IMPACT OF LANDSCAPE FEATURES ON GENETIC AND PHENOTYPIC VARIATION IN A REINTRODUCED POPULATION OF AMERICAN MARTEN (*Martes americana*) IN THE UPPER PENINSULA OF MICHIGAN

By

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ABSTRACT

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American marten (*Martes americana*) were reintroduced into the Upper Peninsula of Michigan and three genetic clusters have developed proximal to release sites with minimal overlap. We used local relative density of a potential competitor and physical landscape features to examine whether distinct genetic boundaries were associated with landscape features. Depending on the scale of analysis, entire UP compared to within and between each genetic cluster, the landscape features most associated with spatial genetic differentiation varied. Spatial patterns in interindividual phenotypic variation were discordant with spatial genetic structure resolved using neutral molecular markers, indicating disparate causes underlying spatial phenotypic and genetic patterns. Variation in skull morphology among descendent marten was associated with sex and age as well as genetic cluster, however skull morphology was not related to lineage. Taken together evidence suggests adaptation to local habitat conditions within the reintroduction area, compared to shared ancestry, has influenced variation in skull shape among genetic clusters. Results contribute to our understanding of how landscape factors influence spatial patterns of genetic and phenotypic variation and improve our ability to target conservation efforts for American marten.

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THESIS INTRODUCTION

Sound and scientifically defensible management of species and the communities and ecosystems in which they live necessitates fundamental knowledge of their biology including understanding of abiotic and biotic factors that impact abundance and distribution. For species that have been formally extirpated from portions of their native range, one commonly used management strategy involves reintroduction. The effectiveness of reintroduction efforts is somewhat controversial and depends in large part on the measures used to evaluate success (Guerrant, 2013). Genetic monitoring is one promising tool for both evaluating and continually monitoring the success of conservation efforts within an ecologically and evolutionary relevant time-frame (Schwartz *et al.*, 2007). The success of reintroduction events is dependent on the ability of reintroduced organisms to establish and maintain genetically diverse interbreeding populations. Populations that become reproductively isolated, due to habitat fragmentation or locations of geographic barriers, may experience limited gene flow and become more susceptible to the potentially negative effects of genetic drift (Frankham, 2002).

Landscape genetics (Manel *et al.*, 2003) is a multi-disciplinary field that has provided important advancements to population genetics and our understanding of how genetic variation is spatially distributed. Emphasis has been placed on assessment of the relative importance of geographic distance and physical landscape features affecting dispersal, gene flow, and genetic variation (Storfer *et al.*, 2010). However, most landscape genetic studies have ignored biotic (e.g., intra- and inter-specific) interactions that can affect patterns of spatial genetic structure (SGS) (exception, James *et al*. 2011). Incorporating species interactions (e.g., competition) is crucial to understanding the SGS of single species and communities of co-distributed species (Johnson and Stinchcombe, 2007).

When populations become separated by a barrier, such as a physical barrier, and gene flow is reduced, selection and genetic drift within isolated subpopulations may enhance phenotypic divergence (Slatkin, 1987). Selection can also influence phenotypic divergence among populations experiencing different local environmental conditions and restrict the success of dispersers immigrating or emigrating among populations (Crispo *et al.*, 2006). Individuals that are able to navigate the habitat matrix between populations may be unable to survive to mate under certain ecological conditions (e.g., unable to acquire food resources, difficulty competing for resources with residents) (Rundle and Nosil, 2005).

In this thesis, I examine the factors influencing patterns of morphological and genetic variation in American marten (*Martes americana)* in the Upper Peninsula of Michigan. Prior to settlement of Michigan by Europeans in the early 1800s, martens were found throughout the Upper Peninsula and throughout the northern Lower Peninsula (perhaps even in low densities throughout the southern half of the Lower Peninsula) (Baker, 1983). Large portions of the state were deforested as human population increase lead to increases in timber harvest and wildfires (Brewer, 1991). Efforts to re-establish a sustainable population in Michigan began in 1955 with the release of eight animals into the Porcupine Mountain Wilderness (Switzenberg, 1955).

Several other introductions (number individuals=276) and translocations (number individuals=85) in the UP followed with a combination of live-trapped and animals from breeders (Baker, 1983). Most individuals came from several areas in Ontario, although some individuals came from as far as Colorado and Minnesota (Williams *et al.*, 2007).

Three genetically differentiated clusters of individuals developed in regions proximal to release sites, suggesting founders from independent releases had colonized different regions with minimal overlap (Williams and Scribner, 2010). The variation in cluster area and lack of evidence for admixture among members of different genetic clusters may be associated with the location of cryptic dispersal barriers, potentially attributed to the degree of landscape permeability and location and contiguity of suitable habitat. Marten are well studied in terms of habitat preferences, dispersal capabilities and biotic interactions with co-distributed species. Marten tend to prefer coniferous or deciduous forest, composed primarily of mature trees with a fairly closed canopy (Buskirk and Powell 1994, Koehler and Hornocker 1977; Marshall 1951). The most suitable forest habitat has a high degree of structural complexity in the understory with an abundance of coarse woody debris that can be used as refuge sites and to forage for small mammal prey during the winter (Chapin *et al.* 1998). Marten tend to avoid large openings of clear-cut land (Chapin *et al.* 1998) or paved highways when foraging or dispersing from natal sites. Fisher (*Martes pennanti*) may influence marten movement and dispersal, because fisher can prey on marten (Krohn *et al.* 1997) or may act as a source of interference competition; a factor affecting marten habitat selection and movements (Krohn *et al.* 1995, Fisher *et al*. 2013). My research considers both physical habitat features and the spatial distribution of a co-occurring species that may influence American marten gene flow.

In addition to influences on spatial patterns of neutral genetic variation, landscape features may select for locally adapted phenotypes and lead to phenotypic divergence among populations of marten. If patterns of phenotypic variability are discordant with spatial genetic structure resolved using molecular markers, this incongruence may indicate disparate evolutionary factors underlying spatial phenotypic and neutral genetic patterns. The features associated with phenotypic divergence among populations have been explored in numerous taxa and traits (Spurgin *et al.* 2014 Wang and Summers, 2010, Reale *et al.* 2003). Skull morphology has been shown to undergo rapid adaptive divergence (Marroig and Cheverud, 2004). Different foraging strategies may be required when individuals expand into novel environments associated with different food resources (Grant 1981, Badyaev 2009, 2010, Melero *et al.* 2008, Wolf *et al*. 2008). Phenotypic variation in skull shape may also be related to limited dispersal due to geographic distance between individuals (isolation by distance; Wright 1943) or dispersion of landscape features (isolation by landscape resistance; McRae 2006). In addition to information on habitat preferences and genetic variation, I have obtained skulls from the University of Michigan collections for a subset of the same individuals that have been genotyped within the UP, and skulls from source populations in Ontario from the Royal Ontario Museum collections. Studies that consider the impact of selection as well as limited dispersal can improve our understanding of the factors shaping spatial genetic and phenotypic variation (Spurgin *et al*. 2014).

CHAPTER I: INFLUENCE OF LANDSCAPE FEATURES AT MULTIPLE SPATIAL SCALES ON SPATIAL GENETIC STRUCTURE OF AMERICAN MARTEN (*Martes americana*)

Quantifying the influence of landscape features on genetic connectivity is a major goal in conservation biology. When landscape features disrupt successful dispersal, genetic discontinuities may arise. The location of genetic cluster boundaries may be related to specific landscape features influencing gene flow. Reintroductions provide a useful context to study contributions of environmental variables and genetic variation among source populations to emergence of spatial patterns in genetic variation among descendents. The reintroduction of American marten (*Martes americana)* in the Upper Peninsula of Michigan originated from multiple genetically differentiated source populations. Three spatially distinct genetic clusters developed in regions proximal to release sites, suggesting founders from independent releases had colonized different regions with minimal overlap (Williams and Scribner 2010). We used landcover data and localized harvest density of a congener (fisher, *Martes pennanti*) to examine whether genetic boundaries were associated with landscape features based on least cost paths estimated using physical landscape and biotic cost surfaces. We used multi-locus microsatellite genotypes to calculate inter-individual relatedness. Across the entire UP we found that the presence/absence of roads was the most supported model explaining inter-individuals genetic variation. Inter-individual comparisons within and between genetic clusters revealed that proportion of forested area or both proportion forested area and percent overhead cover were most closely associated with genetic distance. Reduction in forested area and/or an increase in landscape fragmentation by roads may further decrease genetic connectivity among American marten.

INTRODUCTION

Quantifying the relative influence of landscape features on demographic and genetic connectivity is a major goal in conservation biology. Landscapes can either impede or facilitate movement and genetic exchange among groups of individuals (Manel et al. 2004). When landscape features disrupt successful dispersal, genetic discontinuities may arise whereby genetic clusters of individuals are spatially contiguous, but with little evidence for interbreeding (Williams and Scribner 2010). The location of cluster boundaries may be related to specific landscape features potentially influencing movement, dispersal and genetic connectivity (Lowe and Allendorft 2010). Although there are many methods and software programs implementing these methods for detection of genetic discontinuities (e.g. WOMBLING – Womble 1951**,** STRUCTURE – Pritchard *et al.* 2000, GENELAND – Guillot *et al.* 2005 A) simulation studies have shown that spatial Bayesian clustering methods, as implemented in program GENELAND, have the highest success at detecting boundaries when they exist and detected barriers most quickly (e.g., over fewer generation times) (Landguth *et al 2010,* Blair *et al.* 2012).

Landscape genetics has provided important advancements in quantifying the relative influence of landscape features to population or individual connectivity (Storfer *et al.* 2010). Most landscape genetic studies consider physical landscape features. Often, the degree of landscape permeability is associated with the distribution of physical landscape features (e.g., cover type), climatic factors (e.g., precipitation) and human alterations (e.g., roads) (Storfer *et al.* 2010). Studies generally have not evaluated hypotheses of how the distribution of potentially interacting species could impact dispersal and gene flow (but see James *et al*. 2011). The abundance and distribution of sympatric species (e.g., predators and prey or competitors) can also be landscape-dependent and can affect dispersal and development of spatial genetic structure (e.g., Wiens *et al*. 1993, Danielson and Gaines 1987, Leibold *et al.* 2004, Hauzy *et al.* 2007). Consequently, consideration of both physical landscape features and distribution and relative abundance of co-occurring species (e.g., competitors or predators and prey) can be important for quantifying the impact of landscape composition and configuration on dispersal.

Another important issue in landscape genetics is tied to the scale of analysis (Anderson *et al.* 2010). For continuous or widely distributed organisms, individual-based analyses have been shown to have the most success detecting the effect of specific landscape features on spatial genetic structure in heterogenous landscapes (e.g**,** Cushman and Landguth 2010). In a simulation-based approach, authors were able to detect the effects of a barrier on spatial genetic structure within 1-15 generations by analyzing pairwise genetic distances among individuals using partial Mantel tests (Landguth *et al* 2010). Across large spatial scales in hetergenous landscapes, different landscape features may be influencing dispersal depending on the region of interest and the use of only one spatial scale may obscure the impact of landscape features affecting gene flow (Anderson *et al.* 2010). For example, Murphy *et al.* (2010) found that land cover variables were correlated with genetic distance at finer scales, while ridgelines were significant at broader scales.

Reintroductions and translocations are important conservation tools for extirpated species. Although widely-used, there is considerable variability in the success of reintroduction programs as evidenced by different rates of population increase and geographic range expansion (Griffith *et al.* 1989). Because dispersal is difficult to measure directly, genetic data are frequently used to assess population connectivity (e.g, Koenig *et al.* 1996). Introductions and reintroductions provide a useful context to study the relative contributions of environmental variables and genetic variation among source populations to emergence of genetic variability among descendents (Griffith *et al*. 1989).

The American marten (*Martes americana*) is a useful species for studies of how landscape features influence dispersal. Marten are well studied in terms of habitat preferences, dispersal capabilities and biotic interactions with co-distributed species. Marten tend to avoid open areas with no overhead cover to provide protection from aerial predators (Drew, 1995). Canopy cover (Can) is often associated with prey resources (Thompson and Colgan, 1994) and with sites of marten subnivean entry (Corn and Raphael, 1992). Contiguous mature forested areas (For) are preferred habitat for marten where they have access to high quality prey items including red squirrels (*Tamiscuirus hudsonicus*) and denning/resting sites (Coffin *et al*. 1997, Zielinski and Duncan 2004, Steele 1998, Wilson and Ruff 1999). Marten tend to avoid large openings (Chapin *et al.* 1998) or roads (Rd) when foraging or dispersing from natal areas. Roads represent a potential source of mortality and landscape feature marten tend to avoid (Robitaille and Aubry, 2000).

In addition to physical landscape features, the distribution of heterospecifics can also influence dispersal and colonization success (e.g., Danielson and Gaines 1987). The presence of fisher (*Martes pennanti;* MP) may impede American marten movements, because fisher can prey on marten (Krohn et al 1997)or may act as a source of interference competition and factor affecting marten habitat selection and movements (Krohn *et al.* 1995, Fisher *et al*. 2013). Depending on whether a region offers sufficient prey resources and territories for both species, the presence of one congener may result in exclusion of the other (Fisher *et al* 2013).

