

Conservation Genetics of the Genus *Martes*

Assessing Within-Species Movements,
Units to Conserve, and Connectivity
across Ecological and Evolutionary Time

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ABSTRACT

Understanding the physical and temporal factors that structure *Martes* populations is essential to the conservation and management of the 8 recognized *Martes* species. Recently, advances in 3 distinct subdisciplines in molecular ecology have provided insights into historical and contemporary environmental factors that have created population substructure and influenced movement patterns of several *Martes* species. Intraspecific phylogenetics has allowed us to understand the role of large-scale historical events, such as the last glacial maxima and their associated refugia, in the ecology of at least 5 *Martes* species in North America, Europe, and Asia (*M. americana*, the American marten; *M. martes*, the European pine marten; *M. melampus*, the Japanese marten; *M. pennanti*, the fisher; *M. zibellina*, the sable). In addition, population genetics has examined how *Martes* populations are connected within species across space and, in some cases, how this level of connectivity has changed over recent time. These studies have been conducted on *M. americana*, *M. martes*, and *M. pennanti*. More recently, several landscape genetic analyses, including graph-theoretic and least-cost-path approaches, have been used to evaluate the correlation between landscape features and genetic relatedness among individuals across a landscape. These new approaches are showing promising results for understanding the ways in which multiple habitat features at multiple scales promote or reduce connectivity. Different forms of this landscape-genetics approach have been applied to *M. americana*, *M. martes*, and *M. pennanti* in portions of their ranges. In this chapter, we review the intraspecific phylogenetic, population genetic, and landscape genetic studies conducted on *Martes* populations; discuss commonalities found among

species; and identify knowledge gaps for understanding movements and substructuring in the genus *Martes*.

Introduction

Understanding the biotic and abiotic forces that influence the movements of animals has been a central focus of wildlife management for nearly a century. This topic has come to the forefront of wildlife biology in recent years, as the perils of habitat fragmentation and climate change are becoming clearer and more pronounced. Habitat areas that were once extensive and connected are now becoming small, degraded, or completely isolated. In fact, we can consider these habitat changes on a gradient: from those that completely eliminate the movement potentials of animals, to those that marginally limit the probability of a successful dispersal of an individual, to those that have no effect. For example, urbanization of a once-forested area may act as a complete barrier to movements and isolate populations, whereas various forest-thinning treatments may remove the cover necessary for *Martes* species to disperse, thus exposing them to predation risks that they did not historically face in unmanaged forests.

Some habitat changes are tied to natural cycles occurring on a temporal scale of centuries or millennia, while others are functions of short-term natural or human-induced landscape changes. Understanding whether the contemporary distributions and substructure patterns of animal populations result from long-term influences, such as glaciations, or have been caused by more recent landscape uses is critical for managing and conserving wildlife. Only by disentangling historical from contemporary factors can we determine whether barriers to movements are part of the natural history of the species or are caused by recent human activities. From these understandings, we can also determine whether management actions, such as corridor protection or habitat improvement, will increase animal movement and gene flow within a species' range.

In this chapter, we first provide a basic primer of the principles, methods, and tools of molecular ecology. Then, we discuss recent findings on movements and substructure for each species based on intraspecific phylogenetic studies, which have been conducted on only 5 of the 8 currently recognized *Martes* species: *M. americana*, the American marten; *M. martes*, the European pine marten; *M. melampus*, the Japanese marten; *M. pennanti*, the fisher; and *M. zibellina*, the sable. In general, such information is obtained by extracting information from mitochondrial DNA sequences, which mutate at a much slower rate than many of the nuclear DNA regions commonly used (e.g., microsatellites). Thus, changes in the sequence occur at a slower rate and differences among sequences reflect more-ancestral splits (Avise et al. 1987).

Table 17.1. List of intraspecific phylogenetic, population genetic, and landscape genetic studies conducted on *Martes* species

Species	Intraspecific phylogenetic	Population genetic	Landscape genetic
<i>M. americana</i>	Carr and Hicks 1997; Stone and Cook 2002; Stone et al. 2002; Dawson 2008; Stanson et al. 2009	McGowan et al. 1999; Kyle et al. 2000; Kyle and Strobeck 2003; Small et al. 2003; Broquet et al. 2006a,b; Swanson et al. 2006; Swanson and Kyle 2007; Williams and Scribner 2007	Broquet et al. 2006b; Wasserman et al. 2010
<i>M. flavigula</i>			
<i>M. foina</i>			
<i>M. guatkinsii</i>			
<i>M. martes</i>	Dawson et al. 2001; Perroldi et al. 2008b; Ruiz-González 2011	Kyle et al. 2003; Mergsey 2007; Perroldi et al. 2008a; Ruiz-González 2011	Mergsey 2007; Ruiz-González 2011
<i>M. melampus</i>	Hosoda et al. 1999; Kurose et al. 1999; Murakami et al. 2004; Sato et al. 2009b; Inoue et al. 2010		
<i>M. pennanti</i>	Williams et al. 2000; Drew et al. 2003; Vinkey et al. 2006; Schwartz 2007; Knaus et al. 2011	Williams et al. 2000; Kyle et al. 2001; Wisely et al. 2004; Carr et al. 2007a,b; Hapeman et al. 2011	Carr et al. 2007b; Galimov et al. 2008
<i>M. zibellina</i>	Hosoda et al. 1999; Kurose et al. 1999; Murakami et al. 2004; Inoue et al. 2010; Malychuk et al. 2010; Rezhnev et al. 2010		

Notes: To date, there have been no genetic studies conducted on *M. flavigula*, *M. foina*, or *M. guatkinsii*, and no detailed population or landscape genetic studies on *M. melampus* or *M. zibellina*. The table is shaded to indicate areas where there has been much research (white), some research conducted in limited geographic areas (light gray), and no research conducted (dark gray). Many of these studies are reviewed in the text.

Intraspecific phylogenetic information is used to compare among populations or different parts of the geographic range of a single species, often to make phylogeographic inferences regarding the importance of geographic features for structuring populations in evolutionary time scales. This is in contrast to *interspecific* phylogenetic information, which evaluates the relations among species. We do not include interspecific phylogenetic information that has helped reveal the evolutionary history of the genus *Martes*, as this subject is well covered by Koepfli et al. (2008) and Hughes (this volume). Next, under each species subheading, we follow our intraspecific synthesis by reviewing published population and landscape-genetic studies for the 3 species that have been studied in this regard: *M. americana*, *M. martes*, and *M. pennanti*. To date, no genetic studies have been conducted on *M. flavigula*, the yellow-throated marten; *M. foina*, the stone marten; or *M. gwatkinsii*, the Nilgiri marten (Table 17.1). Population genetic methods use highly variable nuclear DNA markers for evaluating contemporary patterns of gene flow and genetic variability. An extension of the population genetics analysis is the landscape genetics approach (Manel et al. 2003), which combines landscape ecology methods and population genetics data to examine, at a finer resolution, how specific landscape features structure extant populations. Finally, we conclude the chapter with a synthesis of the lessons molecular data has taught us about the genus *Martes*.

The Role of Conservation Genetics

Genetic Diversity and Inbreeding

During the last 2 decades, the role of genetics in conservation biology, and in ecology in general, has been steadily growing (for reviews, see Frankham 1995, 2005; Allendorf and Luikart 2007; Pertoldi et al. 2007; Ouborg et al. 2010a), partly because changes in genetic diversity can help quantify the status of endangerment of a given population, species, or group of species. The assessment and monitoring of genetic diversity in endangered animals is now pervasive (Schwartz et al. 2007), because powerful DNA-analysis methods have become increasingly available to infer the causes of the spatio-temporal dynamics of populations, and to estimate genetic diversity within populations and the organization of genetic diversity among populations (Allendorf and Luikart 2007).

Genetic diversity (also called genetic variability) can be quantified in several ways; we will focus on genetic diversity quantified by molecular genetic methods. These are expressed as the proportion of polymorphic loci (or proportion of polymorphic sequence sites), the proportion of heterozygous loci, and the number and frequency of alleles at these loci. An implicit assumption often made as a first principle of conservation genetics is that of the causal

relation between genetic variability and both the short- and long-term persistence of a population or species (Ouborg et al. 2010a,b). One such measure of genetic variability, called expected heterozygosity (H_E), can provide an indication of the immediate evolutionary potential of the population, although this measure has no deterministic relation to its future value.

Given an initial pool of unrelated founder genes, the potential change and loss of genetic diversity can be assessed by the increase in relatedness. The initial genetic variability of a population is reduced as relatedness among individuals increases. The decline is proportional to the reduction in heterozygosity or increase of average inbreeding coefficient (F) of the parents, caused by inbreeding and genetic drift (change in gene frequencies in a population between generations due to random sampling). The degree of inbreeding within an isolated population is quantified by F_{IS} (the proportion of the variance in the subpopulation [S] contained in an individual [I]), which varies from -1 to 1 , with 0 representing a non-inbred population with random mating (Holsinger and Weir 2009). F_{IS} is greater than 0 when observed heterozygosity (H_O , the level of heterozygosity measured in a sample) is smaller than the level of heterozygosity that would be observed with the population in genetic equilibrium. This occurs when populations are inbred. Inbreeding depression, the reduction in a fitness trait due to inbreeding, is one of the principal concerns of conservation geneticists.

On the contrary, increased genetic divergence between the parents may be an advantage to the offspring by increasing their heterozygosity, which could cause a *heterosis* or *hybrid-vigor* effect, but only until the genetic distance between the parents reaches a limit. Beyond this limit, the divergence and differences in co-adaptation between the parents may reduce fitness in the offspring because of outbreeding depression (Tallmon et al. 2004). Therefore, genetic divergence between the parents of an individual lies on a continuum, with varying fitness consequences for the offspring.

