous, while some animals may be of *M. americana* descent or hybrid *M. americana/M. martes*.

The most likely source of *M. americana* in the North of England are martens that escaped or were released from commercial mink farms. This raises the possibility of hybridization between species in captivity. Unfortunately, there are no official records for farms in Northumberland prior to the 1962 Mink (keeping) regulations, although the farms were present from the 1920s. From 1962, the number of farms in Northumberland varied between two and six, though none are present now. Interestingly, with mink the original stock animals came from the Hudson Bay area, but they were later superseded by a heavier strain from north of the Yukon River and Alaska (Kevin O'Hara, pers. comm.). This may be suggestive of where *M. americana* fur farm stock originated from, although this is speculative. There were also many 'back garden' farmers prior to 1962 from which martens may have escaped or been released. The first reports of wild-living American martens in Northumberland date to the late 1960s (Colin Simms, pers. comm.).

Comparison of mtDNA and microsatellite population structure

A study by Davison et al. (2001) revealed significant mtDNA structuring among the continental populations of *M. martes*, possibly due to a low level of maternal gene flow, or arising from the post-glacial colonisation of Europe. Similarly, using nuclear markers we have uncovered moderate to high levels of genetic structuring among the continental populations. The study by Davison et al. (2001) also revealed two continental European mtDNA lineages, one found throughout Europe, with another found only in Finland and Sweden. It is likely that the latter lineage arose by historical introgression from *M. zibellina* (Davison et al. 2001). The microsatellite results may provide some support for a distinct Fennoscandian group, with elevated genetic distance values between Fennoscandia (especially Finland) and the central European populations.

Units for conservation

Populations of *M. martes* were significantly differentiated by mitochondrial type (Davison et al. 2001), microsatellite genetic variation and population structure, and could be considered separate Management Units (*sensu* Moritz 1994). It is unclear, however, if the genetic differentiation is the result of recent population fragmentation and bottlenecks or if more homogenous genotypic frequencies once existed among populations of *M. martes*, as found between Canadian populations of *M. america* (Kyle et al., submitted). If martens were once more continuously distributed throughout mainland Europe, then the suggestion that less genetic structure historically existed would be reasonable given the potential dispersal of the species. For a similar circumstance in wolves, Vila et al. (1999) suggested that individuals from neighbouring or closely related populations can justifiably be used as a source for re-introduction or population augmentation. Although *M. martes* haplotype frequencies have probably been less affected by population fragmentation and bottlenecks compared with wolves, we believe such a system could be applied to martens. However, the precautionary principle suggests that animals should not be translocated unless absolutely necessary, especially to island populations.

References

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

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.

Bright P, Halliwell E, Mitchell-Jones T (2000) *Return of the Pine Marten to England: Proposed Recovery Programme for one of Britan's Rarest Mammals*. Public consultation document by

The People's Trust for Endangered Species and English Nature, London.

Carmichael LE, Nagy JA, Larter, NC, Strobeck C (2001) Prey specialization may influence patterns of gene flow in wolves of the Canadian Northwest. *Mol. Ecol.*, **10**, 2787–2798.

Caro TM, Laurenson MK (1994) Ecological and Genetic Factors in Conservation – A Cautionary Tale. *Science*, **263**, 485–486.

Crandall KA, Bininda-Edmonds ORP, Mace GM, Wayne, RK (2000) Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.*, **15**, 290–295.

Dallas JF, Piertney SB (1998) Microsatellite primers for the Eurasian otter. *Mol. Ecol.*, **7**, 1248–1251.

Davis CS, Strobeck C (1998) Isolation, variability, and cross-species amplification of polymorphic microsatellite loci in the family Mustelidae. *Mol. Ecol.*, **7**, 1776–1778.

Davison A, Birks JDS, Brooks RC et al. (2001) Mitochondrial phylogeography and population history of pine martens, *Martes martes*, compared with polecats, *Mustela putorius*. *Mol. Ecol.*, **10**, 2479–2488.