Considerable information is available regarding American marten (*Martes americana*) reintroduction history into portions of their former native range. The reintroduction of American martens into the UP of Michigan originated from multiple genetically differentiated source populations (Williams *et al.*, 2007). Efforts to re-establish a sustainable population in Michigan began with the release of 29 marten into the Porcupine Mountains Wilderness State Park (27 individuals from Crown Chapleau Game Preserve (CCGP), Ontario and 2 individuals originally from British Columbia but raised on a fur farm in Delta County, Michigan; Michigan Department of Conservation 1957; Harger and Switzenberg 1958). Ninety-nine marten from the former Port Arthur Ontario Ministry of Natural Resources District (now Thunder Bay District, TB), Ontario, were released into Delta County in 1969 and 1970 (Michigan Department of Natural Resources 1970). One hundred forty eight individuals from Algonquin Provinical Park (APP) were released at four sites in the west-central UP (Churchill *et al*. 1981). From 1989 to 1992, three secondary translocations were made in eastern and northern portions of the UP. In 1989 and 1990, 20 marten from Iron County and 27 marten from the Hiawatha National Forest West Unit were released into the Tahquamenon Bay Area of the Hiawatha National Forest East Unit. In 1992, 19 marten from southern Houghton County were released in southeastern Keweenaw County (William *et al.* 2007).

Three genetically differentiated clusters of individuals developed in regions proximal to release sites, suggesting founders from independent releases had colonized different regions with minimal overlap (Williams and Scribner 2010) (Figure 1.1). Distances and direction of dispersal from release locales by descendents of different source populations varied among genetic sources (Williams *et al*. 2007). Distances of effective gene flow inferred based on spatial autocorrelation also vary greatly among clusters; 90km for the Eastern UP Hiawatha cluster, 40km for the Central Huron Mountain cluster, and 30km for the Western Porcupine Mountain cluster (Williams and Scribner 2010). Variation in cluster area and spatial extent of spatial genetic structure (i.e., non-zero genetic autocorrelation) may reflect differences in landscape permeability at and between initial release sites. Consequently, consideration of each region occupied by a distinct genetic cluster is necessary to correctly identify landscape features influencing the development and maintenance of spatial genetic structure within areas of landscape heterogeneity and distinct founding sources. The degree of spatial clustering and locations of relatively distinct boundaries between different inferred genetic clusters for marten contrasts greatly with fisher that were reintroduced in the UP during the same time period as marten, but are spatially genetically panmictic (Williams 2006).

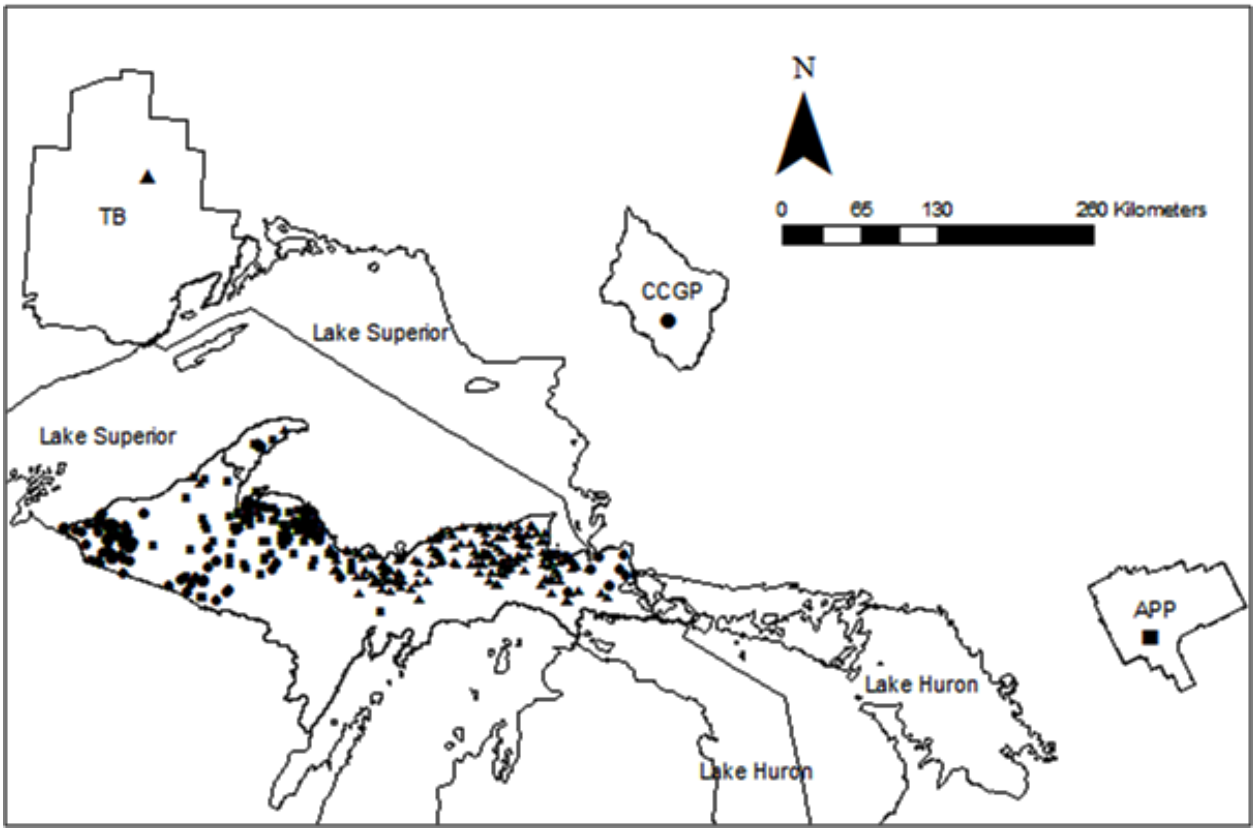


Figure 1.1. Location of American marten genetic clusters within the reintroduction area (Upper Peninsula, UP, of Michigan, MI). Individual membership to each spatial genetic association based on posterior probability estimated using program BAPS 4.1 (Williams and Scribner 2010) is indicated by a unique symbol. Triangles = HIA (Hiawatha), squares = HM (Huron Mountain) and circles = PM (Porcupine Mountain). The location where the majority of individuals from putative source populations came from is indicated by corresponding symbols. Triangle = TB (Thunder Bay) introduced into the south-eastern UP, square = APP (Algonquin Provincial Park) introduced into the south-central and north-central UP, circle = CCGP (Crown Chapleau Game Preserve) introduced into the western UP.

Our objective was to quantify the relative influence of landscape features on spatial genetic structure in different regions of Michigan's UP. Specifically, we used least cost paths and causal modeling to evaluate Euclidean distance (Euc) and each of the four (Can, For, Rd, MP) above-mentioned landscape features associated with patterns of spatial genetic structure at the individual level within and between three regions where marten were reintroduced. To assess the impact of landscape features locally affecting spatial genetic structure among members of different genetic clusters, we quantified support for models parameterized using different suites of landscape features for pairs of individuals (joins) that were either within (intra-cluster) or between (inter-cluster) each of the three genetic clusters. In this way we could assess the importance of landscape features on spatial genetic structure for individuals with shared founding history (and co-occupancy of the same regional landcover; intracluster analyses) and descendents from genetically distinct source populations (intercluster analyses. Results contribute to understanding the relative importance of physical habitat features and species interactions on gene flow during contemporary periods without confounding effects of historical reintroduction events (Blair *et al* 2012).

METHODS

Study Site and Sample Collection

The Upper Peninsula of MI consists primarily of a mix of deciduous/coniferous forest in the central and western regions and broader diversity of cover types (e.g., forest as well as wetlands, agricultural area) in the east. Marten currently occur across much of the UP's ~44,000km^2. Following reintroductions (1955-1992), population abundance rapidly increased allowing for sustainable harvest. A fur-trapping season was opened in 2000. From 2000-2004, the harvest season occurred between December 1st and 15th with a bag limit of 2 for Native Americans and 1 for all other fur-takers across the Upper Peninsula (Frawley, 2001 MI DNR). Muscle tissue samples were collected from marten harvested in the UP of Michigan during the 2000-2004 trapping season. The location of each harvested marten was reported to the level of the section (1 section = 1mi^2).

Genetic Data

Our data consisted of 495 harvested marten that had been genotyped at eleven highly variable microsatellite loci (see Williams and Scribner, 2010 for complete description of sample acquisition and molecular techniques). Marten harvest location was reported to the level of a section (1mi^2) and marten were assigned Cartesian coordinates based on the location of the centroid of a section. Pair-wise estimates of inter-individual genetic distance were calculated for all marten using the A_r measure (Rousset 2000) in program SPAGeDi ver 1.4 (Hardy and Vekemans 2002). Genetic distance between individuals (A_r)

is analogous to the F_{st} /1-F_{st} measure of genetic differentiation between populations. Microsatellite loci have high mutational rates making them appropriate genetic markers for examining relatively recent changes in contemporary patterns of connectivity (Pearse and Crandall 2004). Previous research based on aspatial (STRUCTURE; Pritchard *et al.* 2000) and spatial (BAPS 4.1, Corander *et al.* 2006) clustering analyses demonstrated the presence of three genetic clusters and assigned individuals to one of three clusters based on posterior probabilities (Williams and Scribner 2010). We were specifically interested in the landscape features at the boundaries between genetic clusters. Based on simulations from Blair *et al.* 2012, spatial Bayesian clustering methods, such as GENELAND, have the greatest probability of detecting barriers after relatively few generations of population separation. Thus, individuals were assigned to a genetic cluster for subsequent regional analyses based on the cluster with the highest posterior probability of assignment (Guillot *et al*. 2005a; Guillot *et al.* 2005b).

Boundaries between genetic clusters identifying cluster membership were identified based on the spatial model option in program Geneland (Guillot *et al*. 2005a; Guillot *et al.* 2005b, Blair *et al.* 2012). The spatial model incorporates X and Y (longitude and latitude) coordinates with each individual's multilocus genotype to determine genetic cluster boundaries that we hypothesized may be attributed to limited gene flow associated with physical (e.g., landscape features or roads) or biotic (e.g., inter-specific interactions) landscape features that are barriers to dispersal. For our model, we included coordinate uncertainty (1km) associated with marten harvest locations because the location of harvested marten was likely recorded with unknown error. The number of genetic groups (K), was estimated using a Markov Chain Monte Carlo (MCMC) algorithm. Based on author recommendations (Guillot *et al.* 2005b), we first allowed K to vary and then ran the algorithm again with K fixed at the K most supported by the data (number of K with highest average posterior probability). We ran the model four times, allowing K to vary, with the following parameters: 250000 MCMC iterations, maximum rate of Poisson process at 495 (i.e., equal to the number of individuals as suggested by Guillot *et al.* 2005a), coordinate uncertainty of 1km, minimum K at 1, maximum K at 5, maximum number of nuclei of the Poisson-Voronoi tessellation at 1500, close to three times the number of individuals in our dataset (i.e., 3 times the maximum rate of Poisson process as suggested by Guillot *et al*. 2005a). We used an uncorrelated allele frequency model (i.e., asymptotic distribution of allele frequency following a Dirichlet distribution) based on the increased performance over the correlated allele frequency model available in Geneland (Guillot *et al.* 2005a). We then re-ran the MCMC algorithm 10 times (thinning=10) with K fixed at 3 (previously inferred number of genetic clusters when allowing K to vary), a burn-in of 25000 iterations, and other parameters as described above (Frantz *et al*. 2009). Cluster boundaries were used to define which individuals were considered in intra and intercluster regional causal modeling analyses (described in Causal Modeling methods section).

Landscape Models

To quantify associations between landscape features and spatial genetic structure among individuals within and between clusters, we calculated total cost distances using the Least Cost Path (LCP) tool in the Landscape Genetics extension for ArcGIS9.3 (Etherington 2011). The LCP method calculates a single path of least resistance between individual marten based on hypothesized relationships between specific landscape features within a given grid cell and the ability of an organism to move through landscape features (Moore *et al.* 2011). To derive the grids, we selected landscape features that we predicted would be relevant to American marten dispersal based on relevant literature and expert opinion (Table 1.1). For all cost surfaces, we classified grid cells at a 100m resolution. Previous research exploring the impact of scale-dependent inference in landscape genetics has shown that models of connectivity are usually robust to increasing pixel grain (cell size) (Cushman and Landguth 2010).