All metrics described above are estimated using genetic markers (also called loci) that are unlikely to be under selection pressure (neutral markers). Another form of genetic variation is the quantitative variation that results from natural selection, which can produce, for example, a continuous distribution of a phenotypic trait (e.g., size, weight). Quantitative genetics is correlated to the population's adaptive potential, or its capacity to adapt via changes in allele frequencies in the gene pool. The correlation between neutral and quantitative variation is weak and, therefore, divergence among populations at neutral loci is potentially uninformative (Lynch 1996). Given that we are unaware of any quantitative genetic studies on wild *Martes* populations, this topic will not be covered extensively in this review, although we expect that new molecular-genetic approaches will make the study of genes under selection an important pursuit in the near future (Ellegren 2008; Ouborg et al. 2010a,b).

Connectivity and Genetic Diversity

Generally, small fragmented populations are genetically depauperate. This loss of genetic variability has 2 potential consequences: (1) low genetic variability can reduce their adaptive potential to changing environmental conditions, including disturbed habitats, and (2) small, fragmented, and isolated populations can suffer from inbreeding depression due to increasing relatedness among individuals. One of the most common practical conservation strategies to offset concerns about inbreeding depression is to increase the level of connectivity and, thus, gene flow among populations; however, high levels of gene flow can also reduce or impede the capacity for adaptation to a stressor (Lenormand 2002; Tallmon et al. 2004; Postma and van Noordwijk 2005). The actual degree of adaptation is a dynamic interaction between the selective pressures acting on the population and gene flow.

Gene flow can be estimated using methods based on genetic differentiation among populations, quantified with the use of neutral (non-adaptive) regions of the genome. One common metric is F_{ST} , which is a measure of genetic divergence among subpopulations (S) compared to the total population (T) (Allendorf and Luikart 2007; Holsinger and Weir 2009). F_{ST} is a higher hierarchical level than F_{IS} , mentioned earlier, which compares the individual to the subpopulation. F_{ST} ranges from 0 (no genetic differentiation among populations) to 1 (complete genetic differentiation or no gene flow). Alternatively, there are suites of metrics that assign genotypes to populations based on the frequency of alleles in each population (Manel et al. 2005). These metrics have been used in several *Martes* species to quantify movement rates and to identify immigrants (e.g., Kyle et al. 2001; Carr et al. 2007b).

Population genetic data have been used to delineate substructure, identify isolated populations, and define units of conservation. By definition, these data rely on group or population statistics. This poses a challenge when species appear to be distributed continuously across a landscape, and groups are not readily apparent. Although some elements of population genetics, such as measures of between-population genetic distance, are inherently spatial, they do not specifically take the landscape into account. The field of landscape genetics is an extension of population genetics that uses either individual or population genetic data, explicit spatial information, and associated covariates (e.g., elevation, forest type, distance to roads) to identify environmental variables that influence the species' movement patterns.

Landscape genetic approaches are relatively new, but since the term was coined in 2003 (Manel et al. 2003), >500 papers have been published that reference or use these methods. The most common landscape genetics approach is to compare ecological distances among individuals or populations to a matrix of genetic distances (or the inverse, genetic relatedness). These ecological distances are measured along streams, and through forest cover,

riparian zones, nonhuman habitations, savanna, steppe, or any other environmental variable deemed important to the organism's life history, survival, and ability to disperse. This approach becomes more complicated when the landscape is a mosaic of habitat patches, and there is not a continuous path within the ecological covariate of choice, forcing populations or individuals to move through nonoptimal habitats to interact. Here the standard landscape genetics approach has been to impose cost values on habitats of different quality and type, and conduct least-cost-path modeling to derive a matrix of least-cost paths among individuals (or populations). Given that a specific cost per habitat type is rarely known, multiple models with different cost penalties are often created. These multiple models are then evaluated by comparing the many matrices of least-cost paths to the matrices of genetic distances (using Mantel tests as described above). In more complex models, these resistance values can be an aggregation of costs imposed by multiple variables or can be evaluated using multiple matrix regression models, where each covariate's influence on genetic relatedness can be evaluated (Baikenhol et al. 2009).

In addition to least-cost-path modeling, there have been several graph-theoretic approaches developed for landscape genetic analyses. These approaches allow identification and prioritization of important locations and populations for maintaining connectivity. The most widely used graph-theoretic approach is one based in electrical circuit theory and incorporated into the program CIRCUITSCAPE (McRae and Beier 2007; McRae and Shah 2009; Schwartz et al. 2009). This model simultaneously considers all possible paths connecting individuals or populations based on resistance distances. This approach is similar to least-cost-path modeling, but it can provide different results because it simultaneously evaluates contributions for multiple dispersal pathways, which can identify areas where connectivity is most tenuous (i.e., "pinch-points"; McRae and Shah 2009).

Genetic Monitoring

Recently, many research efforts have used molecular markers to monitor wild populations of fish, wildlife, and plants (Boulanger et al. 2004; Schwartz et al. 2007; Grivert et al. 2008; Jacob et al. 2010; Palstra and Ruzzante 2010). These methods use diagnostic molecular markers either to monitor changes in estimated parameters such as abundance, using traditional wildlife biology tools, or to monitor changes in population genetic metrics (Schwartz et al. 2007; McComb et al. 2010). These approaches may be particularly useful for the study of *Martes* species, given the animals' secretive nature. Deciding on the best strategy for monitoring will depend largely on the number of markers available for the species of interest and the power associated with various metrics. To detect trends in population numbers, Tallmon et al. (2010) have

shown that monitoring population genetic metrics may be as powerful as, or potentially more powerful than, monitoring changes in abundance.

One common population genetic metric to monitor is effective population size, or N_E , formally defined as the size of an ideal population with the same rate of change of allele frequencies or heterozygosity as the observed population. N_E has been considered the most important and critical surrogate parameter to describe the status of small populations. In populations with an N_E smaller than a few hundred individuals, natural selection is not very effective and is easily overpowered by genetic drift; hence, a small N_E reduces the population's potential to adapt to environmental changes. Populations with large N_E have the potential to react to the selective pressures generated by environmental changes, if their genetic variability is high enough and if the speed of the environmental changes is not too high (i.e., if the rate of adaptive evolution at least matches the rate of environmental change; Allendorf and Luikart 2007). Therefore, the N_E of a population can predict its capacity to survive in a changing environment more reliably than the population size; furthermore, N_E determines the speed at which genetic variability is lost (Schwartz et al. 1999; Luikart et al. 2010; Hare et al. 2011).

To predict the long-term persistence of animal populations, accurate estimates of population size are also necessary. Thus, abundance has been a commonly monitored metric. Census methods based on direct counts can be inaccurate if individuals are difficult to detect. New molecular techniques for the analysis of noninvasive genetic samples (feces or hairs) typed for diagnostic genetic markers allow counts of individuals in a population by determining the number of unique genotypes in the population (Luikart et al. 2010; Marucco et al. 2011). This possibility has created a relatively new discipline, noninvasive genetics, which is a set of field, laboratory, and analytical techniques that enable researchers to study the biology of natural populations without observing or capturing individuals (Long and MacKay, this volume).

Phylogenetic Inference within Species

One important aspect of conservation genetics is the use of molecular genetic data to understand the ways in which geographic features influence long-term connectivity among groups of individuals or clades (where a *clade* is an organism and its descendants). This is the field of phylogeography (Avise 2004). By understanding phylogeographic relations, it is now possible to investigate geographic variation using different molecular markers and to deduce the phylogenetic relations of populations within *Martes* species (e.g., Davison et al. 2001; Stone et al. 2002; Pertoldi et al. 2008a,b; Ruiz-González 2011). Intraspecific phylogenetic approaches also allow us to identify the genetic legacy of species translocations (e.g., Drew et al. 2003; Vinkey et al.

2006), elucidate taxonomic uncertainties or the validity of subspecies (e.g., Drew et al. 2003; Knaus et al. 2011), delineate conservation units (e.g., Stone et al. 2002; Sato et al. 2009b; Slauson et al. 2009), reconstruct postglacial colonization histories (e.g., Davison et al. 2001; Ruiz-González 2011), understand range expansions and contractions, and even explore temporal changes in genetic variation through the use of historical DNA (e.g., Pertoldi et al. 2001; Schwartz 2007; Pertoldi et al. 2008a).

Molecular Markers

Many ecological questions can be answered with molecular genetic data; however, no single molecular tool (i.e., molecular marker) is best for all questions. The choice of the molecular marker depends largely on the question being addressed, available laboratory facilities, and prior research conducted with that category of marker on the target species. For example, most studies of intraspecific phylogenetics are based on variation in mitochondrial DNA (mtDNA) because of its properties, which include maternal transmission, extensive intraspecific variation, general lack of interspecific variation, and absence of genetic recombination. Thus, intraspecific studies conducted to date on *Martes* species have largely involved analyses of mtDNA sequences (Table 17.2). Because mtDNA has a relatively fast rate of nucleotide divergence, it is well suited to examining events that occurred during the last few million years.