Davison A, Birks JDS, Brooks RC et al. (2002) On the origin of faeces: Morphological versus molecular methods for surveying rare carnivores from their scats. *J. Zool.*, **257**, 141–143.

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

Kyle CJ, Strobeck C (2001) Genetic structure of North American wolverine (*Gulo gulo*) populations. *Mol. Ecol.*, **10**, 337–348.

Kyle CJ, Strobeck C (2002) Connectivity of Peripheral and Core Populations of North American Wolverines. *J. Mammal*, **83**, 0000–0000.

Kyle CJ, Strobeck C (submitted) Genetic homogeneity of Canadian mainland marten populations underscores genetic distinctiveness of Newfoundland pine martens(*Martes americana atrata*). *Can. J. Zool.*

Kyle CJ, Davis CS, Strobeck C (2000) Population genetic structure of martens (*Martes americana*) from the Northwest Territories and Yukon. *Can. J. Zool.*, **78**, 1150–1157.

Kyle CJ, Robitaille JF, Strobeck C (2001) Genetic variation and structure of fisher (*Martes pennanti*) populations across North America. *Mol. Ecol.*, **10**, 2341–2347.

Lande R, Shannon S (1996) The role of genetic variation in adaption and population persistence in a changing environment. *Evolution*, **50**, 434–437.

Langley PJW, Yalden DW (1977) Decline of rarer carnivores in Great Britan during the 19th century. *Mamm. Rev.*, **7**, 95–116.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Can. Res.*, **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 a Small Carnivore: Human Interactions* (ed. Griffiths HI), pp. 217–230. Backhuys, Leiden.

Mills LS, Allendorf FW (1996) The one-migrant-per-generation rule in conservation and management. *Conserv. Biol.*, **10**, 1509–1518.

Mitchell-Jones AJ, Amori G, Bogdanowicz W, Krystufek B, Reijnders PJH, Spitzenberger F, Stubbe M, Thissenn JBM, Vohralik V, Zima J (1999) *The Atlas of European Mammals*. T and AD Payser Ltd., London.

Moritz C (1994) Defining evolutionarily significant units for conservation. *TREE*, **9**, 373–375.

Nei M (1972) Genetic distances between populations. *Am. Nat.*, **106**, 283–292.

Nei M, Roychoudhury AK (1974) Sampling variances of heterozygosity and genetic distance. *Genetics*, **76**, 379–390.

O'Sullivan PJ (1983) The distribution of the Pine marten (*Martes martes*) in the Republic of Ireland. *Mamm. Rev.*, **13**, 39–44.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Mol. Ecol.*, **4**, 347–354.

Paetkau D, Waits LP, Clarkson PL, Craighead L, Strobeck C (1997) An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. *Genetics*, **147**, 1943–1957.

Paetkau D, Waits LP, Clarkson LP et al. (1998) Variation in genetic diversity across the range of North American brown bears. *Con. Bio.*, **12**, 418–429.

Raymond M, Rousset F (1995) GENEPOP (version 3.1d): Population genetics software for exact tests and ecumenicism. *J. Hered.*, **86**, 248–249.

Sokal RR, Rohlf FJ (1995) *Biometry*, 3rd edn. W.H. Freeman and Company, New York.

Strachan R, Jefferies DJ, Chanin PRF (1996) *Pine Marten Survey of England and Wales 1987–1988*. Joint Nature Conservation Committee, Peterborough, UK.

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

Walker CW, Vila C, Landa A, Linden M, Ellegren H (2001) Genetic variation and structure in Scandinavian wolverine (*Gulo gulo*) populations. *Mol. Ecol.*, **10**, 53–63.

Waser PM, Strobeck C (1998) Genetic signatures of interpopulation dispersal. *TREE*, **13**, 43–44.

Webster JA (2001) A Review of the Historical Evidence of the Habitat of the Pine Marten in Cumbria. *Mamml. Rev.*, **31**, 17–31.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.