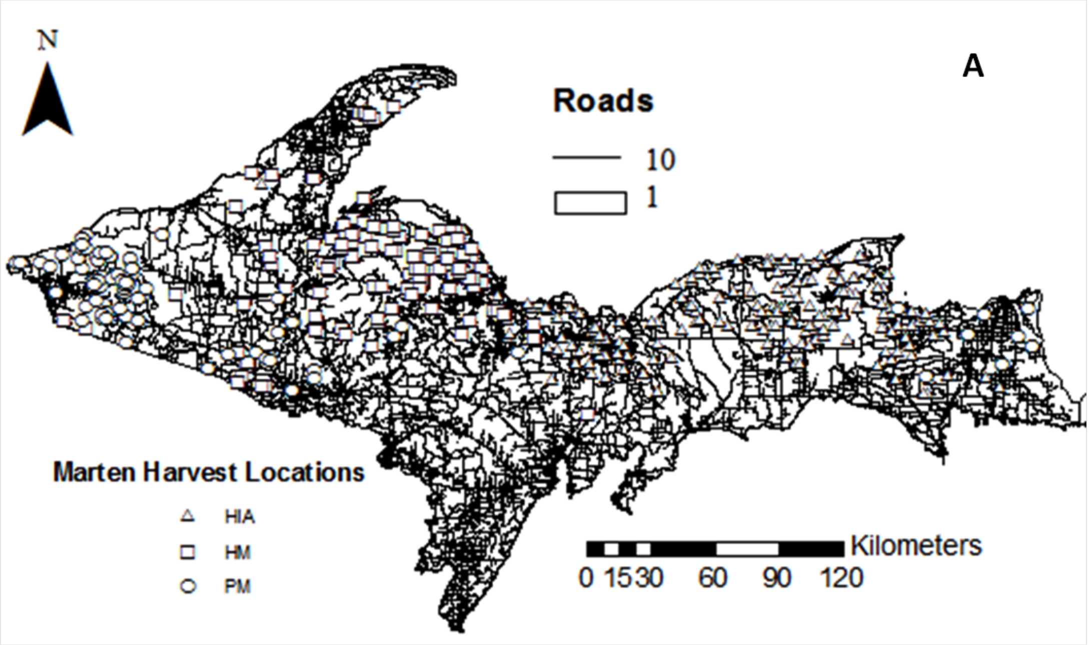
In the isolation by distance model, we assumed a uniform surface whereby each grid cell had the same resistance (one), assuming no effect of landscape features on spatial genetic structure. The underlying hypothesis was that genetic relationships among individuals were associated with straight-line geographic distance (Wright 1943). We represented two different levels of roads (Rd) as categorical cost functions. Roads represent a potential source of mortality and landscape feature marten tend to avoid (Robitaille and Aubry 2000). Roads have been shown to affect spatial genetic structure in other mustelid landscape genetic studies (e.g., fisher – Garroway *et al* 2011). For one model, we tested for the influence of the presence/absence of roads on spatial genetic structure. Using the Michigan Geographic Framework All Roads dataset we classified grid cells at a 100m resolution. We assigned a higher cost (higher grid cell value) to roads versus grid cells without roads (Figure 1.2 A). For our second model of roads, we classified grid cells based on the Michigan Geographic Framework All Roads and Michigan Department of Transportation National Functional Classification codes (NFC), with higher cost (higher grid cell value) assigned to major roadways (freeways and principle arterials) versus other roads (such as unpaved county roads) versus grid cells without roads. Results from sensitivity analyses showed almost no difference between Mantel correlations for our Rd models so we proceeded with the model of 2 road levels (Appendix 1.1). For our canopy cover model (Can), we evaluated the effect of percent overhead cover on marten spatial genetic structure. Canopy cover (Can) is often associated with prey resources (Thompson and Colgan, 1994) and is associated with sites of marten subnivean entry (Corn and Raphael, 1992). Using the 2001 National Land Cover Database Percent Tree Canopy dataset, we considered percent canopy cover as a continuous floating point raster. Higher cost values were assigned to grid cells with lower canopy cover (Figure 1.2 B). For proportion of forested area (For), we used the 2001 GAP LandCover dataset derived from Landsat satellite to quantify the proportion of each 100 x 100m grid cell composed of forest and nonforested areas (Figure 1.2C). Contiguous mature forested areas (For) are preferred habitat for marten where they have access to high quality prey items including red squirrels (*Tamisciurus hudsonicus*) and denning/resting sites (Coffin *et al*. 1997, Zielinski and Duncan 2004, Steele 1998, Wilson and Ruff 1999). For our fisher model (MP), we hypothesized that areas of higher localized fisher harvest density would impede dispersal. Our assumption was that areas of higher or lower harvest density would provide a surrogate measure of comparatively higher or lower fisher densities. For example, Bowman *et al.* (2007) demonstrated that density of trapped ranch mink explained the largest component of variation in annual mink harvest by trappers. Raphael (1994) used harvest numbers as a proxy for population status of furbearers (marten and fisher). Cattadori *et al*. (2003) used grouse harvest abundance as an index of grouse abundance.

We used fisher harvest locations in the UP for individuals harvested during 2000-2004 to create kernel density function (Silverman 1986) grids in ArcGIS 9.3. We then reclassified grids into categories ranging from 1-10 (low to high relative harvest density). Relative harvest density estimates were adjusted for unequal harvest efforts (bag limit of 1 for Management Unit B and a bag limit of 3 across both Units) by weighting the density estimates for each geographic area by the percentage of harvest that occurred in each Management Unit (Bales and Self 1993). Management Unit A encompasses the western UP (coincident with region occupied by Porcupine Mountain genetic cluster) and management unit B encompasses the eastern/central UP (coincident with region occupied by Huron Mountain and Hiawatha clusters) as well as the Keewenaw Peninsula. We then created a median density grid by calculating the median values over the 5 yearly density grids (Figure 1.2D).

For all cost surfaces, we tested several different weighting schemes (1 to 10, 1 to 100, 1 to 1000) using the significance of Mantel correlations to determine how sensitive our results were to different cost values (Appendix 1.1, Rayfield *et al.* 2010). Because weighting schemes did not influence the significance of Mantel correlations at the scale of the entire UP, we performed causal modeling with all variables on a scale of 1 to 10. We assumed a linear relationship between increasing cost values and increasing raw raster values (Garroway *et al.* 2011). For example, for our cost surface of canopy cover, cost increased from 1 (100% canopy cover) to 10 (0% canopy cover).

Table 1.1. Model variables used to create cost surfaces for individual American marten in the Upper Peninsula of Michigan.

Variable	Type	Description	Rationale	Data Source	Weights
Roads	Categorical	Roads	Behavioral avoidance and potential source of mortality (Robitaille and Aubry, 2000)	MI Geographic Framework All Roads dataset (http://www.mcgi.state.mi.us/)	1, 10, 1,100, 1,1000
Proportion Forested Area	Continuous	Percentage of grid cell containing forest stands	Marten prefer forested areas of coniferous or deciduous and mixed coniferous/deciduous stands (Coffin *et al.* 1997, Zielinski and Duncan 2004, Steele 1998, Wilson and Ruff 1999, Potvin *et al.* 2000) where there may be a higher abundance of prey resources, resting sites, and escape from possible predators.	2001 GAP Landcover dataset derived from Landsat satellite imagery (http://gapanalysis.usgs.gov/gaplandcover/data/), 30m resolution	1-10, 1-100, 1-1000
Canopy Cover	Continuous	Percent of a given area occupied by overhead cover	Protection from aerial predators (Drew, 1995), associated with subnivean resting access (Corn and Raphael, 1992), and prey (Thompson and Colgan, 1994)	National Landcover Database 2001 Percent Tree Canopy dataset (http://www.mrlc.gov/nlcd2001.php), 30m resolution	1-10, 1-100, 1-1000
Fisher Harvest Density	Continuous	Density of harvested fishers	Fisher may predate on marten (Raine 1987) and represent a source of indirect competition for food resources, particularly small mammal prey (Krohn et al. 1997) and denning sites (Clem 1977).	Derived from Michigan Department of Natural Resources fisher harvest locations during 2000-2004 and ESRI ArcGIS 9.3 Kernel Density Tool	1-10, 1-100, 1-1000



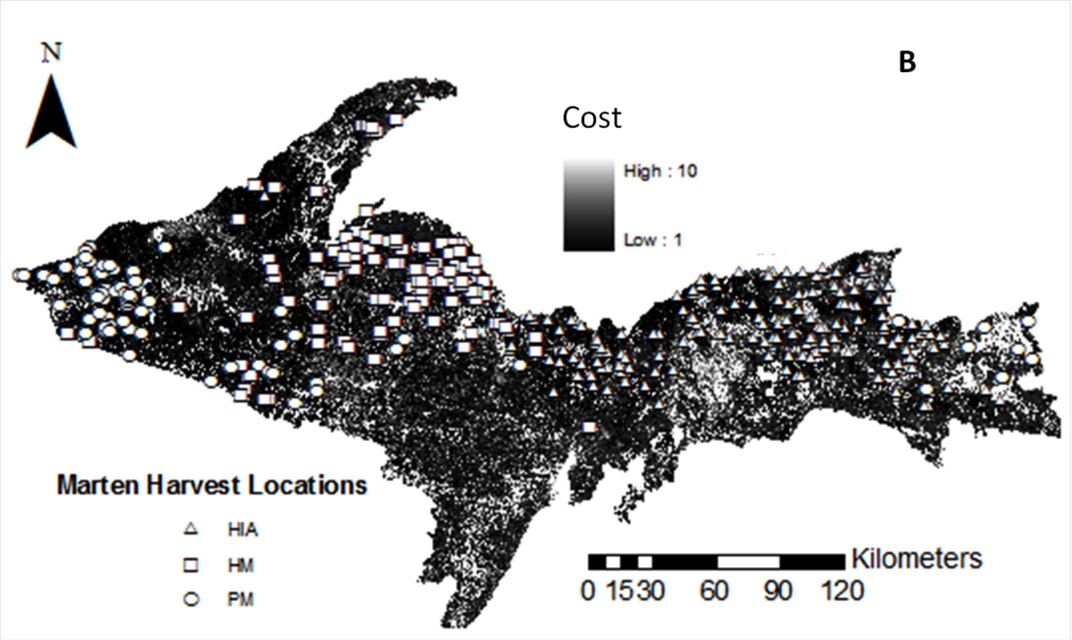
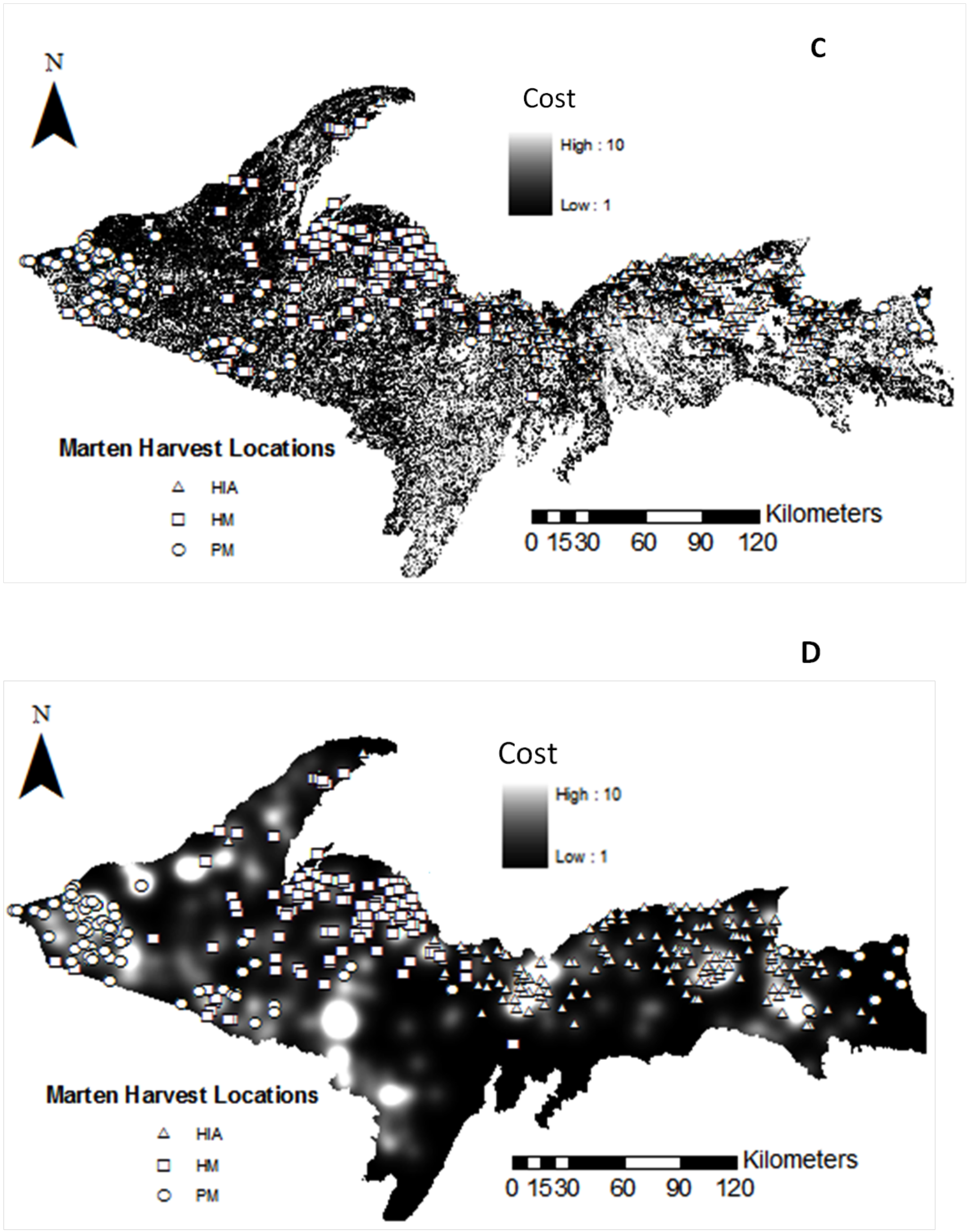


Figure 1.2 A-D. Hypothesized relationship between specific landscape features and American marten gene flow. A) Road grid cells assigned a cost of 10, non-road grid cells a cost of 1. B) Grid cells with low percent canopy cover (white) assigned higher cost and grid cells with high percent canopy cover (black) assigned a lower cost. C) Grid cells with low percent forested area (white) assigned higher cost and grid cells with high percent forested area (black) assigned a lower cost. Grid cells with low localized fisher harvest density (black) assigned lower cost and grid cells with high localized fisher harvest density (white) assigned a higher cost. Symbols indicate American marten harvest location and affiliation to one of three spatial genetic clusters

Figure 1.2 (cont'd)



Causal Modeling

We used causal modeling based on partial Mantel tests (Yang *et al.* 2013; Cushman *et al.* 2006) to determine the relative support for each of our models (Euclidean distance or least cost distance calculated for landscape variables described above). Causal modeling relies on a series of partial Mantel tests between matrices of inter-individual genetic distance and geographic distance. Although partial Mantel tests have low Type II error rates (false negatives; Cushman *et al.* 2013), they have inflated Type I error rates (false positives particularly when there is spatial autocorrelation in genetic or landscape data; Guillot and Rousset 2011, Meirmans 2012, Amos *et al.* 2012, Cushman *et al.* 2013) and this may lead to spurious correlations in analyses examining associations of alternative models of landscape feature cost distance and genetic distance. Consequently, Cushman *et al.* 2013 suggested evaluating the relative support for landscape models (based on the correlation coefficient between cost distance and genetic distance) rather than relying on hypothesis testing based on p values (significance of the correlation between cost distance and genetic distance). We evaluated the relative support for each landscape model based on the magnitude of r values from partial Mantel tests. Specifically, we calculated the difference between partial Mantel r values for correlations between genetic distance and a focal model, while accounting for variables in the alternative models (Test 1). We then estimated correlations between genetic distance and landscape features under the alternative models, while accounting for variables in the focal model (Test 2). Positive values for the difference in partial Mantel r-values provide evidence for model support (Step 1 of Causal Modeling). Models with support (positive values) are formally evaluated (Step 2 of Causal Modeling) by examining the Mantel r values from Test 1 and Test 2. For a model to be fully supported in this second step, it must have positive values for all partial Mantel tests in Test 1, and all partial Mantel r values must be zero or negative in Test 2.