Two mitochondrial molecular markers have been used mainly on intraspecific phylogenetic studies of *Martes* species: the control region or displacement (D-) loop and cytochrome *b* (cyt *b*). Historically, the sequence length examined for both of these markers has been short (300–500 bp) due to logistical constraints, although this is rapidly changing (Morin et al. 2010). A few studies have used both cyt *b* and the control region to obtain greater resolution, whereas others have complemented analysis of a single region with data on restriction fragment length polymorphisms (RFLPs) or with additional mtDNA markers, such as the NADH dehydrogenase subunit 2 gene, with several transfer RNA (tRNA) markers, or with internal spacer regions of the nuclear ribosomal DNA (rDNA). Several earlier studies on the intraspecific phylogenetics of *Martes* species have not detected clear structuring, possibly because they used a small fragment of mtDNA that did not provide enough resolution to identify intraspecific patterns.

Each DNA sequence has its own genealogy, and these genealogies may evolve at different rates. Furthermore, various methods of analysis probe different aspects of molecular and spatial histories. Consequently, to reconstruct a species' phylogeographic history, one would ideally use a range of sequences (e.g., nuclear, cytoplasmic, sex-linked, autosomal, conserved, neutral, high and low mutation-rate DNA fragments) and apply a suite of pertinent

Table 17.2. Information on the molecular marker and length of DNA sequences examined for understanding intraspecific phylogenetic relationships in *Martes* species

Species	Intraspecific phylogenetic data	Molecular marker (number of basepairs)	References
<i>M. americana</i>	Yes	Cyt <i>b</i> (441 bp) Cyt <i>b</i> (1140 bp); ald C (241 bp) Cyt <i>b</i> (441 bp) and complete cyt <i>b</i> (1140 bp) in combination with RFLPs 1428 bp cyt <i>b</i> (1140 bp); tRNA-Pro (25 bp); D-loop (263 bp) mtDNA; 14 microsatellites	Carr and Hicks 1997 Stone and Cook 2002 Stone et al. 2002 Slauson et al. 2009 Dawson 2008
<i>M. flavigula</i>	No	—	—
<i>M. foina</i>	No	—	—
<i>M. gwatkinsii</i>	No	—	—
<i>M. martes</i>	Yes	D-loop (321 bp) D-loop (350 bp) Cyt <i>b</i> ; tRNA-Thr; tRNA-Pro; complete D-loop; rDNA 12S (1608 bp)	Davison et al. 2001 Pertoldi et al. 2008b Ruiz-González 2011
<i>M. melampus</i>	Yes	Cyt <i>b</i> (402 bp); RFLPs of rDNA Cyt <i>b</i> (1140 bp) Cyt <i>b</i> ; tRNA-Thr; tRNA-Pro; D-loop (521–524 bp) Cyt <i>b</i> ; D-loop; NADH-2 (2814 bp) D-loop (535–537 bp)	Hosoda et al. 1999 Kurose et al. 1999 Murakami et al. 2004 Sato et al. 2009b Inoue et al. 2010
<i>M. pennanti</i>	Yes	Isoenzymes D-loop (301 bp) D-loop (301 bp); cyt <i>b</i> (428 bp) Entire mitochondrial genome	Williams et al. 2000 Drew et al. 2003 Vinkey et al. 2006; Schwartz 2007 Knaus et al. 2011
<i>M. zibellina</i>	Yes, parts of range	Cyt <i>b</i> (402 bp); RFLPs of rDNA Cyt <i>b</i> (1140bp) Cyt <i>b</i> ; tRNA-Thr; tRNA-Pro; D-loop (521–524 bp) Cyt <i>b</i> (702 bp) D-loop (535–537 bp) D-loop (495 bp)	Hosoda et al. 1999 Kurose et al. 1999 Murakami et al. 2004 Malyarchuk et al. 2010 Inoue et al. 2010 Rozhnov et al. 2010

analytical approaches (Sato et al. 2009b; Balloux 2010). New techniques are becoming available to sequence entire genomes (Morin et al. 2010), promising a multitude of new data and greater insights into the intraspecific relations of *Martes* species (Knaus et al. 2011).

Microsatellites, which are regions of the genome with high mutation rates, have been widely used in conservation genetics investigations, and many software programs are available for analysis of these data (Excoffier and Heckel 2006). Microsatellites, considered neutral genetic markers, have been useful for estimating N_E , abundance, gene flow, hybridization, and genetic diversity in natural populations. They are highly variable in nearly all vertebrates, which enables the identification of individuals in a population. They also have many advantages compared with other genetic markers. First, microsatellite loci are codominant, which means alleles from both the chromosome pairs in diploid organisms (e.g., mammals) are detected. Next, microsatellites are believed to be largely selectively neutral, thus conforming to many theoretical population genetic models. Third, the ability to inexpensively develop microsatellites for a particular species or adapt those already developed for related taxa has made microsatellites popular for many investigations of wildlife genetics (Schwartz and Monfort 2008). In fact, there have been nearly 20 studies of *Martes* species that used microsatellites to infer gene flow or estimate abundance and effective population size (e.g., Kyle et al. 2003; Wisely et al. 2004; Hapeman et al. 2011).

Recently, a surge of new molecular genetic tools will enable researchers to examine molecular markers under selection. One of the most promising is the use of single nucleotide polymorphisms (SNPs). SNPs represent the most widespread source of sequence variation within genomes (Brumfield et al. 2003; Chen and Sullivan 2003; Ellegren 2008; Wang et al. 2009) and have the potential to significantly expand our ability to survey both neutral variation and genes under selection in natural populations, because SNPs have the very advantageous characteristic of being detectable throughout the genome. The use of SNPs involves amplification of very short fragments of DNA, which makes them particularly attractive for noninvasive genetic monitoring projects, where DNA is often degraded (Seddon et al. 2005). Furthermore, SNP genotypes based on single nucleotide changes are universally comparable and do not require standardization across laboratories, which can be a problem when comparing other genetic data (e.g., microsatellites) produced by different laboratories (Vignal et al. 2002). The enhanced opportunities for collaboration among laboratories located in different countries or continents will aid our ability to understand the population dynamics of *Martes* species and the influence of those dynamics on genetic variation. The main obstacle remaining for the use of SNPs is the difficulty of identifying them in non-model organisms (Smith et al. 2004; Ryyänen et al. 2007), but that situation is changing rapidly.

The Conservation Genetics of *Martes* Species

In this section, we synthesize the results of intraspecific phylogenetic, population genetic, and landscape genetic studies on each *Martes* species. We review species from west to east, starting with *M. americana* with its western extent in Alaska (USA), and moving to *M. melampus* in Japan.

Martes americana—American Marten

The intraspecific phylogeny of *M. americana* has been debated for several decades, with clear doubts about subspecific and specific status. As many as 14 subspecies of *M. americana* have been described (*M. a. abieticola*, *M. a. abietinoides*, *M. a. actiosa*, *M. a. americana*, *M. a. atrata*, *M. a. brumalis*, *M. a. caurina*, *M. a. humboldtensis*, *M. a. kenaiensis*, *M. a. nesophila*, *M. a. origenes*, *M. a. sierra*, *M. a. vancouverensis*, and *M. a. vulpina*; reviewed in Dawson and Cook, this volume). Traditionally, these have been placed into 2 morphologically distinct groups, *americana* and *caurina* (Merriam 1890; Clark et al. 1987). Although several studies (e.g., Merriam 1890; Anderson 1970; Hall 1981; Clark et al. 1987; Carr and Hicks 1997; Stone and Cook 2002; Stone et al. 2002; Small et al. 2003; Dawson 2008) have corroborated the separation of *M. americana* into these 2 groups, the level of distinctiveness between them has been vigorously debated. Before 1953, these 2 groups of martens were recognized as distinct species: *M. americana* and *M. caurina*, the Pacific marten (Merriam 1890). Yet, on the basis of intergradation in British Columbia (Canada) and Montana (USA), Wright (1953) proposed they be considered a single species. Since then, they have been synonymized under *M. americana*, which is reflected in currently accepted taxonomy (e.g., Wilson and Reeder 2005); however, the presence of 2 distinct clades has continued to be acknowledged by many researchers. Preliminary molecular data corroborated the distinction of *caurina* and *americana* as 2 monophyletic mitochondrial clades (Carr and Hicks 1997; Stone et al. 2002), but subsequent studies gave only subspecific status to the *caurina* and *americana* clades (Stone and Cook 2002; Stone et al. 2002).

These 2 lineages are largely allopatric. The *americana* clade is widespread from interior Alaska south to Montana and eastward to Newfoundland (Canada) and New England (i.e., northwestern, north-central, and northeastern North America) with little or no geographical structure present among populations. In contrast, the *caurina* clade occurs in western North America, extending from Admiralty Island in southeastern Alaska south through 2 large peninsular extensions, the first in the Cascade and Coast ranges to California (USA), and the other through the Rocky Mountains to Colorado (USA; Wright 1953; Hall 1981; Carr and Hicks 1997; Stone et al. 2002). Within *caurina*, there is strong structuring throughout its distribution, with several haplotypes confined to single populations (Stone et al. 2002). These 2 lineages

appear to have diverged as a result of isolation in distinct southern glacial refugia; one in eastern and the other in western North America (Stone et al. 2002). The authors hypothesized that the individuals belonging to the *caurina* clade represent an early Holocene colonization northward along the west coast as coastal ice receded at the end of the last glaciation, whereas *americana* populations represent a later colonization from continental source populations that expanded through river corridors and traversed the coastal mountains. Interbreeding between these 2 lineages at contact zones has been shown (Stone and Cook 2002; Stone et al. 2002).

Microsatellite studies corroborated the patterns of population structure derived from sequences of the mitochondrial *cyt b* gene fragment (Carr and Hicks 1997; Stone et al. 2002), the nuclear aldolase *C* gene fragment (Stone and Cook 2002), and earlier morphological comparisons (Merriam 1890; Anderson 1970). Consistent with the occurrence of 2 distinct species of martens in North America, Small et al. (2003) showed that northern populations of *M. caurina* have greater genetic differences among populations and lower within-population genetic diversity than northern populations of *M. americana*, likely caused by the longer periods of isolation in coastal forests that were fragmented during the early Holocene period. The lack of differences among *M. americana* populations has been attributed to either continued gene flow or a more recent expansion throughout the range (Small et al. 2003). These results are consistent with a previous study that used randomly amplified polymorphic DNA markers (RAPD) to examine substructure among martens in Canada from southern British Columbia (*caurina*), Northwest Territories (*americana*), Labrador (*americana*), and the island of Newfoundland (*americana*; McGowan et al. 1999). They found that genetic distances were small among *M. americana* populations throughout Canada, yet large among all comparisons between *americana* and *caurina*, supporting the phylogenetic findings of Carr and Hicks (1997). This was again confirmed with larger sample sizes and microsatellite DNA by Kyle et al. (2000), who showed little substructure among populations of *M. americana* from the Yukon to the Northwest Territories in Canada.

Most recently Dawson (2008) and Dawson and Cook (this volume) reviewed previous molecular studies and developed a more-detailed view of genetic differentiation throughout the range of North American martens. These authors concluded that there are 2 distinct clades in North American martens that are consistent with species-level differences, and that *M. americana* and *M. caurina* are valid species that parallel their original taxonomic descriptions.

The subspecific status of several marten populations in the *caurina* clade from Oregon (USA) and California was investigated by Slauson et al. (2009). These authors evaluated the subspecific identity of a rediscovered population of martens in northern California that was within the historical range of a

subspecies presumed to be extinct—*M. a. humboldtensis*, the Humboldt marten. They compared the mtDNA (1428 bp) sequence diversity of contemporary specimens within the presumed historical range of *M. a. humboldtensis*, including samples from neighboring populations of *M. a. caurina* and *M. a. sierrae*, and a historical museum specimen of *M. a. humboldtensis*. The museum specimen shared 1 haplotype with martens from both the rediscovered population in California and from coastal Oregon. This result suggests that the rediscovered population descends from a relictual population that previously existed in coastal California, Oregon, or both. They also concluded that the subspecific boundary between *M. a. humboldtensis* and *M. a. caurina* is questionable, because the historical haplotype from *M. a. humboldtensis* was shared with contemporary populations in both coastal Oregon and coastal California; consequently, extant marten populations in these regions should be managed collectively. One additional finding from Slauson et al. (2009) was that *M. a. sierrae* differed substantially from both *M. a. humboldtensis* and *M. a. caurina*, suggesting that marten populations were not a single large, genetically homogeneous population throughout the Pacific states (Washington, Oregon, and California) historically, and that this divergence may have occurred in separate glacial refugia.

Kyle and Strobeck (2003) studied the effects of habitat configuration on genetic variability and differentiation among *M. americana* populations in Canada. In agreement with previous studies, they observed little genetic structure in the northern regions, where habitat is homogeneous and few barriers to dispersal are thought to exist, compared with the more-fragmented southern region. Contrary to their expectations, no strong breaks in gene flow were found between any of the 35 sampled regions, regardless of the degree of fragmentation, with the exception of the insular Newfoundland population (*M. a. atrata*). This lack of genetic structure suggests a very large N_E for these populations and that, at larger spatial scales, dispersal by *M. americana* is not limited by landscape features, as was believed previously.

Although marten populations on the mainland appear to have large N_E values, there has been much concern about the N_E and genetic variation of *M. americana* on large islands. The abundance of *M. a. atrata* decreased from an estimated 630–875 animals in 1986 to only 300 animals in 1995 (Snyder 1986; Forsey et al. 1995). Such a drastic and rapid population decline raised concerns that inbreeding could affect the average fitness of this population (i.e., lead to inbreeding depression; Forsey et al. 1995). Population genetics studies on Newfoundland are consistent with this bottleneck, because *M. a. atrata* had the lowest mean number of alleles per locus and expected heterozygosity among the 25 populations sampled throughout Canada (Kyle et al. 2003). Whether this is a natural property of insularity (similar to *M. martes* in Ireland and Scotland; Kyle et al. 2003) or the result of recent declines in abundance or habitat fragmentation is unclear. It will be important to con-

duct genetic monitoring of *M. a. atrata* to monitor changes in genetic variation in this subspecies.

In addition to genetic substructure analyses, population genetic analyses have also provided insights into hybridization among *Martes* species. Microsatellite data have confirmed earlier mtDNA data that suggested the hybridization of *M. americana* (introduced) and *M. martes* (native) in England (Davison et al. 2001; Kyle et al. 2003), although several native *M. martes* populations in Great Britain show no sign of genetic introgression. Hybridization has also been confirmed between *M. caurina* and *M. americana* in 2 places—on Kuiu Island in southeastern Alaska and in the northern U.S. Rocky Mountains (Small et al. 2003). To date, no studies have examined the extent of the hybrid zone or the fitness of these *M. caurina* × *M. americana* hybrids relative to the parental populations.

Population genetic data have also been used to evaluate the success of reintroductions of *M. americana*. Swanson et al. (2006) and Swanson and Kyle (2007) examined reintroduced populations in Michigan (USA) and found high levels of genetic variation, possibly due to the use of multiple source locations or the temporal separation of the reintroductions, which occurred over a 24-year period (Swanson and Kyle 2007; Williams and Scribner 2007).

Although the field of phylogenetics can assess large-scale evolutionary time patterns, and population genetics can make general inferences about population substructure, the field of landscape genetics promises to provide managers with more-detailed information on the specific habitat conditions that influence the movements of *Martes* species. Broquet et al. (2006a) tested whether *M. americana* in the boreal forests of Ontario (Canada) showed isolation-by-distance (i.e., genetic distance positively correlated with geographic distance) and found no significant relation. The samples were collected in 11 different habitat patches that could be categorized as logged (regenerating tree stands of different ages and types) and unlogged old-growth forest (>80 years old). Examining patterns of isolation-by-distance revealed a significant pattern in unlogged landscapes, but not in the logged replicates. This suggests that dispersal by *M. americana* differs in suboptimal, logged habitats compared with intact environments, and is consistent with demographic studies on movements by this species (Broquet et al. 2006a). In a follow-up study, the authors used least-cost-path modeling to confirm these results, which showed that their findings were partially dependent on the resolution of the maps they used, with intermediate grid-cell sizes producing the strongest associations (Broquet et al. 2006b). The authors explain this result by suggesting that the largest grid-cell sizes (coarsest resolution) may miss important landscape features to which martens are sensitive, whereas the smallest grid cells (finest resolution) would require more complex parameterization of habitat features to produce more-accurate model results (Broquet et al. 2006b). The studies by Broquet et al. (2006a,b) demonstrate that, not

only are movements by *M. americana* reduced in suboptimal, logged habitats, but gene flow (movement plus mating) is also impacted.

Least-cost-path modeling has also been conducted on *M. americana* in Idaho (USA); Wasserman et al. (2010) examined the effects of distance, geographic barriers, canopy closure, roads, tree size-class, and overall habitat models on gene flow. *Martes americana* selected high-elevation areas for movements, where there were deep winter snowpacks and moist montane forests characterized by subalpine fir (*Abies lasiocarpa*) and Engelmann spruce (*Picea engelmannii*). Yet, the most important finding was that the features associated with movements (i.e., gene flow) were different from the features the animals selected for primary habitat. Habitat selection was most influenced by forest fragmentation and road density at broad spatial scales, and old-growth forest at fine scales (Wasserman 2008). These variables were not important, however, for predicting gene flow at any scale. This finding demonstrates the importance of considering habitat selection for persistence (daily use) and habitat selection for dispersal separately. Wasserman et al. (2010) demonstrate how newer landscape genetic metrics can investigate gene flow using habitat variables and spatial scales that are concordant with management actions on the ground.

Martes pennanti—Fisher

Martes pennanti is endemic to North America and has 3 recognized subspecies: *M. p. pacifica* on the Pacific coast, *M. p. columbiana* in the Rocky Mountains, and *M. p. pennanti* in the eastern and midwestern United States (Goldman 1935; Hall 1981). Intraspecific phylogenetic research on *M. pennanti* has been conducted largely to examine the genetic consequences of past translocations, to examine the validity of morphologically based subspecies designations, and to provide information for future reintroductions.

The first research to examine the genetics of *M. pennanti* translocations was limited to its eastern range, and showed little overall genetic subdivision among native populations (Williams et al. 2000), probably because of the low variability of the molecular marker used. Yet, reintroduced subpopulations, even those that shared a source, had substantial subdivision. Subsequently, Drew et al. (2003) considered the conservation status of *M. pennanti* and evaluated the potential genetic consequences of past translocations by examining population-level variation of control-region sequences. They sampled populations throughout the species' range in North America (Figure 17.1) including 5 populations unaffected by translocations, and 2 where reintroductions or augmentations had occurred. Populations in Oregon and in the northern U.S. Rocky Mountains (Montana and Idaho) had received translocated animals from distant source populations and, as a result, these populations showed greater similarities to source populations than to adjacent ones. Additional sequences obtained from museum specimens collected prior to any



Figure 17.1. Geographic range of *Martes pennanti*, the fisher, adapted from Knaus et al. (2011) and Reid and Helgen (2008). The area in light gray is the presumed current range; labels indicate locations discussed in the text.

translocations suggested historical gene flow among populations in British Columbia, Washington, Oregon, and California (Drew et al. 2003). The authors concluded that anthropogenic impacts have greatly reduced and isolated extant populations in Oregon and California and, consequently, that British Columbia would be the most appropriate source population for future translocations to recover *M. pennanti* in Washington and some localities in Oregon and California. This conclusion was confirmed by Warheit (2004) using the same molecular markers, as reported by Lewis and Hayes (2004).