Depending on the spatial scale of landscape genetic analyses, different landscape features may be important in generating and maintaining spatial genetic structure (Anderson *et al.* 2010). Additionally, genetically distinct source populations were used in reintroduction efforts in different regions of the UP (Figure 1.1, Williams and Scribner 2010). To avoid confounding effects of ancestry we conducted causal modeling within (intra-cluster) each region associated with a genetic cluster. Analyses were then conducted using inter-individual comparisons between members of different genetic clusters (inter-cluster). Consequently, we restricted analyses to inter-individual comparisons of marten that were within (intra-cluster) or between (inter-cluster) genetic groups assigned based on genetic cluster boundaries identified using GENELAND. For example, one series of partial Mantel tests would consist of intra-cluster comparisons for individuals from the Eastern Hiawatha UP genetic cluster and another series of partial Mantel tests would consist of only inter-cluster comparisons for individuals from the Eastern Hiawatha and Central Huron Mountain UP cluster. Because our interest was in quantifying the impact of landscape features on spatial genetic structure across what Williams and Scribner (2010) described as cluster ‘boundaries’ versus within the three genetic clusters, we excluded individuals that were assigned to a specific spatial genetic cluster that was not the cluster from where they were harvested (n = 25).

RESULTS

Genetic Structure of American Marten in the UP

The most supported number of clusters across the Upper Peninsula was K=3 (Figure 1.3). GENELAND identified boundaries consistent with the three genetic clusters identified from STRUCTURE (nonspatial) (Pritchard *et al*. 2000) and BAPS 4.1 (spatial) (Corander *et al.* 2006) in Williams and Scribner (2010). The three clusters correspond with a western UP cluster (Porcupine Mountain, PM), central UP cluster (Huron Mountain, HM) and eastern UP cluster (Hiawatha, HIA) (Figure 1.3). High posterior probability of assignment for each cluster is represented in white, low posterior probability of assignment for each cluster is represented in red (Figure 1.3). Contour lines that are more closely spaced together are indicative of steep spatial gradients in genetic cluster membership suggesting reduced gene flow (Figure 1.3). Areas of most reduced gene flow were coincident with the spatial location of genetic cluster boundaries (Figure 1.3).

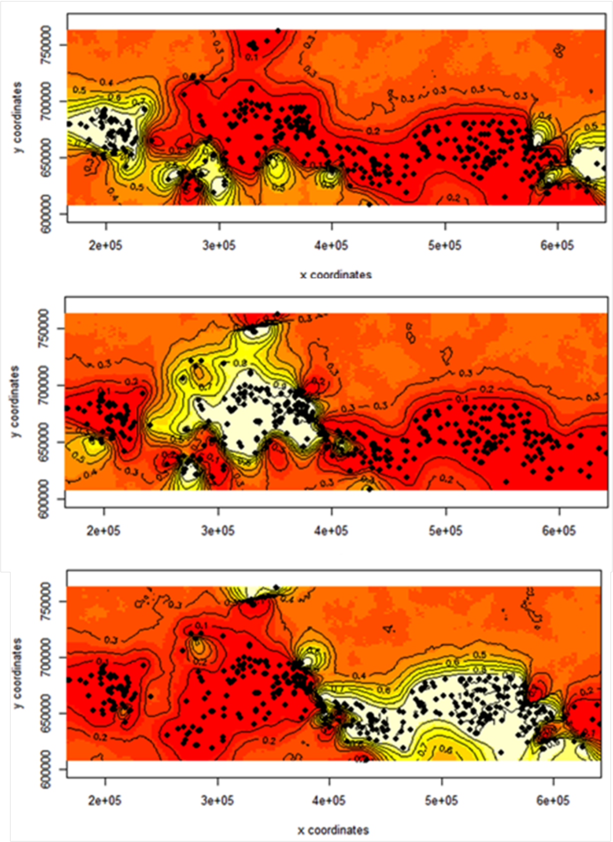


Figure 1.3. Contour map from spatial genetic analysis showing the probability of assignment to a genetic cluster for K=3 (scale units in meters) from program Geneland. Each circle represents an individual marten. Posterior probability maps were used to determine boundaries between clusters.

Causal Modeling Across the entire UP

The most supported landscape model varied depending on the scale of analysis. When considering individuals from across the entire Upper Peninsula, regardless of affiliation to a genetic cluster, we found two landscape features had support compared to most alternative hypotheses (positive values for the difference between Test 1 – Test 2, Appendix 1.2). Specifically, roads (Rd) and Euclidean distance (Euc) were supported compared to nearly all alternative models (Appendix 1.2). Only Rd had a positive partial Mantel r value relative to Euc indicating that Rd had support independent of Euc at this spatial scale of analysis, whereas Euc was not supported independently of Rd (Table 1.2).

Intra-cluster Analysis: Causal Modeling of Landscape features within regions associated with each genetic cluster.

When considering individuals from within the Hiawatha cluster, percentage canopy cover (Can) and proportion forested area (For) were supported compared to all alternative models (Appendix 1.2). There remained a positive correlation with genetic distance for each variable, relative to the other, indicating that neither Can nor For were supported independently (Table 1.2). This suggests that neither the proportion forest area nor percentage overhead cover is a single sufficient explanation of the genetic differences among individuals within the Hiawatha Cluster. When considering individuals from within the Porcupine Mountain and Huron Mountain cluster, percentage canopy cover (Can) and proportion forested area (For) were supported compared to all alternative models (Appendix 1.2). Only For had a positive partial Mantel r value relative to Can indicating that For had support independent of Can at this spatial scale of analysis, whereas Can was not supported independently of For (Table 1.2).

Inter-cluster Analysis: Causal Modeling of Landscape features between regions associated with each genetic cluster.

Inter-cluster analyses for individuals associated with the Hiawatha and Huron Mountain clusters showed that roads (Rd) and proportion forested area (For) were supported compared to nearly all alternative models (Appendix 1.2). Only For had a positive partial Mantel r value relative to Rd indicating that For had support independent of Rd at this spatial scale of analysis, whereas Rd was not supported independently of For (Table 1.2). Inter-cluster analyses for individuals associated with the Porcupine Mountain and Huron Mountain clusters showed that percentage canopy cover (Can) and proportion forested area (For) were supported compared to nearly all alternative models (Appendix 1.2). Only For had a positive partial Mantel r value relative to Can indicating that For had support independent of Can at this spatial scale of analysis, whereas Can was not supported independently of For (Table 1.2).

Table 1.2. Partial Mantel r values for the most supported hypotheses for each regional and UP wide analysis. The most supported hypotheses are those with positive values for the difference in partial Mantel r for Test 1 (genetic distance ~ focal model | alternative model) and Test 2 (genetic distance ~ alternative model | focal model). All=all American marten regardless of cluster affiliation across the reintroduction range, HIA=Only individuals within Hiawatha genetic cluster, HM=Only individuals within Huron Mountain genetic cluster, PM=Only individuals within Porcupine Mountain genetic cluster, HIA-HM = individuals on either side of the Hiawatha/Huron Mountain cluster boundary, HM-PM=Only individuals on either side of the Huron Mountain Porcupine Mountain cluster boundary. Can=Canopy Cover, Euc=Euclidean Distance, Rd = Roads, For= Percent Forested Area. Bolded values indicate outcomes consistent with expectations of reciprocal causal modeling (Cushman *et al.* 2013).

All	Euc	Rd
Test 1		
Euc	x	**0.009**
Rd	-0.005	x
Test 2		
Euc	x	**-0.005**
Rd	0.009	x

PM	Can	For
Test 1		
Can	x	**0.095**
For	-0.083	x
Test 2		
Can	x	**-0.083**
For	0.095	x

HIA	Can	For
Test 1		
Can	x	**0.005**
For	**0.029**	x
Test 2		
Can	x	0.029
For	0.005	x

HIA-HM	Rd	For
Test 1		
Rd	x	**0.034**
For	-0.019	x
Test 2		
Rd	x	**-0.019**
For	0.034	x

HM	Can	For
Test 1		
Can	x	**0.084**
For	-0.074	x
Test 2		
Can	x	**-0.074**
For	0.084	x

HM-PM	Can	For
Test 1		
Can	x	**0.055**
For	-0.047	x
Test 2		
Can	x	**-0.047**
For	0.055	x

DISCUSSION

Our results indicate the presence of genetic discontinuities within the UP and that the relationship between specific landscape features and inter-individual genetic distance is dependent on the spatial scale (regional versus entire UP) of the analysis. Across the entire UP, of the variables evaluated only the model including roads (Rd) was associated with spatial genetic structure relative to other variables. Analyses conducted within regions showed support for models associated with proportion of forested area (For) or both proportion of forested area and percentage of overhead cover (Can). Although associations between genetic differentiation and landscape features in each of our models were low based on partial Mantel r values, partial correlations are similar to findings of other studies of American marten (Wasserman *et al.* 2010, Broquet *et al.* 2006).

Large scale landscape genetics (Entire UP)

When analyses were conducted based on all individuals, regardless of affiliation to a genetic cluster, roads were the landscape feature most strongly associated with inter-individual genetic differentiation. Roads represent a potential source of mortality and landscape feature marten tend to avoid (Robitaille and Aubry 2000) and have been implicated in other systems as a human feature of landscapes that affects spatial genetic structure (e.g., Balkenhol and Waits 2009**,** Forman and Alexander 1998). When considering all individuals across the entire study area, there are a large number of inter-individual pairwise comparisons that cross roads within the Upper Peninsula (Figure 1.2A). Roads stretch over large spatial extents and are often implicated as having broad scale effects on spatial genetic structure (Balkenhol and Waits 2009). This result is consistent with some landscape genetic analyses for mustelids at large spatial scales in areas similar to the UP (fisher in Ontario – Garroway *et al.* 2011). Identifying the causal mechanism associated with roads is difficult, as roads may represent a number of things including direct mortality (Potvin and Breton 1997), increased trapping pressure because of access by trappers along roads within forested areas (marten - Hodgman *et al.* 1994), and habitat fragmentation and degradation (review in Balkenhol and Waits 2009). In much of the contiguous forested area in the Upper Peninsula, residential development is limited and so the impact of roads on marten movement is most likely attributable to trapping mortality associated with easier access into forest interiors (Robitaille and Aubry 2000). Even if marten are able to move across roads, roads may still decrease gene flow through impacts on the development of territories and home-range boundaries along roadways negatively influencing reproductive success (bobcat and coyotes along freeways - Riley *et al.* 2006). The result is in contrast to other mustelid landscape genetic analyses (marten in Northern Idaho - Wasserman *et al.* 2010), in which researchers found that elevation was the primary driver of genetic differentiation across the study area. However, this system is much different from the UP with respect to elevation (variation in elevation much greater in Northern Idaho than the UP) and so we would not expect elevation to be a major factor contributing to genetic differentiation in our system.

Within-region Analyses of Landscape Features and Spatial Genetic Structure

Intra-cluster Analyses within the Hiawatha Cluster

For individuals within the Hiawatha cluster in the eastern UP, percent overhead cover (Can) and proportion forested area (For) were the primary features associated with spatial genetic structure. Neither landscape feature had support independent of the other suggesting genetic differentiation within this region is jointly influenced by variation in percent overhead cover and forested area (Table 1.2). Within the eastern portion of the UP, there are large areas with low percent of overhead cover and proportion of forested area that would incur a high cost of moving through them (Figure 1.2C). Most notably is the variation in overhead cover within the eastern portion of the UP compared to other regions with more contiguous stretches of high percent canopy cover (Figure 1.2C). Canopy cover was also supported more strongly in this region relative to forest (Can partial Mantel r= 0.029, For partial Mantel r= 0.005), although as previously mentioned not independently of forest. Marten tend to avoid open areas with no overhead cover to provide protection from aerial predators (Drew, 1995). Canopy cover (Can) is often associated with prey resources (Thompson and Colgan, 1994) and with sites of marten subnivean entry (Corn and Raphael, 1992).

Intra-cluster Comparison within the Porcupine Mountain and Huron Mountain Cluster and Inter-cluster Comparisons

For individuals within the Porcupine Mountain and Huron Mountain genetic cluster, the most supported model of association between landscape features and inter-individual genetic distance was the proportion of forested area (For) (Table 1.2). Similarly, for inter-cluster comparisons of individuals from Hiawatha and Huron Mountain or Huron Mountain and Porcupine Mountain clusters, the most supported model of association between landscape features and inter-individual genetic distance was the proportion of forested area (For) (Table 1.2). Within the areas encompassed by each of these clusters (with the exception of within the Hiawatha cluster) and cluster boundaries, there is a high prevalence of forested areas and thus it may seem counter-intuitive that proportion of forested area was the most highly supported model (Figure 1.2B). However, in other landscape genetic analyses, it is the spatial distribution of landscape features compared to the prevalence that affects genetic differentiation (Emaresi *et al.* 2011). There is considerable variation from one grid cell to the next in terms of proportion forested area (Figure 1.2B) and this variation appears to be influencing the cost of movement and consequently genetic differentiation among individuals occupying these regions. Mature forested areas are preferred habitat for marten where they have access to high quality prey items including red squirrels (*Tamiscuirus hudsonicus*) and denning/resting sites (Coffin *et al*. 1997, Zielinski and Duncan 2004, Steele 1998, Wilson and Ruff 1999). In contrast to UP-wide analyses where roads are the landscape feature most closely associated with genetic differentiation, there are relatively fewer interindividual pairwise comparisons that cross roads in each of our regional analyses (Figure 1.2A). Consequently although roads are important at a broad scale, the heterogeneity in proportion forested area at regional scales is more closely associated with genetic distance (Table 1.2).