Recently, more-detailed studies of *M. pennanti* have been conducted in the U.S. Rocky Mountains. It was assumed that *M. pennanti* occurring in the U.S. Rocky Mountains in the late 20th century were all descended from reintroduced stocks. However, Vinkey et al. (2006) reported that mtDNA (428 bp of cytochrome *b* and 301 bp of D-loop) haplotypes found only in *M. pennanti* from west-central Montana were likely derived from a relict population that persisted despite intensive fur harvesting in the early 20th century. Using the same molecular markers as Vinkey et al. (2006), Schwartz (2007) compared *M. pennanti* in west-central Montana with samples from north-central Idaho and found no differences. One museum specimen, collected in north-central Idaho in 1896, before any known translocation, had the same haplotype as the “native Montana haplotype” discovered by Vinkey et al. (2006). Thus, *M. pennanti* in north-central Idaho and west-central Montana are the only confirmed native populations in the U.S. Rocky Mountains, although many of these individuals have likely interbred with translocated animals. *Martes pennanti* from Idaho and Montana are not all descendants of translocated individuals, but are also the descendants of those that survived early 20th-century trapping.

A recent study by Knaus et al. (2011) has re-examined some of the results reported by Drew et al. (2003) and Vinkey et al. (2006), using the complete mitochondrial genome (16,290 bp). The most striking result was that the full-genome analysis identified patterns that were obscured by using only the control region; for example, Drew et al. (2003) showed that both northern and southern California shared a common haplotype, suggesting gene flow, yet the full-genome analysis revealed that these geographic areas each had unique haplotypes, concordant with microsatellite data (Wisely et al. 2004) and consistent with long-term isolation. Furthermore, similar to findings about *M. martes* and *M. americana*, Knaus et al.’s (2011) work on *M. pennanti* has shown expansion from refugia following the last glacial maximum; they suggest that *M. pennanti* expanded from an Eastern refugium and radiated westward within the past 16,700 years (range: 9000–31,300 years ago). The full-genome analysis also confirmed the uniqueness of the native Montana haplotype (Knaus et al. 2011).

Kyle et al. (2001) used microsatellite DNA to show relatively high levels of genetic structuring ($F_{ST}=0.14$; range: 0.028–0.261) of *M. pennanti* in Canada

compared with that of *M. americana* ($F_{ST} = 0.020$). Despite this high level of substructure, the populations maintained high genetic variability ($H_E = 62\%$). The greater amount of genetic structure in *M. pennanti* could be a reflection of philopatry and the large demographic changes that affected many populations after European settlement. Wisely et al. (2004) and Aubry et al. (2004) also found high levels of genetic substructure among *M. pennanti* populations (F_{ST} varied from 0.11 to 0.60) from southern British Columbia to the southern Sierra Nevada in California. Unlike Kyle et al. (2001), however, Wisely et al. (2004) found much lower values of H_E for *M. pennanti* in the fragmented populations along the Pacific coast (H_E range: 0.16–0.42) associated with relatively high estimates of F_{IS} , suggesting inbreeding. This pattern of reduced heterozygosity follows a north-south gradient, with *M. pennanti* populations in the southern part of the Sierra Nevada having the lowest levels of genetic variation in western North America (Wisely et al. 2004), a pattern that Wisely et al. (2004) suggest is due to the peninsular shape of the distribution in the Pacific states.

Population substructure in *M. pennanti* has been observed in eastern North America in both nuclear (microsatellite) and mitochondrial DNA (288 bp section of D-loop). MtDNA indicated 4 refugial populations in the northeastern United States and Canada: the Adirondack Mountains in New York, White Mountains in New Hampshire, the Moosehead Plateau in Maine in the United States, and the Cumberland Plateau in New Brunswick, Canada (Hapeman 2006). Microsatellite analyses provided evidence of the same 3 distinct populations in the United States (New Brunswick was not analyzed), corresponding to the last known remnants of *M. pennanti* in the east by the end of the 1930s. There is strong evidence, however, of range expansions and subsequent contact among these 3 populations (with limited gene flow) in the narrow corridor between Lake George and Great Sacandaga Lake near the New York-Vermont border (Hapeman et al. 2011). In addition, in the United States there are remnant genetic signals of reintroductions of *M. pennanti* from Maine to Vermont, from the Adirondacks to the Catskill Mountains in New York, and from Vermont and New Hampshire to Connecticut (Hapeman et al. 2011).

Population genetic data have also been used to evaluate the success of *M. pennanti* reintroductions. Williams et al. (2000) showed that older reintroductions had significant allele-frequency differences from their source populations. Although some of these differences may be due to initial sampling error because reintroductions typically involve relatively few individuals, the fact that recent introductions of *M. pennanti* show no significant allele-frequency differences from source populations suggests that the differences are more likely due to genetic drift (Williams et al. 2000). Drift can occur rapidly in small populations, especially for species such as *M. pennanti* that exhibit a polygynous mating system (Allendorf and Luikart 2007).

Several new landscape genetics approaches have been used to evaluate a recolonizing population of *M. pennanti* in southern Ontario (Carr et al. 2007a,b; Garroway et al. 2008). Initial research tested the idea that Algonquin Provincial Park was the source population for this expanding population of *M. pennanti* by examining microsatellite profiles of 35 sites (groups of samples, or “populations”) surrounding the park (Carr et al. 2007a). The authors found that these 35 sites could be clustered into 5 discrete genetic groups, suggesting multiple origins for *M. pennanti* in Ontario; thus, the origin of *M. pennanti* in Ontario was not Algonquin Park, as predicted initially, but rather remnant populations in Ontario and Quebec in Canada and New York in the United States. Carr et al. (2007a) also showed that these populations were rapidly homogenizing along their expansion fronts. Subsequent research used assignment tests to infer the proportion of immigrants into each of the 5 genetic clusters and relate the proportion of immigrants to habitat variables including snow depth, coniferous forest cover, deciduous forest cover, mixed-wood forest cover, and nonforest (Carr et al. 2007b). Carr et al. (2007b) showed a positive association between snow depth and the proportion of immigrants, and a negative association between the proportion of coniferous forest in the landscape and the proportion of immigrants. The best regression model included both snow depth and proportion of coniferous forest, suggesting that the most suitable landscapes for *M. pennanti* had low snowfall and large expanses of coniferous forest (Carr et al. 2007b).

Finally, this same dataset was used in a graph-theoretical framework to examine network structure for evaluating habitat quality, gene flow, and population substructure (Garroway et al. 2008). The graph-theoretical framework is a new approach for landscape genetics that can be used to evaluate complex systems of connectivity that lead to system-level properties not readily discerned by examining relations among populations. This analytical approach has been adopted in the fields of social-network analysis, neurobiology, and transportation efficiency-network analysis (Costa et al. 2007). Basically every complex network, in this case a network of connectivity among populations of *M. pennanti*, has very specific topological features that typify its connectedness and its responses to perturbations (Costa et al. 2007). Garroway et al. (2008) showed that the network for *M. pennanti* in Ontario displayed high levels of clustering, and short mean-path lengths connecting pairs of nodes (populations). Using the graph-theoretic approach also allowed the authors to explore the effect of removing populations (nodes) on system connectivity and resilience. Garroway et al.’s (2008) removal analysis suggested that trapper harvest (i.e., removal of nodes) is unlikely to affect genetic connectivity among *M. pennanti* populations, given current conditions. In addition, they demonstrated a negative association between measures of node connectivity and both the proportion of immigrants into a node and snow depth, confirming Carr et al.’s (2007b) previous results.

Martes martes—European Pine Marten

Martes martes is well distributed throughout Europe (Proulx et al. 2004; Figure 17.2). It is a habitat specialist confined to mature deciduous and coniferous forests (Domingo-Roura 2002; Ruiz-González 2011), has a limited dispersal ability compared with other mustelids (Kyle et al. 2000), and has a slow reproductive rate, potentially rendering it vulnerable to habitat changes (Bright 2000; Webster 2001). Traditionally, *M. martes* has been subdivided into at least 8 subspecies based on coat color and geographic range (*M. m. borealis*, *M. m. latinortum*, *M. m. lorenzi*, *M. m. martes*, *M. m. minoricensis*, *M. m. notialis*, *M. m. ruthena*, and *M. m. uralensis*; Amori et al. 1996; Mitchell-Jones et al. 1999), although support for recognizing all these subspecies may be limited.

The phylogeography of *M. martes* was investigated initially using a small fragment (321 bp) of the mtDNA control region (Davison et al. 2001). This study suggested that extant populations of *M. martes* in central and northern Europe are the result of colonizations from 1 or more glacial refugia and subsequent mixing. The fragment sizes of DNA used were too small though to identify the specific locations of the refugia, or the process of postglacial recolonization of central Europe. Moreover, the scarcity of samples from a suspected Mediterranean refugium (1 specimen from the Iberian Peninsula, 3 from Italy, and 2 from the northern Balkans) has left the recolonization hypothesis open. Interestingly, Davison et al. (2001) reported evidence of genetic introgression (i.e., widespread historical or contemporary hybridization) of *M. martes* with *M. zibellina*, the sable, in Fennoscandia, along with mtDNA and morphological evidence of introgression with *M. a. caurina* in England, which was later confirmed with microsatellite data (Kyle et al. 2003).

More recently, Ruiz-González (2011) investigated unresolved questions posed by Davison et al. (2001) and re-examined phylogeographic patterns of *M. martes* throughout its current range. With the advantage of newer technologies and methods, this study was more comprehensive in terms of the number of specimens included and the length of the mtDNA sequence examined (1600 bp). Sampling also covered a larger portion of the species range, including individuals from Scandinavia in the north, the Russian Federation in the east, and the Iberian Peninsula in the southwest. Ruiz-González (2011) revealed the presence of 69 haplotypes for *M. martes* (and 11 for *M. zibellina*), which are split into 2 major clades: the European-Mediterranean and Fennoscandian-Russian clades. The first clade, including all *M. martes* samples collected from throughout its current European range, is further subdivided into 2 subclades that connect haplotypes in central-northern Europe and the Mediterranean region. Surprisingly, haplotypes in the Mediterranean subclade apparently did not contribute to the postglacial recolonization of most of the Palearctic range of the species. It appears that central-northern

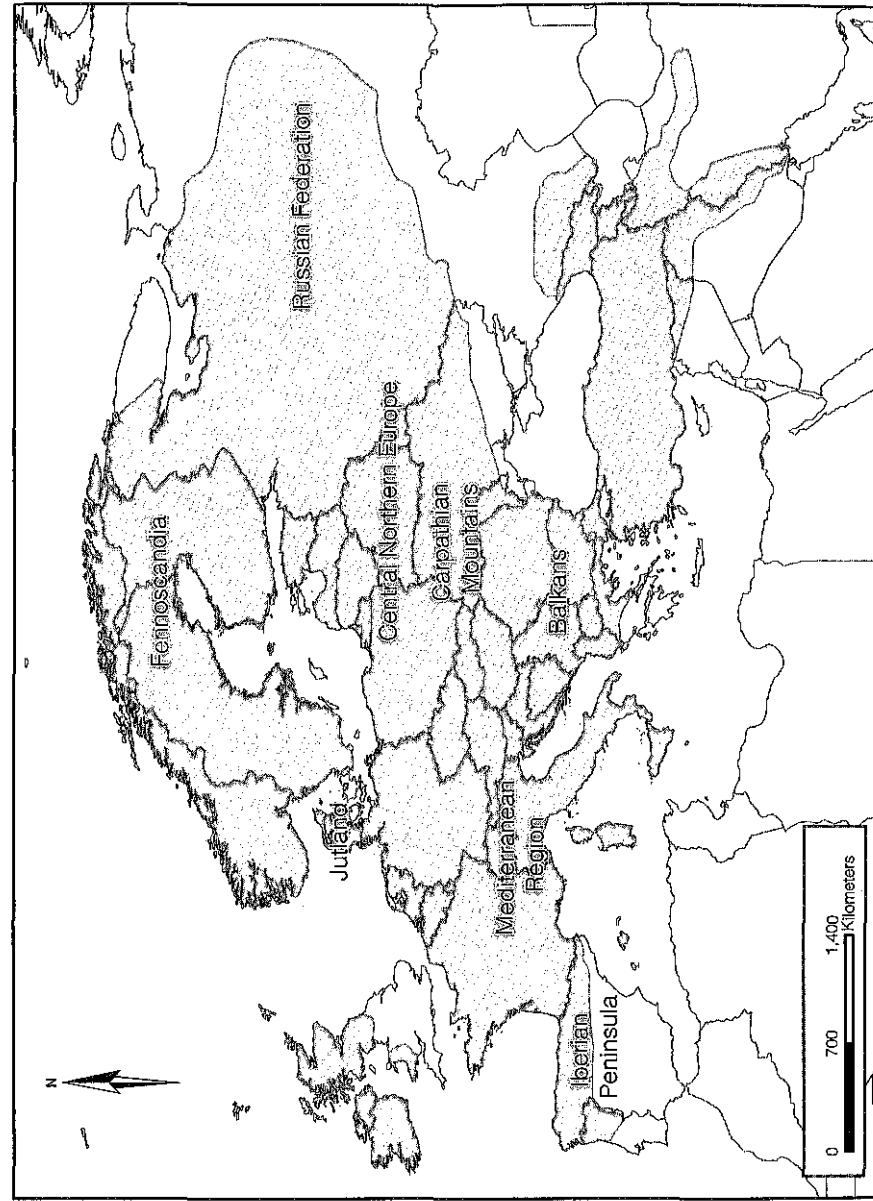


Figure 17.2. Geographic range of *Martes martes*, the European pine marten, adapted from Kranz et al. (2008). The area in light gray is the presumed current range; labels indicate locations discussed in the text.

Europe was recolonized by a population of *M. martes* that survived the last glaciation in an undetermined central-European refugium (possibly the Carpathian Mountains), as was suggested previously by paleontological data (Sommer and Benecke 2004). In addition to this complex recolonization of Europe, genetically differentiated populations of *M. martes* in Fennoscandia and Russia are introgressed with mtDNA of *M. zibellina*, highlighting the complex phylogeographic history of *M. martes* (Ruiz-González 2011).

In a more spatially restricted study, Pertoldi et al. (2008b) studied genetic differentiation of *M. martes* in 3 isolated geographic regions in northern Europe (Jutland and Sealand in Denmark, and southern Scania in southernmost Sweden) by sequencing the hypervariable region of the mtDNA D-loop (350 bp). Pertoldi et al. (2008b) found 8 haplotypes, with 2 shared by individuals from all 3 regions, yet with unique haplotypes found in all localities. This subdivision was likely due to the insular and peninsular nature of the northern European landscape. By comparing these data with previous haplotype analyses (Davison et al. 2001), Pertoldi et al. (2008b) confirmed the presence of 2 primary clades in central and northern Europe, with samples in southern Scania being well differentiated from those in central Sweden. Altogether, these studies point to 3 themes in the phylogeography of *M. martes*: (1) survival in multiple refugia during the last glacial period in the Mediterranean, central-northern European, and Fennoscandian-Russian regions; (2) postglacial recolonization of northern Europe by the central-northern European clade; and (3) recent genetic drift caused by isolating factors, such as major waterways and peninsulas.

Martes martes populations have been shown to have a higher level of genetic structure (with an overall F_{ST} value of 0.18, range: 0.016–0.330) and lower genetic variation (H_E range excluding the insular populations: 53.8–63.8%) than their North American sibling species, *M. americana*, the American marten, sampled throughout Canada (average H_E : 63.6% excluding the Newfoundland island population; Kyle and Strobeck 2003; Kyle et al. 2003). The level of genetic differentiation among *M. martes* populations is correlated with the geographic distance among populations ($r = 0.31$, $P = 0.11$; D_S : $r = 0.55$, $P = 0.007$, D_{LR} : $r = 0.91$, $P = 0.00006$; Kyle et al. 2003; Figure 17.3); thus, *M. martes* in Europe appears to have greater substructure per unit distance than *M. americana*. It is difficult to exclude more ancient processes (e.g., the influence of glaciations) as a cause of the differences observed, but it may be related to the greater level of persecution and habitat fragmentation experienced by *M. martes* (Kyle et al. 2003). At a more local level, *M. martes* in northern Spain are also highly substructured, with F_{ST} values of 0.057–0.172 in a 250-km² area (Ruiz-González 2011). Although the distribution of samples is nearly continuous, substructure in at least 1 of 3 genetically identified clusters corresponds to the presence of anthropogenic influences, such as reservoirs, high road densities, and urbanization (Ruiz-González 2011). Of

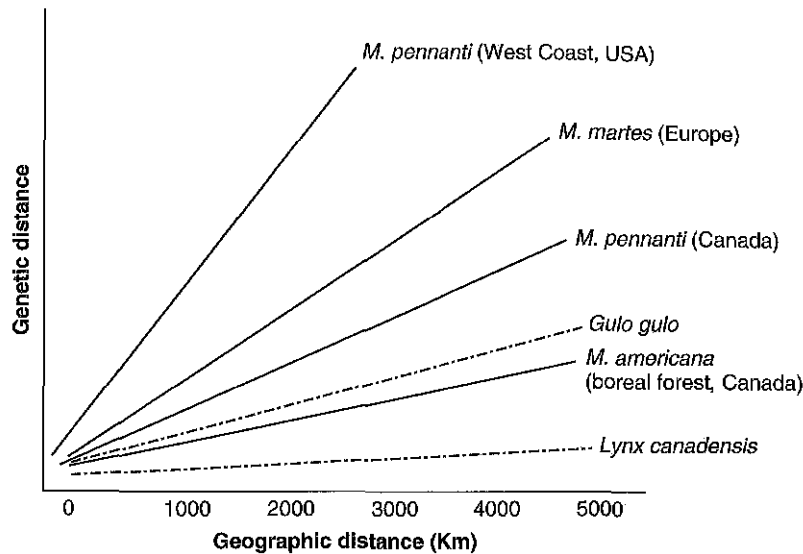


Figure 17.3. A schematic showing the approximate relation between genetic distance and Euclidean distance for several *Martes* species. Dotted lines show the approximate relation for other mid-sized carnivores as a reference. Adapted from Kyle and Strobeck (2003).

particular interest is the potential that 1 of the areas in northern Spain is structured by interspecific competition with *M. foina*, the stone marten (Ruiz-González 2011).