Regional analyses are consistent with some landscape genetic analyses of American marten (Broquet *et al.* 2006), but in contrast to other landscape genetic analyses of American marten (Wasserman *et al*. 2010, Koen *et al*. 2012). As previously mentioned, in the Wasserman *et al*. study, elevation was found to be the primary driver of genetic differentiation for marten in Northern Idaho. The Koen *et al.* (2012) study found that American marten genetic distance was most closely associated with the Euclidean distance compared to effective distance based on suitable habitat. However, the methods used in the Koen *et al.* (2012) study differed from ours as they based their analysis on genetic differentiation among sites compared to among individuals.

Although there is variation across the UP in the localized fisher harvest density (Figure 1.2D), this model was never supported independently of all alternative models (Table 1.2, Appendix 1.2). Fisher may predate on marten (Raine 1987) and are potential competitors for food resources, particularly small mammal prey (Krohn et al. 1997) and denning sites (Clem 1977). Studies of inter-specific competition in other systems have demonstrated that gene flow between genetic assemblages may be disrupted by competition between species at different life stages (Cangi *et al.* 2013). There are areas within and near cluster boundaries with relatively high fisher density (Figure 1.2D), however these are localized within considerably larger regions of low fisher density (Figure 1.2D). Consequently marten may not be restricted during dispersal by the spatial distribution of fisher. Within the area occupied by the Porcupine Mountain genetic cluster, there is a large area of high fisher harvest density (Figure 1.2D). Although marten and fisher have been shown to exhibit spatial segregation at small scales ($0.3 mi^2$ - Fisher *et al*. 2013) at the scale of the UP marten and fisher occur together (Figure 1.2D). However, as previously mentioned it is not always the prevalence but rather the spatial variation in landscape features most associated with genetic differentiation

(Emaresi *et al.* 2011). Additionally, fisher density in combination with other landscape features could be associated with genetic distance among marten. Thus, consideration of multivariate cost surfaces would be useful in determining how combinations of landscape features influence genetic differentiation.

The spatial scale of analysis is an important issue to consider in landscape genetic analyses (Anderson *et al* 2010). Combining data over large spatial scales and spatially heterogeneous landscape surfaces can preclude identification of landscape features associated with spatial genetic structure at microgeographic scales. Within the Upper Peninsula, previous research has identified areas where gene flow of descendents of reintroduced individuals has been interrupted and individuals associated with a region populated by descendents of one source population are more closely related than individuals from adjacent regions and genetic clusters (Williams and Scribner 2010). If analyses were not conducted at spatial scales consistent with the degree of spatial variance in landscape features analyses would have failed to identify important landscape features associated with marten spatial genetic structure.

Fisher were reintroduced (1961-1990) into the UP coincident with reintroductions of marten (1961-1992) (Williams *et al.* 2007). However, fisher exhibit isotropic dispersal from release sites and panmixia over the reintroduction area (Williams 2006). Genetic data contrast with marten that exhibit strong signatures of spatial genetic structure (Williams and Scribner 2010). Dispersal distance tends to be proportional to home range size in mustelids (Bowman *et al.* 2002). Reported home range areas for marten in Ontario vary from 1.0–12.7 km^2 for females and 3.3–11.2 km^2

for males (Thompson and Colgan 1987). Reported home ranges of fisher in Ontario found male home ranges were approximately 11 ± 4.4km^2 and females had home ranges of 2.4 ± 0.8km^2 (Koen *et al* 2007). Taken together, this suggests that although different marten source populations were used in reintroduction events into different areas of the UP, differences among source individuals is not sufficient to explain the spatial genetic structure exhibited by marten.

Other factors not measured in this study could also contribute to the development of genetic clusters. One possibility is positive assortative mating or mate avoidance by individuals from distinct genetic clusters. Positive assortative mating, whereby individuals of similar phenotype or quality mate more often than expected by chance (Burley 1983) has been documented in many taxa including mammals (e.g., Farrell *et al.* 2011). Positive assortative mating among individuals or descendents of individuals from the same source population could result in an accumulation of coancestry over time and establishment of spatial genetic structure.

Within the Upper Peninsula of MI, marten are highly genetically structured as seen in the existence of genetic clusters reflecting spatial discordance in regions occupied by descendents of sources used in reintroductions. The specific landscape features most strongly associated with genetic relationships among individuals varied depending on the spatial scale of the analysis. At a UP-level analysis, roads were most associated with genetic differentiation, while regional analyses revealed that either proportion forested area or a combination of forested area and canopy cover were most associated with genetic differentiation. Our study highlights how consideration of multiple spatial scales is important to correctly identify all the landscape features influencing genetic differentiation across a large and heterogenous study area.

APPENDICES

APPENDIX 1.1 Sensitivity analysis

Table A1.1 Sensitivity analysis. Results from sensitivity analysis to determine the influence of weighting scheme on the resulting cost distance from least cost path (LCP) analysis for each landscape feature.

Model	Weights	Mantel r	P value
Geographic Distance (Euc)	1	0.199	0.002
Roads (2 levels) (Rd)	1, 10	0.199	0.002
	1,100	0.197	0.002
	1, 1000	0.190	0.002
Road (3 levels) (Rd)	1, 5, 10	0.199	0.002
	1, 50, 100	0.198	0.002
	1, 500, 1000	0.195	0.002
Canopy Cover (Can)	1 to 10	0.190	0.002
	1 to 100	0.190	0.002
	1 to 1000	0.185	0.002
Proportion Forested Area (For)	1 to 10	0.184	0.002
	1 to 100	0.128	0.002
	1 to 1000	0.052	0.035
Fisher Density (MP)	1 to 10	0.198	0.002
	1 to 100	0.180	0.002
	1 to 1000	0.162	0.002

APPENDIX 1.2 Causal models

Table A1.2. Causal models. Causal modeling procedure evaluating the strength of support for each hypothesis. The Cluster column represents which individuals from genetic clusters are included in the partial Mantel tests. All=all American marten regardless of cluster affiliation across the reintroduction range, Hiawatha=Only individuals within Hiawatha genetic cluster, Huron Mountain=Only individuals within Huron Mountain genetic cluster, Porcupine Mountain=Only individuals within Porcupine Mountain genetic cluster, Hiawatha-Huron Mountain = individuals on either side of the Hiawatha/Huron Mountain cluster boundary, Huron Mountain-Porcupine Mountain=Only individuals on either side of the Huron Mountain Porcupine Mountain cluster boundary. The Diff column represents the difference between the r value for Test 1 (correlation between genetic distance and focal model, while accounting for alternative models) and Test 2 (correlation between genetic distance for the alternative models, while accounting for the focal model). Models are outlined with a black box if they have support relative to most alternative models (positive difference for the r value of Test 1 – Test 2). Only models with support relative to alternative models are selected for step 2 of causal modeling whereby the r values from Test 1 and Test 2 for each supported model are compared to one another.

Cluster	Number of Interindividual Comparisons	Focal Model	Alternative Model	partial Mantel r	p value	Diff
All	87746	Euc	Rd	-0.005	0.403	-0.013
		Euc	Can	0.086	0.002	0.148
		Euc	MP	0.021	0.175	0.005
		Euc	For	0.102	0.003	0.169
		Rd	Euc	0.009	0.322	0.013
		Rd	Can	0.091	0.002	0.159
		Rd	MP	0.022	0.185	0.006
		Rd	For	0.105	0.003	0.177
		Can	Euc	-0.062	0.009	-0.148
		Can	Rd	-0.068	0.001	-0.159
		Can	MP	-0.011	0.334	-0.071
		Can	For	0.066	0.021	0.112
		MP	Euc	0.017	0.240	-0.005
		MP	Rd	0.016	0.261	-0.006
		MP	Can	0.060	0.009	0.071
		MP	For	0.080	0.067	0.106
		For	Euc	-0.067	0.010	-0.169
		For	Rd	-0.072	0.002	-0.177
		For	Can	-0.046	0.002	-0.112
		For	MP	-0.026	0.434	-0.106

Table A1.2 (cont'd)

Cluster	Number of Interindividual Comparisons	Focal Model	Alternative Model	partial Mantel r	p value	Diff
Hiawatha	21945	Euc	Rd	-0.079	0.004	-0.161
		Euc	Can	-0.066	0.061	-0.166
		Euc	MP	0.110	0.002	0.178
		Euc	For	-0.023	0.093	-0.098
		Rd	Euc	0.083	0.003	0.161
		Rd	Can	-0.059	0.085	-0.152
		Rd	MP	0.118	0.003	0.193
		Rd	For	-0.017	0.159	-0.085
		Can	Euc	0.100	0.014	0.166
		Can	Rd	0.093	0.013	0.152
		Can	MP	0.141	0.002	0.222
		Can	For	0.029	0.014	-0.067
		MP	Euc	-0.068	0.009	-0.178
		MP	Rd	-0.075	0.009	-0.193
		MP	Can	-0.081	0.014	-0.222
		MP	For	-0.063	0.009	-0.191
		For	Euc	0.074	0.050	0.098
		For	Rd	0.068	0.086	0.085
		For	Can	0.005	0.052	0.067
		For	MP	0.128	0.002	0.191

Table A1.2 (cont'd)

Cluster	Number of Interindividual Comparisons	Focal Model	Alternative Model	partial Mantel r	p value	Diff
Huron Mountain	11628	Euc	Rd	-0.050	0.081	-0.102
		Euc	Can	-0.113	0.022	-0.243
		Euc	MP	0.071	0.044	0.124
		Euc	For	-0.132	0.014	-0.283
		Rd	Euc	0.052	0.072	0.102
		Rd	Can	-0.115	0.023	-0.247
		Rd	MP	0.077	0.032	0.135
		Rd	For	-0.134	0.010	-0.287
		Can	Euc	0.130	0.011	0.243
		Can	Rd	0.131	0.016	0.247
		Can	MP	0.125	0.009	0.223
		Can	For	-0.074	0.016	-0.157
		MP	Euc	-0.053	0.076	-0.124
		MP	Rd	-0.059	0.059	-0.135
		MP	Can	-0.097	0.027	-0.223
		MP	For	-0.114	0.023	-0.258
		For	Euc	0.151	0.012	0.283
		For	Rd	0.153	0.018	0.287
		For	Can	0.084	0.035	0.157
		For	MP	0.144	0.009	0.258

Table A1.2 (cont'd)

Cluster	Number of Interindividual Comparisons	Focal Model	Alternative Model	partial Mantel r	p value	Diff
Porcupine Mountain	5671	Euc	Rd	-0.041	0.164	-0.084
		Euc	Can	-0.121	0.020	-0.262
		Euc	MP	0.087	0.040	0.149
		Euc	For	-0.149	0.016	-0.320
		Rd	Euc	0.043	0.163	0.084
		Rd	Can	-0.126	0.015	-0.270
		Rd	MP	0.090	0.023	0.155
		Rd	For	-0.153	0.013	-0.328
		Can	Euc	0.141	0.012	0.262
		Can	Rd	0.145	0.016	0.270
		Can	MP	0.138	0.011	0.238
		Can	For	-0.083	0.024	-0.178
		MP	Euc	-0.062	0.059	-0.149
		MP	Rd	-0.065	0.058	-0.155
		MP	Can	-0.101	0.017	-0.238
		MP	For	-0.125	0.028	-0.287
		For	Euc	0.171	0.013	0.320
		For	Rd	0.175	0.013	0.328
		For	Can	0.095	0.035	0.178
		For	MP	0.162	0.009	0.287

Table A1.2 (cont'd)

Cluster	Number of Interindividual Comparisons	Focal Model	Alternative Model	partial Mantel r	p value	Diff
Huron Mountain-Porcupine Mountain	16371	Euc	Rd	-0.003	0.419	-0.009
		Euc	Can	-0.047	0.151	-0.108
		Euc	MP	0.018	0.326	0.020
		Euc	For	-0.060	0.078	-0.137
		Rd	Euc	0.005	0.433	0.009
		Rd	Can	-0.052	0.137	-0.117
		Rd	MP	0.019	0.332	0.021
		Rd	For	-0.065	0.056	-0.146
		Can	Euc	0.061	0.110	0.108
		Can	Rd	0.065	0.080	0.117
		Can	MP	0.048	0.148	0.069
		Can	For	-0.047	0.164	-0.103
		MP	Euc	-0.002	0.486	-0.020
		MP	Rd	-0.002	0.491	-0.021
		MP	Can	-0.021	0.314	-0.069
		MP	For	-0.031	0.338	-0.092
		For	Euc	0.077	0.077	0.137
		For	Rd	0.081	0.039	0.146
		For	Can	0.055	0.236	0.103
		For	MP	0.061	0.192	0.092

Table A1.2 (cont'd)

Cluster	Number of Interindividual Comparisons	Focal Model	Alternative Model	partial Mantel r	p value	Diff
Hiawatha - Huron Mountain	32130	Euc	Rd	-0.049	0.048	-0.099
		Euc	Can	-0.008	0.386	-0.028
		Euc	MP	0.040	0.101	0.064
		Euc	For	-0.024	0.118	-0.063
		Rd	Euc	0.050	0.036	0.099
		Rd	Can	0.046	0.491	0.034
		Rd	MP	-0.001	0.082	0.030
		Rd	For	-0.019	0.188	-0.052
		Can	Euc	0.020	0.305	0.028
		Can	Rd	0.012	0.370	-0.034
		Can	MP	0.042	0.103	0.064
		Can	For	0.032	0.414	-0.008
		MP	Euc	-0.024	0.194	-0.064
		MP	Rd	-0.031	0.135	-0.030
		MP	Can	-0.021	0.277	-0.064
		MP	For	-0.030	0.145	-0.076
		For	Euc	0.039	0.093	0.063
		For	Rd	0.034	0.133	0.052
		For	Can	0.041	0.304	0.008
		For	MP	0.054	0.057	0.076

CHAPTER II: DOES PHENOTYPIC VARIATION REFLECT SIGNATURES OF SPATIAL GENETIC STRUCTURE AND ENVIRONMENTAL HETEROGENEITY?