Ruiz-González (2011) also used landscape genetic approaches to evaluate how *M. martes* responds to various vegetation types and topographic and hydrologic features. He found that gene flow and connectivity were strongly reduced by croplands, wetlands, roads, urban areas, and reservoirs (Ruiz-González 2011). Intact forests and scrublands acted as corridors for connecting populations, whereas urban areas acted to reduce gene flow (Ruiz-González 2011). Similar work in Ardennes, La Bresse, and L'Isère in France has shown the importance of forest structure for dispersal of *M. martes* across large landscapes (Mergéy 2007).

Martes zibellina—Sable

Martes zibellina exhibits substantial interpopulation variation in morphological characters and a multiplicity of local forms, complicating the study of intraspecific taxonomy (Monakhov 1976; Pavlinov and Rossolimo 1979). Based on phenotypic and geographic differences, as many as 16 subspecies have been designated for *M. zibellina* (*M. z. angarensis*, *M. z. arsenjevi*, *M. z. averini*, *M. z. brachyura*, *M. z. ilimpiensis*, *M. z. jakutensis*, *M. z. kamtschadalis*, *M. z. obscura*, *M. z. princeps*, *M. z. sahalinensis*, *M. z. sajanensis*, *M.*

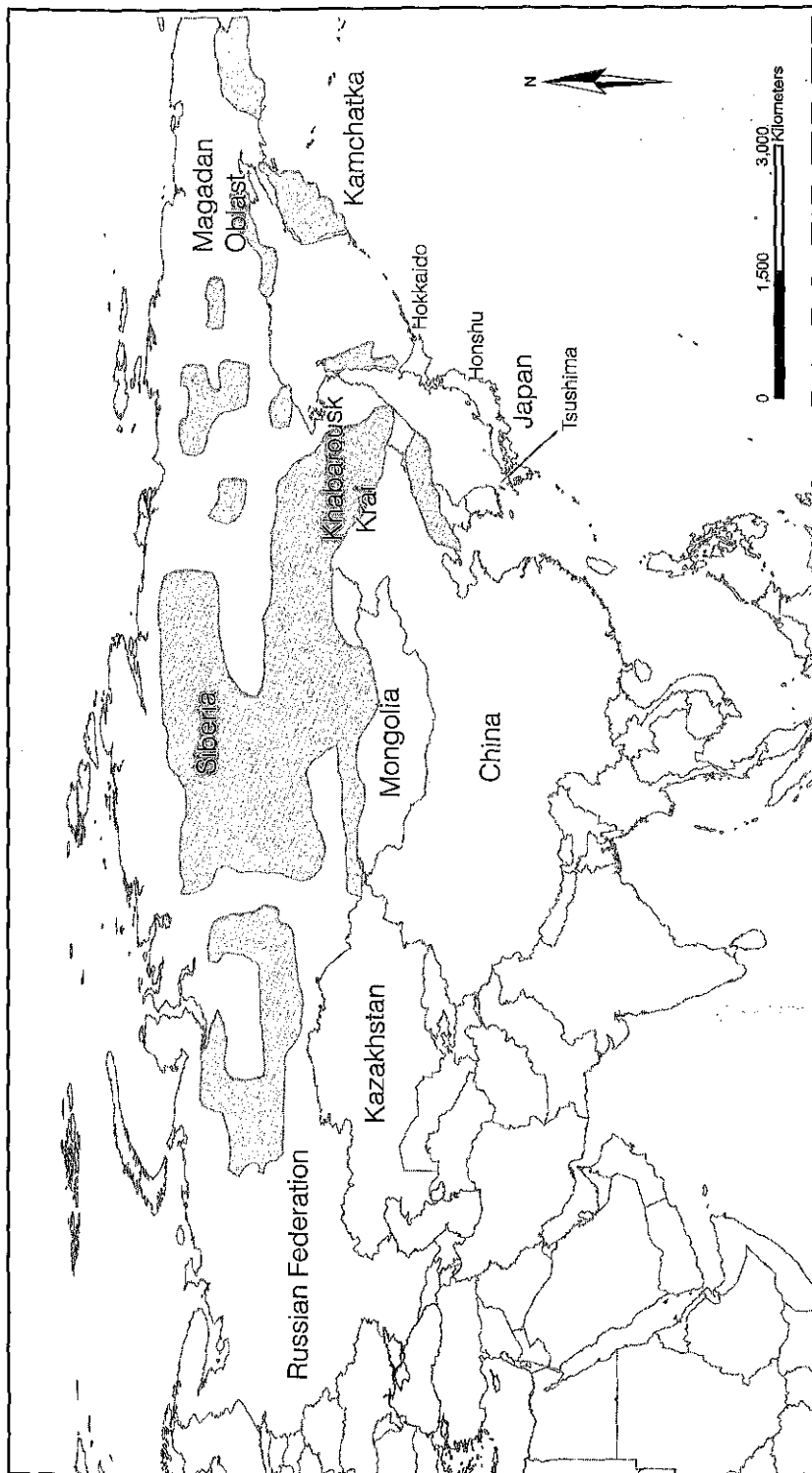


Figure 17.4. Geographic range of *Martes zibellina*, the sable, adapted from Abramov and Wozencraft (2008). The area in light gray is the presumed current range; labels indicate locations discussed in the text.

M. z. schantaricus, *M. z. tomensis*, *M. z. tungussensis*, *M. z. yeniseensis*, and *M. z. zibellina*; Ognev 1925; Kurose et al. 1999; Miyoshi and Higashi 2005; Wilson and Reeder 2005). Yet, untangling the intraspecific phylogenetic history of *M. zibellina* has been complicated by the massive number of human-mediated introductions and reintroductions throughout most of its range in Russia during the 20th century (Figure 17.4; Monakhov 2001; Powell et al., this volume).

Initial analyses of population-level genetic variation in the mtDNA *cyt b* gene fragment of *M. zibellina* from Russia pointed to the existence of high levels of intraspecific heterogeneity (Balmysheva and Solovenchuk 1999a,b; Petrovskaya 2007). Populations of *M. zibellina* from Siberia and the Far East showed the prevalence of 3 different haplotypes, probably representing 3 monophyletic clades (Balmysheva and Solovenchuk 1999a,b; Petrovskaya 2007). To confirm these findings, fine-scale analysis of mtDNA variation was recently conducted by Malyarchuk et al. (2010). This study focused on the analysis of phylogenetic relations using mtDNA *cyt b* sequences in 17 *M. zibellina* from Magadan Oblast, Kamchatka, and Khabarovsk Krai in Russia (Figure 17.4), and data published previously on *M. z. brachyura* from Hokkaido Island, Japan. Malyarchuk et al. (2010) identified 2 phylogenetic clades: the first was represented predominantly by *M. zibellina* from different regions of Northeast Asia, including Kamchatka, Khabarovsk Krai, and the Magadan Oblast, together with samples from Hokkaido, and the second consisted of haplotypes from Magadan Oblast and Khabarovsk Krai, without haplotypes represented in populations in Kamchatka or Hokkaido (Figure 17.4). Interestingly, a specimen of *M. martes* from Sweden clustered with this group, supporting evidence reported previously for historical introgression of *M. martes* with *M. zibellina* in Fennoscandia (Davison et al. 2001; Ruiz-González 2011), although contemporary hybridization has also been suggested (Rozhnov et al. 2010). In the Magadan Oblast, individual *M. zibellina* from both clades have been found, which has been explained by the introduction there of *M. zibellina* from Kamchatka and Khabarovsk, starting in the 1950s (Petrovskaya 2007; Malyarchuk et al. 2010).

Overall, there is strong evidence of at least 2 clades of *M. zibellina* in Russia, both with high sequence divergence that probably resulted from the impact of Pleistocene glaciations. During the Holocene, these 2 clades were reunited in a new contact zone (Malyarchuk et al. 2010). Interestingly, *M. zibellina* on the island of Hokkaido do not form a unique clade, which would be expected if Hokkaido served as a glacial refugium. Thus, it appears that divergence between the populations in Hokkaido and the Russian Far East has been a recent process, probably because Hokkaido was periodically connected to the mainland of Asia by a land bridge as recently as 10,000 years ago (Oshima 1990; Hosoda et al. 1999). Hokkaido may still have been a refugium for 1 of the *M. zibellina* clades during the last glaciation, with recent

postglacial expansion into the mainland of Asia (Kurose et al. 1999). An examination of population genetic structure will be necessary to further understand the complex intraspecific phylogeography of *M. zibellina*, and how contemporary landscape features structure extant populations.

Martes melampus—Japanese Marten

Martes melampus is endemic to Japan, where it occurs on the main Japanese islands of Honshu, Shikoku, and Kyushu, but it is not endemic to Hokkaido Island (Masuda 2009), where *M. zibellina* occurs (Murakami et al. 2004; Murakami 2009). There are at least 3 recognized subspecies of *M. melampus*, based on differences in coat color: *M. m. corensis*, *M. m. melampus*, and *M. m. tsuensis* (Anderson 1970). *Martes melampus* was introduced to Hokkaido from Honshu and is currently expanding its range in southern Hokkaido, whereas the native *M. zibellina* is distributed in central and eastern Hokkaido. The contact zone between the 2 species is in central Hokkaido (Murakami et al. 2004; Masuda 2009). *Martes melampus tsuensis* is restricted to Tsushima Island in the Korea Strait (Figure 17.4), and *M. m. corensis* is thought to occur on the Korean Peninsula, but its existence and identity are controversial (Hosoda et al. 1999).