Landscape features can act as agents of selection, favoring locally adapted phenotypes and limiting the likelihood of successful dispersal. Here I used geometric morphometrics to quantify variation in American marten (*Martes americana*) skull morphology of descendent individuals and their putative source populations. Marten were reintroduced into the Upper Peninsula (UP) of Michigan from genetically distinct source populations. Since colonization, three distinct genetic clusters have developed proximal to release sites. I investigated whether differences in skull shape among descendent individuals were consistent with cluster membership and thus, whether differences in skull shape could be attributed to shared ancestry. Alternatively local ecological conditions could be associated with variation in skull shape. Landscape mediated dispersal limitation could also localize genetic changes of descendents. Skull shape for descendent individuals differed from source individuals, but not in relation to lineage. Within the reintroduction area, descendent skull shape was related to genetic cluster. Patterns of skull shape variation among descendents were distinct from spatial genetic structure based on neutral molecular markers, suggesting different causes underlying spatial morphological and genetic patterns. Morphological differences were also not consistent with hypotheses of dispersal limitation, as evidenced by a lack of association between measures of inter-individual variation in skull morphology and by Euclidean distance or landscape resistance. Taken together evidence suggests that habitat features that vary among spatially distinct genetic clusters, rather than shared ancestry, are influencing regional differences in marten skull shape.

INTRODUCTION

Environmental conditions can act as agents of selection, favoring locally adapted phenotypes and limiting the likelihood of successful dispersal among populations (Clobert *et al.* 2009, Manel *et al* 2010). For example, immigrant phenotypes may be more prone to predation (Nosil *et al.* 2005), less likely to successfully mate with resident individuals (Bensch *et al.* 1998) or may be generally maladapted to new environments (Nosil *et al.* 2005). Fitness of different phenotypes will likely differ across a species' range that encompasses a wide range of environmental conditions (Via *et al.* 1995). Over longer periods of time, phenotypic differences may accrue over microgeographic or macrogeographic scales resulting in adaptive radiation and speciation (Dobeli and Dieckmann 2003).

The features associated with genetic and phenotypic divergence among populations have been explored in numerous taxa and traits (Spurgin *et al.* 2014 – birds, Wang and Summers, 2010 – amphibians, Reale *et al.* 2003 - mammals). Skull morphology has been shown to undergo rapid adaptive divergence (Marroig and Cheverud, 2004). Different foraging strategies may be required when individuals expand into novel environments associated with different food resources (birds– Grant 1981, Badyaev 2009, 2010; mammals – Melero *et al.* 2008, Wolf *et al*. 2008). For example, in Galapagos sea lions (*Zalophus wollebaeki*) stable isotope analysis revealed that colonies inhabiting different islands of the Galapagos archipelago used different food resources, and differences in resource use correlated with divergence in skull morphologies associated with feeding behavior (Wolf *et al.* 2008). Although phenotypic differences are most often associated with local adaptation (isolation by adaptation, Wang and Summers 2011, Orr 1998), similar to studies of population genetic differentiation at neutral loci (e.g., Orsini *et al.* 2013, Lee and Mitchell-Olds 2011), phenotypic divergence may be investigated as a function of isolation by dispersal limitation including physical distance among individuals of shared or different ancestry (isolation by distance, Wright 1943) and relative landscape permeability, that effects how freely organisms move through the landscape (isolation by landscape resistance, McRae 2006). The relative importance of selection, isolation by distance, and isolation by landscape resistance to phenotypic variation are not well understood. Therefore, studies that simultaneously consider each of these features and their potential interactions are required in order to understand phenotypic divergence (Wang and Summers 2011).

Yet another factor influencing phenotypic variation among populations is colonization or introduction history (e.g., Spurgin *et al* 2014). Genetic and phenotypic differences among individuals may reflect differences among putative source populations or a combination of factors including selection mediated by novel local environmental conditions at release sites and limited dispersal and drift due to small founding numbers (Merilä and Crnokrak, 2001, Kolbe *et al.* 2007, Spurgin *et al.* 2014). Genetic and phenotypic variation among source populations may or may not be observed as differences at the genotypic (Clegg *et al.* 2002a, Estoup and Clegg 2003) and/or phenotypic level among descendent individuals (Clegg *et al.* 2002b, 2008). Introductions and reintroductions provide a useful context to study the relative contributions of environmental variables and genetic/phenotypic variation among source populations to emergence of genetic and phenotypic variability among descendents released into novel environments. Colonization history has been implicated as a factor contributing to divergence among descendents of introduced mustelids (Melero *et al.* 2008). For example, invasive Mediterranean populations of American mink (*Mustela vison*) exhibited morphological variation that was related to morphological features of founder individuals (i.e., animals that have escaped from fur farms), and adaptation to local environmental conditions (Melero *et al.* 2008).

In the Mammalian Order Carnivora, variation in skull morphology has been attributed primarily to selection on features associated with acquisition and processing of food (Dayan and Simberloff 1994, Loy *et al.* 2004). Most members of Carnivora are predatory species (Dayan and Simberloff 2005), however there is large variation in skull morphology associated with dietary breadth (Raia 2004). Within the genus *Martes* (Family Mustelidae), individuals exhibit marked differences in skull morphology associated with feeding behavior. Cranial features of stone marten (*Martes foina*) are adapted to a strictly carnivorous diet. Relative to the pine marten (*Martes martes)*, stone marten have a more narrow post-orbital constriction allowing for a larger anterior portion of the temporalis muscle associated with generating bite force in addition to reduced check teeth dentition. Pine marten skull shape reflects a reliance on a more omnivorous diet (e.g., invertebrates, plant material and fruits; Loy *et al.* 2004).

Many species in the Family Mustelidae, including American marten, are opportunistic feeders. The abundance and distribution of available mustelid food resources is influenced by environmental conditions; specifically habitat features

(Macarthur and Macarthur 1961; Murdock 2000, Zielinski and Duncan 2004). Consequently, selection for morphological traits related to food acquisition and mastication may vary with spatial variation in the distribution of specific landscape features. Differences in landscape features associated with the distribution of food resources may select for different skull shapes (coyotes – Sears *et al* 2003). Most notably perhaps are differences in skull shape related to the size of prey items. In Carnivora, species that consume larger prey items have an increased bite force relative to their size (Christiansen and Wroe 2007). Bite force is related to gape angle (Dumont and Herrel 2003) and surface area for temporal muscle attachment (LaCroix *et al*. 2011) and so, individuals incorporating different sized prey items may differ in skull shape. For example, in areas with a mix of lowland forest and agricultural cover types, marten may feed more heavily on small prey such as shrews (e.g., pygmy shrew, *Sorex hoyi*; Baker 1983) as well as berries and plant matter (Caryl *et al.* 2012). In upland coniferous forest areas where diets may include a higher proportion of sciurids such as red squirrel (*Tamiasciurus hudsonicus*), and snowshoe hare (*Lepus americanus*) whose distribution varies predominantly with the presence of mature stands of coniferous trees (Steele 1998, Wilson and Ruff 1999). Within the Great Lakes region, American marten inhabit landscapes varying greatly in physical and biotic features. The Upper Peninsula (UP) of Michigan (MI) consists primarily of a mix of deciduous/coniferous forest in the central and western regions and broader diversity of cover types (e.g., forest as well as wetlands, agricultural area). Consequently, marten skull shape may differ depending on regional differences in land-cover associated with food resources across the UP.

Co-distribution of competitor species may affect habitats occupied and concomitantly prey consumed (e.g., sunfish – Werner and Hall 1977). When co-distributed, marten and fisher (*Martes pennant*) overlap in diet. However, due to larger body size, fisher use a wider range of prey, primarily larger mammals such as hares (*Lepus americanus*) (Martin 1994). In the UP, marten and fisher are co-distributed and are often harvested within the same section ($1mi^2$) (Williams *et al.* 2007). During the winter, diet analysis has shown that when co-distributed, marten tend to utilize smaller, arboreal prey (e.g., *T. hudsonicus*) or smaller, subnivean (space between snow and ground) prey (e.g., voles such as genus *Microtus,* and other microtines) compared to fisher (Raine 1987). Consequently, the primary sources of food for marten may vary regionally with variation in *M. pennanti* density. Regional variation in the relative abundance of competitor species in areas co-occupied by both species may lead to regional variation in skull morphology.

Within a species or population, differences in skull morphology may be attributed to differences in habitat occupied by males and females (La Croix *et al.* 2011) and differences in food resources used (Shine *et al*. 2003). For example, male deer use younger more open forest stands as the growing season progresses, compared to females within the same population (Lesage *et al.* 2002). Within the genus *Martes,* sexual dimorphism in skull shape (e.g., differentiation of the masticatory apparatus) (Wiig 1986; Loy *et al.* 2004) is related to food acquisition. Based on gut content analyses, male skulls are specialized for capturing and killing prey whereas female skull features reflect opportunistic feeding habits (Loy *et al.* 2004). Consequently, variation in skull shape among marten may reflect sexual dimorphism related to differential feeding preferences between males and females.

In addition to sexual dimorphism, mammals may exhibit dimorphism in skull shape related to age. Juveniles are significantly handicapped relative to adults when it comes to feeding performance and efficiency (Tanner 2010). Adult skulls differ in shape from juvenile skulls and these shape differences are attributed to dietary differences at each life stage (e.g., LaCroix *et al.* 2011, Segura *et al.* 2013). Previous studies of intraspecific skull ontogeny in mammals have found evidence that adults differ in postnatal development likely related to adaptation to specific features (e.g., food availability) of their habitats (Galatius and Gol'din, 2011, LaCroix Dissertaion 2011).

Within the Upper Peninsula of Michigan, American marten have been extirpated and several reintroductions have reestablished marten in this region. Marten were extirpated from the UP by the late 1930s (Manville 1948), coincident with an increase in habitat degradation from logging and fires, and unregulated trapping during the 19th and 20th century (Berg 1982). Once a sufficient amount of continuous forested habitat in the UP was re-established, reintroduction efforts to establish a viable population in Michigan began began with the release of 29 marten into the Porcupine Mountains Wilderness State Park (27 individuals from Crown Chapleau Game Preserve (CCGP), Ontario and 2 individuals originally from British Columbia but raised on a fur farm in Delta County, Michigan; Michigan Department of Conservation 1957; Harger and Switzenberg 1958). Ninety-nine marten from the former Port Arthur Ontario Ministry of Natural Resources District (now Thunder Bay District, TB), Ontario, were released into

Delta County in 1969 and 1970 (Michigan Department of Natural Resources 1970). One hundred forty eight individuals from Algonquin Provinical Park (APP) were released at four sites in the west-central UP (Churchill *et al*. 1981). From 1989 to 1992, three secondary translocations were made in eastern and northern portions of the UP. In 1989 and 1990, 20 marten from Iron County and 27 marten from the Hiawatha National Forest West Unit were released into the Tahquamenon Bay Area of the Hiawatha National Forest East Unit. In 1992, 19 marten from southern Houghton County were released in southeastern Keweenaw County (William *et al.* 2007). The majority of source individuals originated from three locations in Ontario, although there were several reintroduction events using individuals from Colorado and Minnesota. Fortuitously, the location of reintroduction events differed among sources (see Williams *et al.* 2007 for a full account of reintroduction and translocation history). The detailed information available for Michigan regarding stocking history, genetic variation among source populations and descendents of source populations within the reintroduction area (Williams et al. 2007) make the UP-mustelid complex a particularly useful system for investigating factors associated with incipient divergence in morphological features for descendent individuals.

Analyses based on neutral genetic markers indicated no significant genetic bottlenecks for martens from any source or within the reintroduction area in the UP (Williams 2006), however marten exhibit strong signatures of spatial genetic structure and genetic divergence, as evidenced by three distinct genetic clusters within the UP (Williams and Scribner 2010). Genetic ‘clusters’ are located in regions proximal to reintroduction release sites, suggesting founders from distinct source populations and independent releases had colonized different regions with minimal overlap (Figure 2.1, Williams and Scribner 2010). The variation in cluster area, quantified by the spatial genetic autocorrelation for individuals from each genetic cluster (Williams and Scribner 2010), and discontinuities among clusters reflect the relative permeability of specific landscape features to marten dispersal and gene flow (Chapter 1). At the scale of the entire Upper Peninsula, spatial genetic structure is most closely associated with dispersal limitation due to roads (Chapter 1). If patterns of phenotypic variability are discordant with spatial genetic structure resolved using molecular markers, data would indicate selection acting on skull shape through habitat mediated effects on resource composition. Alternatively, variation in skull shape may be related to shared ancestry which could be geographically structured due to dispersal limited by the geographic distance (isolation by distance; Wright 1943) between individuals or dispersion of landscape features (isolation by landscape resistance; McRae 2006). Studies that consider the impact of selection and limited dispersal can improve our understanding of the factors shaping spatial genetic and phenotypic variation.

Our objective was to quantify sources of variation in American marten skull morphology. Using individuals from the reintroduction area and three putative source populations in Ontario, we examined whether lineage (putative source population of descendents) influenced variation in skull shape. We tested the hypothesis that for descendents of different source populations, differentiation among descendent marten was associated with different ‘genetic clusters’ (Williams and Scribner 2010) in the reintroduced range at the genetic and phenotypic levels. Spatial genetic structure for descendent UP marten may be related to landscape ‘permeability’, and so we tested the hypothesis that phenotypic differences are also related to dispersal limited by degree of landscape-mediated ‘connectivity’ or permeability (isolation by landscape resistance) or the Euclidean distance among individuals (isolation by distance). We quantified the influence of sex, age and skull size as ‘nuisance’ parameters on skull shapes among descendent individuals. We also tested the hypothesis that skull shape was related to differences in land-cover, which we hypothesized influenced prey and the relative distribution of food resources. We also tested the hypothesis that skull shape was related to differences in localized fisher harvest density (a surrogate of fisher relative abundance), which we hypothesized would affect the availability of prey through direct or indirect competition. Our results contribute to understanding of the relative importance of dispersal potential, environmental features, and shared ancestry to phenotypic divergence among individuals.

METHODS

Samples and Study Area – The Upper Peninsula (UP) of Michigan (MI) consists primarily of a mix of deciduous/coniferous forest in the central and western regions and broader diversity of cover types (e.g., forest as well as wetlands, agricultural area). Marten currently occur across much of the UP's ~44,000km^2. Following reintroductions (1955-1992), population abundance rapidly increased allowing for sustainable harvest. A fur-trapping season was opened in 2000. From 2000-2004, the harvest season occurred between December 1st and 15th with a bag limit of 2 for Native Americans and 1 for all other fur-takers across the Upper Peninsula (Frawley, 2001 MI DNR). For a previous study on reintroduction success, muscle tissue samples were collected from marten harvested in the UP of Michigan during the 2000-2004 trapping season (n=495) (Williams and Scribner 2010). Marten were genotyped at eleven highly variable microsatellite loci (see Williams and Scribner, 2010 for complete description of sample acquisition and molecular techniques). The location of each harvested marten was reported to the level of the section (1 section = 1mi^2).

Skulls of museum specimens from genotyped descendents of individuals reintroduced into the Upper Peninsula of MI (Table 2.1, Figure 2.1) were digitally photographed in lateral Figure 2.2A) and ventral views (Figure 2.2B). Skulls from individuals harvested during the winters of 2000-2004 were collected by Michigan Department of Natural Resources personnel. Specimens were prepared by, and catalogued into the University of Michigan Museum collection. Specimens were sexed and aged (adult or juvenile; greater or less than 1.5 years old, respectively) based on cementum annuli and fully erupted adult dentition. We also photographed skulls from individuals from each of three putative source populations in Ontario(Table 2.2, Figure 2.1). Skulls from individuals harvested during the years 1944-1978 were collected by the Ontario Ministry of Natural Resources (OMNR) or its predecessor, the Department of Lands and Forests (DLF). Locations of individuals were recorded to the level of township, preserve, or provincial park by OMNR or DLF personnel based on a topographic map of Ontario. Specimens were prepared by, and catalogued into, the Royal Ontario Museum collection.

Table 2.1. Number of American marten specimens from the Upper Peninsula of Michigan for each skull view, genetic cluster within the reintroduced area (HM=Huron Mountain, PM=Porcupine Mountain, HIA=Hiawatha, see Figure 2.1), sex and age class.

View	Descendents' Cluster Affiliation	Males (Adult and Juvenile)	Females (Adult and Juvenile)	Total (Across Sex and Age)
Ventral Cranium	HM	16 and 7	9 and 2	34
	PM	12 and 12	3 and 5	32
	HIA	20 and 17	12 and 9	58
Lateral Cranium	HM	18 and 11	8 and 2	39
	PM	13 and 12	2 and 3	30
	HIA	24 and 19	12 and 8	63

Table 2.2. Number of American marten specimens from Ontario for each skull view, from each of three putative source populations (TB=Thunder Bay/Port Arthur District, APP=Algonquin Provincial Park, CCGP=Crown Chapleau Game Preserve see Figure 2.1), sex and age class.

View	Source Sampling Location	Males (Juvenile and Adult)	Females (Juvenile and Adult)	Unkown (Juvenile and Adult)	Total (Across Sex and Age)
Ventral Cranium	TB	0 and 2	1 and 2	0 and 3	8
	APP	1 and 0	0	0	1
	CCGP	4 and 9	9 and 1	0	23
Lateral Cranium	TB	0 and 2	1 and 2	0 and 3	8
	APP	1 and 0	0	0	1
	CCGP	4 and 8	8 and 2	0	22

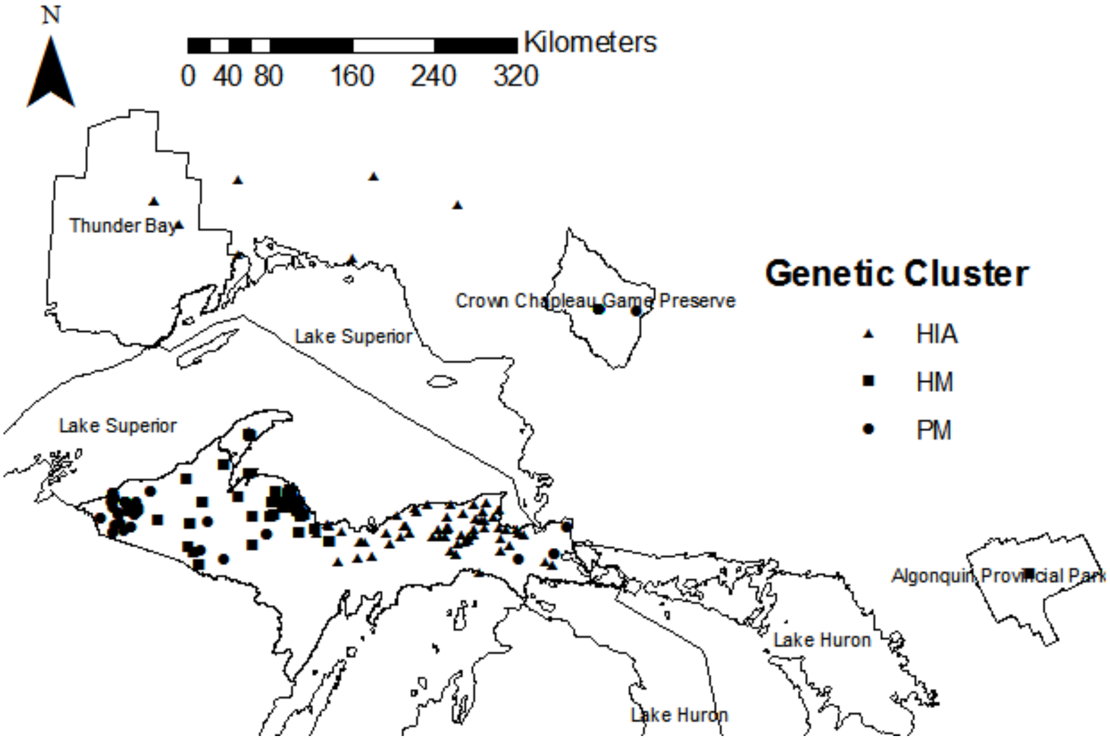
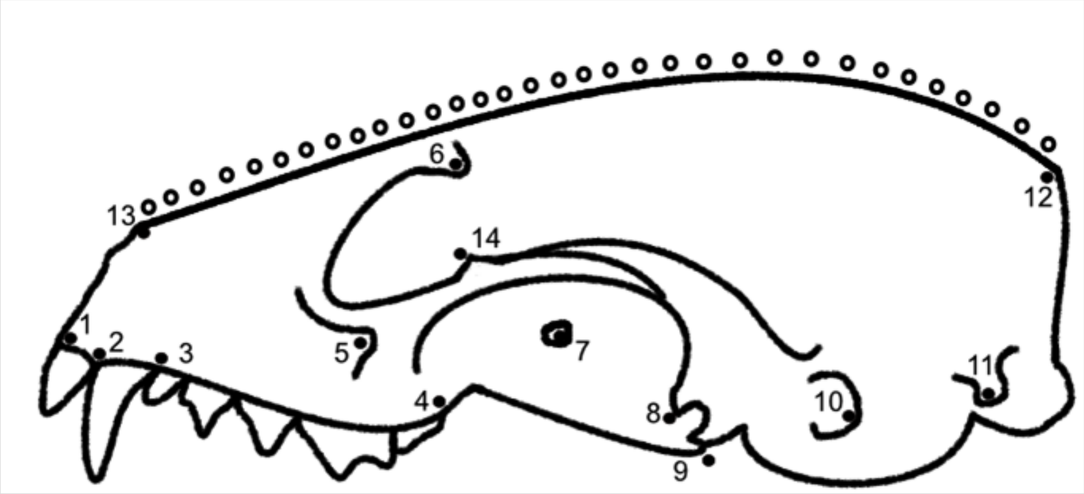
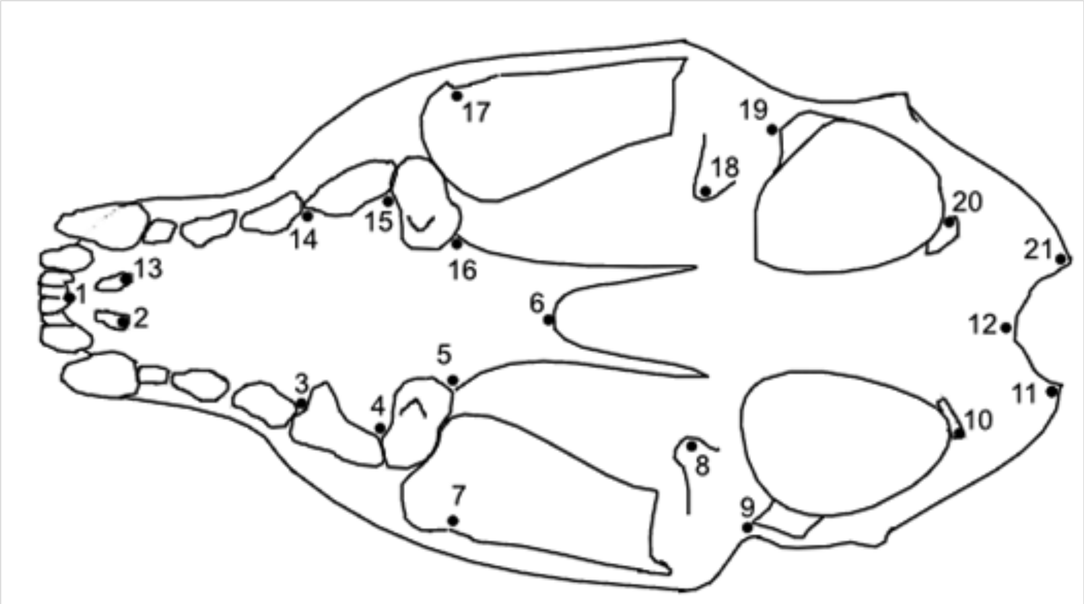


Figure 2.1. Location of skulls collected from harvested American marten individuals in the Upper Peninsula of MI (descendent individuals; Huron Mountain (HM), Porcupine Mountain (PM), and Hiawatha (HIA)) and Ontario (source individuals; Thunder Bay, Crown Chapleau Game Preserve, and Algonquin Provincial Park). Symbols indicate assignment to one of three genetic clusters (Williams and Scribner 2010) within the reintroduction area based on multilocus microsatelitte genotypes and lineage (putative source to descendent individuals).

Data Collection- We used a landmark-based geometric morphometrics approach to quantify variation in skull shape and size (Rohlf 1990, Zelditch *et al.* 2004). To obtain photographs of the lateral view, the skull was oriented so that the sagittal plane was parallel to the photographic plane. Fourteen landmarks and 32 semilandmarks were selected for the lateral view (Figure 2.2A). For the ventral view, skulls were oriented so that the occlusal plane was parallel to the photographic plane. Twenty-one landmarks were selected for the ventral view (Figure 2.2B). A digital camera was used to take images for each view by mounting the camera on a tripod at a fixed distance of 30 cm from each skull. Each skull was placed on a soft bag filled with sand to maintain stability. Photographs were acquired using a Nikon digital camera, with a macro lens and scale was determined with a ruler in millimeters. Selection of landmarks for each view followed the standards proposed by Zelditch *et al.* 2004.



A



B

Figure 2.2. Landmarks (numbered) and semilandmarks (o) shown on the A) lateral cranium and B) ventral cranium of an adult American marten, *Martes americana.* Descriptions of landmarks and semilandmarks are provided in Supporting Information Appendix Table 3.1.

Landmarks and Procrustes Superimposition

Landmarks and semilandmarks were superimposed by Procrustes generalized least squares (GPA) using the gpagen function in R package geomorph to obtain measurements of skull shape with size, position, and orientation effects removed (Adams and Otarola-Castillo, 2013). For semilandmarks, we utilized an approach that involves sliding semi-landmarks to minimize bending energy of the deformation. This procedure adjusts the spacing of semi-landmarks to minimize the potential for shape changes to be attributed solely to the relative positioning of the semi-landmarks (Bookstein 1997, Green 1996). We projected aligned Procrustes residuals into tangent space (equivalent to Euclidean space) for use in subsequent analyses (Dryden 1998). To avoid inflating degrees of freedom, bilaterally homologous landmarks were reflected from one side of the ventral cranium view to the other and averaged following Procrustes superimposition. Reflection and averaging was carried out in the bilat.symmetry in the R package geomorph (Adams and Otarola-Castillo 2013) and only the symmetric component of morphological variation was used in statistical analyses to avoid redundancy and to control for asymmetric diff erences in the skull (Klingenberg *et al.* 2002). We used centroid size, defined as the square root of the sum of square differences between each landmark and the centroid of the landmark configuration, as our measure of skull size for each view (Bookstein 1986, Dryden 1998).