Martes melampus has a complex taxonomic history; moreover, the presence of the closely related *M. zibellina* (Hosoda et al. 1997, 2000; Sato et al. 2003, 2009b; Koepfli et al. 2008) makes intraspecific assignment difficult. Several studies have focused on genetic relations within and between *M. melampus* and *M. z. brachyura* (Hosoda et al. 1999; Kurose et al. 1999). Hosoda et al. (1999) used the restriction fragment length polymorphism (RFLP) of rDNA spacer and the mitochondrial *cyt b* (402 bp) gene-fragment sequences, and Kurose et al. (1999) sequenced the entire *cyt b* (1140 bp) gene to reveal the extent of intra- and interspecific variation in these 2 species. Both studies showed large genetic differences between the species, yet, in both studies, the clustering of haplotypes of *M. melampus* in phylogenetic trees did not correspond with expected geographic relations between populations on different Japanese islands. Only the Tsushima Island populations (*M. m. tsuensis*) showed geographically concordant genetic differentiation. These results suggest that mtDNA introgression between local populations of *M. melampus* may have resulted from incomplete geographic isolation on each island, limited interpretive power of the available sequence, or the fact that *M. melampus* may have recently expanded to the Japanese islands and the genetic signals have not yet had time to become geographically concordant.

One of the most comprehensive phylogenetic studies of *M. melampus* populations was recently published by Sato et al. (2009b). They conducted molecular phylogenetic analyses of 49 individuals sampled from throughout Japan, focusing on 3 mtDNA loci (*cyt b*, control region, and the NADH

subunit 2 gene) and 1 nuclear gene (the growth-hormone receptor gene, including the polymorphic intron regions). Sato et al. (2009b) identified 9 intra-specific groups, not correlated with winter coat color, but consistent with the geography of the Japanese islands; in particular, they demonstrated the monophyly of *M. m. tsuensis*, the Tsushima marten, supporting the view that its genetic distinctiveness and uniformity resulted from a long history of isolation on small islands. This also confirmed earlier studies (and subspecies designations) proposing that the Tsushima Island population is an evolutionarily significant unit. Sato et al. (2009b) also provide support (although with a limited sample size) of the uniqueness of martens in the Iwate region of Honshu (Figure 17.4).

Overall, phylogenetic patterns of *M. melampus* are more complex and more difficult to resolve than for North American and European martens, which exhibit strong patterns of postglacial expansion. For *M. melampus*, the clearest signal is the isolation of island populations (e.g., Tsushima Island), probably since the Pleistocene. Other patterns are obscured by complex phylogeographic events, such as the dynamics of land bridges among islands (e.g., the Seto-Ohashi Bridge) and some recent translocations by the fur industry (Sato et al. 2009b). Additional research on the population genetic structure of *M. melampus* using variable nuclear markers will provide additional insights into these relations.

Synthesis

Each species in the genus *Martes* is a unique product of evolution with a distinct ecological niche, but we have revealed a few general patterns among these species. First, it is clear that the complex glacial histories of Europe, Asia, and North America created refugial populations that are only recently coming back into contact. In Europe, *M. martes* apparently persisted during the last ice age in well-established southern-Mediterranean refugia, and also in central-northern European and Fennoscandian refugium (Ruiz-González 2011). Genetic evidence suggests large-scale expansions across Europe from the central-northern refugium. *Martes americana* was restricted to multiple refugia, as well, with 2 distinct clades (*americana* and *caurina*) persisting in eastern and western forest refugia in the southern parts of North America during past glacial advances. These deep phylogeographic splits produced 2 clades with very distinct evolutionary histories (Stone et al. 2002), consistent with species-level differences. The Eastern clade, much like the central-northern clade of *M. martes*, expanded into a larger area after the last glacial period. The complex recolonization of ice-free forests in North America also produced separate clades below the species level in the *caurina* clade (Slauson et al. 2009). *Martes pennanti* also persisted in a North American refugium, which was likely in the midwestern or eastern United States (Knaus et al.

2011). Colonization of U.S. Rocky Mountain and California forests appears to be from a single source area, although more information is needed to confirm this hypothesis. More recent biogeographic processes have subsequently shaped this species, producing well-supported clades in the U.S. Rocky Mountains and Sierra Nevada.

Less is known about the intraspecific phylogenetic relations of *M. zibellina*, although there is support for at least 2 clades: one largely in the Russian Far East and on Hokkaido Island in Japan, and another farther west. In the areas between these 2 regions, haplotypes from both clades occur, suggesting recent contact. Of particular interest is that the island of Hokkaido was apparently not an isolated refugium, because *M. zibellina* on this island cluster well with samples from the Russian Far East (Malyarchuk et al. 2010). It is possible that Hokkaido Island may have been the refugium from which the Russian Far East samples originated. *Martes melampus* also has a complex evolutionary history, with support for glacial processes contributing to at least 2 clades: one on Tsushima Island in the Korean Strait (Sato et al. 2009b), and another on the Japanese islands (except Hokkaido). Overall, one of the challenges faced by molecular ecologists is that of untangling these deep phylogeographic signals that were created by complex postglacial histories and influenced by contemporary landscape changes.

Although intraspecific phylogenetic information is essential for providing a context for managers to understand the long-term dynamics of individual species, the field of population genetics has been instrumental in examining the relations among populations. Kyle and Strobeck (2003) plotted a linear regression of D_s (an index of genetic distance) and geographic distances for mainland populations of *M. americana*, *M. martes*, and *M. pennanti* (Figure 17.3). The highest levels of genetic structure per unit distance were found for *M. martes* and *M. pennanti*, followed by *M. americana* (Figure 17.3). This suggests that, outside the Canadian boreal forests, managers of both North American and European *Martes* species should not expect rapid recolonization of areas where populations are currently absent, probably because of life-history or dispersal constraints imposed by the more-fragmented southern habitats.

The field of landscape genetics, an extension of population genetics that examines the finer-scale movements of *Martes* species, is currently being used to provide more-detailed examinations of contemporary landscape features to understand the structuring of populations. This finer-scale genetic information will be most useful to managers of these species. One of the most important landscape-genetic findings to date is that habitat selection for daily requirements (e.g., shelter, food, mating opportunities) is different from habitat selection for dispersal (Wasserman et al. 2010). This provides a good example of the way information from molecular ecology studies can augment traditional field biology studies that use other tools, such as radio-

telemetry and habitat suitability analyses, to understand habitat use. These traditional studies excel at providing detailed habitat information, but because of the sometimes rare nature of dispersal, they have difficulty documenting the habitats used for long-distance dispersal (e.g., Aubry et al. 2004).

In a third application of landscape genetics, Carr et al. (2007b) showed that immigration of *M. pennanti* in Ontario was correlated with low snow accumulation and more coniferous forests, landscape elements that now appear key for maintaining connectivity among populations of this species. Similarly, Broquet et al. (2006a,b) showed that logging significantly changed the landscape for *M. americana* and reduced gene flow compared with unlogged landscapes. In Europe, Ruiz-González (2011) found analogous results using a landscape genetic approach to show that gene flow and connectivity for *M. martes* were restricted by croplands, wetlands, roads, urban areas, and reservoirs, and most facilitated by intact forests and scrublands.

In another type of landscape-genetics application, Garroway et al. (2008) examined the impact of removing a population on existing connectivity, and found that fur trapping is unlikely to affect genetic connectivity under current conditions. This type of approach can be readily used by managers to assess the relative impact of trapping regulations in one area compared with another for any *Martes* species.

Overall, we still have an incomplete taxonomic and evolutionary framework for a significant portion of the *Martes* species complex. Unfortunately, research on intraspecific phylogenetics of *Martes* species is limited and biased toward some species (see Tables 17.1 and 17.2). Most research has been conducted on *M. americana*, *M. martes*, *M. melampus*, and *M. pennanti*, whereas other species, including *M. zibellina* (Hosoda et al. 1999; Kurose et al. 1999; Murakami et al. 2004; Inoue et al. 2010; Malyarchuk et al. 2010), have been only partially or insufficiently studied (Tables 17.1 and 17.2). For *M. flavigula*, the yellow-throated marten; *M. foina*, the stone marten; and *M. gwatkinsii*, the Nilgiri marten, intraspecific phylogenetic studies are completely absent from the literature. Similar trends hold for population genetic and landscape genetic information.

With the recent explosion of genomic approaches, we expect significant amounts of molecular genetic data to be generated that will facilitate exploring the intra- and interpopulation dynamics of *Martes* species (Beja-Pereira et al. 2009). Along with these technological advances, concomitant developments are occurring in analytical approaches for inferring demographic histories and evolutionary relationships from molecular genetic data, and testing their statistical significance (Pertoldi and Topping 2004; Bouchy et al. 2005; Nomura 2005; Bach et al. 2006). Overall, we encourage the use of new genomic methods and associated analytical tools to further resolve intraspecific relations among clades within each species. Specifically, we anticipate the use

of new approaches that examine genes under environmental selection. When we understand how these genes are structured across the landscape, we can better delineate appropriate units for conservation (e.g., species, subspecies, management units), better inform reintroduction efforts, and better predict how *Martes* populations will adapt as the climate changes. We also see the field of genomics providing unprecedented power to conduct novel and important population and landscape genetic studies.

Although these newer approaches will refine our understandings of the well-studied *Martes* species, our most urgent recommendation is to initiate sample collection and all levels of genetic studies on *M. flavigula*, *M. foina*, and *M. gwatkinsii*. There is a complete lack of knowledge about their phylogenetic histories and the factors that influence their movements on the landscape. Without this information, we will have no basis for understanding how these species will fare in a rapidly changing world (Hoffmann and Sgrò 2011).

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