Statistical Analyses

Prior to statistical analyses, we quantified measurement error using a Procrustes Analysis of Variance (ANOVA) with the Procrustes distances among specimens (calculated based on the digitized and superimposed landmarks) as our dependent variable and individual (levels = 48) and replicates (levels = 2) as our independent factors. We then compared the amount of variation between individuals versus between replicates within individuals. ANOVA was performed using digitized landmarks from replicate (n=2) photographs of the lateral view of 48 individuals. Individuals were oriented, a single photo was taken and then each individual skull was moved and photographed a second time. In this way we were able to quantify the amount of measurement error (3.5 percent). Procrustes ANOVA was carried out using the procD.lm function in the geomorph package (Adams and Otarola-Castillo 2013). Procrustes ANOVA is appropriate for landmark-based geometric morphometric data because covariance matrices for GPA-aligned Procrustes coordinates are singular, making standard multivariate analyses inappropriate. Instead of using the explained covariance matrices among variables, Procrustes ANOVA uses the Procrustes distance among individuals to conduct a statistical assessment of model covariates. Rows of the shape matrix (Procrustes aligned coordinates projected into tangent space) are randomized relative to the design matrix (constructed from model covariates) and the observed sum of squares are evaluated using permutations.

Comparing Source and Descendent Individuals

To characterize differences among source and descendent individuals, we performed a principal component analysis (PCA) of Procrustes aligned shape coordinates. We plotted the scores from the eigen-analysis for those PCs that explained greater than 10% of the variance in shape.

Variation in Shape among Descendent Individuals

We used a Procrustes ANOVA to quantify variation in skull shape (Procrustes-aligned coordinates) of descendent individuals associated with centroid size, sex (2 levels; male or female), age (2 levels; juvenile or adult), and genetic cluster (cluster (3 levels; Hiawatha (HIA), Huron Mountain (HM), and Porcupine Mountain (PM), Williams and Scribner 2010), localized fisher harvest density and dominant cover type using the procD.lm function in geomorph (Adams and Otarola-Castillo 2013). Interaction terms were not significant and so we present results for main effects only. Centroid size was included in our model, to test for the influence of allometry. The genetic cluster covariate includes information on shared ancestry of descendent individuals and the contemporary geographic location (and associated landscape features) of genetic clusters within the reintroduction area (Williams and Scribner 2010). We wanted to determine whether the skull shape of individuals affiliated with one specific genetic cluster was distinct from individuals affiliated with a different genetic cluster. Differences in shape among genetic clusters may reflect source population traits as a contributing factor to contemporary skull shape among descendents. However, variation in skull shape could also arise because the UP varies spatially with respect to landscape features and localized congener (fisher) harvest density. Accordingly, differences in these physical and biotic landscape variables associated with UP regions where individuals from distinct source populations were reintroduced could also contribute to any variation in skull shape that is found to be associated with genetic cluster.

Landscape variables

The first analysis of descendent individuals allowed us to partition variation in marten skull shape associated with features of the location of harvested individuals (nodes). Land cover varies spatially across the UP (Figure 2.3A), and differences in the dominant cover type could affect the distribution of prey resources and contribute to differences in skull shape among regions of the UP. The abundance and distribution of available mustelid food resources is influenced by environmental conditions; specifically habitat features (MacArthur and MaAarthur 1961; Murdock 2000, Zielinski and Duncan 2004). Consequently, selection for morphological traits related to food acquisition and mastication may vary with spatial variation in the distribution of specific landscape features (e.g., coyotes – Sears *et al.* 2003). To quantify dominant cover type, we used land cover classes from the 2001 IFMAP GAP LandCover dataset derived from Landsat satellite imagery (30m grid cells) (Figure 2.3A). Variation in the distribution of a potential competitor may also influence the availability of food. When co-distributed, fisher may utilize large mammalian prey such as snowshoe hare (*Lepus americanus*) and marten may incorporate a higher percentage of smaller prey items (Martin 1994). In the UP, marten and fisher are co-distributed and are often harvested within the same section (1mi^2) (Williams *et al.* 2007). To obtain a surrogate measure of localized fisher harvest density, we used fisher harvest locations from 2000-2005 to create kernel density function (Silverman 1986) grids in ArcGIS 9.3 and reclassified 30m grid cells into categories ranging from 1-10 (low to high localized fisher harvest density). Local harvest density estimates were adjusted for unequal harvest efforts (bag limit of 3 for

Management Unit 1 and a bag limit of 1 for Management Unit 2) by weighting the density estimates for each geographic area by the percentage of harvest that occurred in each Management Unit (Bales 1993). We then created a median density grid by calculating the median values over the 5 yearly density grids. (Figure 2.3B). We created a 1.61km diameter circular buffer (approximately the area of a section) around each harvest location and extracted the harvest abundance of fisher and majority cover type within each buffer. We included the mean abundance of fisher harvest and majority cover type as covariates to assess whether variation in skull shape not attributed to size, sex and age, or genetic cluster (an indicator of founding source population; Williams and Scribner 2010) was related to features that vary spatially across the UP, that we hypothesized would select for differences in skull shape.

A)

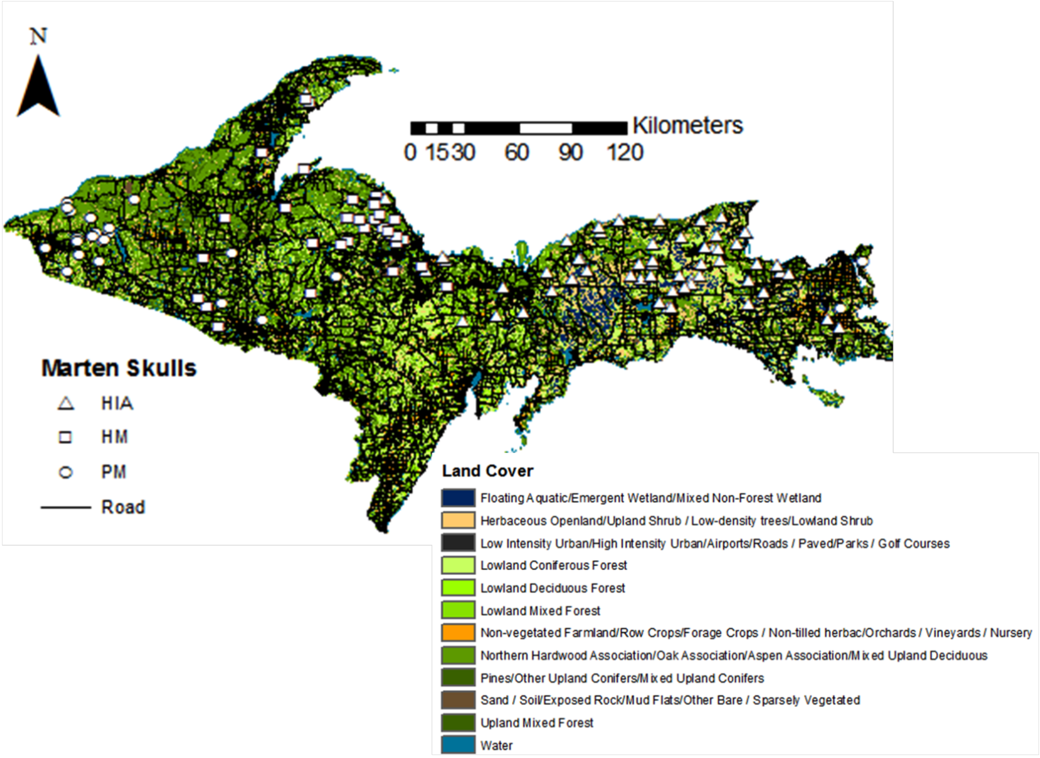
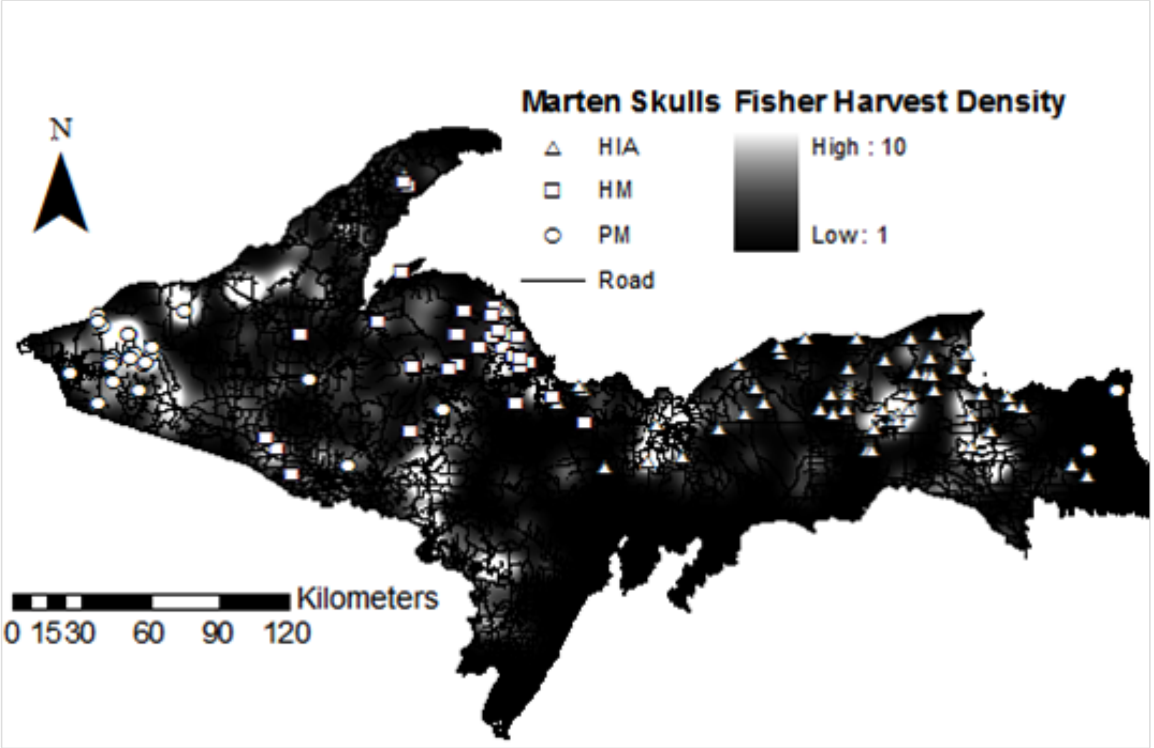


Figure 2.3. A) Landcover from the 2001 IFMAP GAP dataset derived from LandSat Imagery used to quantify dominant cover types associated with each harvested marten (resolution 1mi2, area of a section). B) Estimates of local fisher harvest density associated with section (1mi2) an individual marten was harvested in, was based on locations of harvested fisher during 2000-2004, and was created using a kernel density estimator. Roads are indicated as black lines in both A and B. Symbols indicate assignment of individual marten (*Martes americana*) to one of three genetic clusters (Williams and Scribner 2010) within the reintroduction area based on multilocus microsatelitte genotypes and lineage (putative source to descendent individuals).

Figure 2.3 (cont'd)

B)



Concordance between neutral genetic and phenotypic variation

The genetic cluster covariate used in the above analysis describes at a coarse scale the impact of shared ancestry (putative source populations) and differences in relatedness on marten skull morphology. Even with a significant effect of genetic cluster, morphological variation might be strongly associated with neutral genetic variation among individuals, supporting a hypothesis of neutral divergence (Monteiro *et al.* 2005, Nogueira *et al.* 2005; Perez *et al*. 2009). If patterns of phenotypic variability are discordant with spatial genetic structure resolved using molecular markers, this incongruence may indicate disparate causes underlying phenotypic and spatial genetic patterns. We used the residuals from regression analyses described above of skull shape on size and age (covariates explaining majority of variation in shape) to generate a matrix of pair-wise morphological distances among individuals using the Euclidean distance option in the dist function in the R package vegan (Oksanen *et al*. 2013). Thus, we investigated only variation in skull shape remaining after accounting for variation in size and age.

Genetic data used to create matrices of inter-individual genetic distance consisted of descendent marten for which we also had skull data. Individuals were genotyped at eleven highly variable microsatellite loci including Ma-2, Ma-5, Ma-8, Ma-14, Ma-19, Gg-3, Gg-7, Tt-4 (Davis and Strobeck 1998), Mvis072, Mer022, and Mer041 (Fleming *et al*. 1999). A pair-wise, individual-by-individual measure of genotypic distance was calculated from multilocus genotypes for all marten across the UP using Rousett's A_r (Roussett 2000) estimated using program SPAGeDi ver 1.4 (Hardy 2002). The A_r measure of genetic distance between individuals is analogous to the F_{st} measure of genetic differentiation between populations (Rousset 2000). Microsatellite loci have relatively high mutational rates making them appropriate genetic markers for examining relatively recent changes in contemporary patterns of connectivity (Pearse 2004).

Using matrix regression (Smouse et al. 1986) we quantified associations between inter-individual genetic similarity using neutral genetic markers (relatedness; Roussett 2000) with inter-individual similarity in skull shape to test the hypothesis that variation in phenotypic and molecular variation based on neutral markers were congruent (appropriateness of mantel test for distance matrices - Legendre and Fortin 2010). We performed this analysis using the mantel.test function in package ncf in R (Ottar 2012). Non-significant correlations would indicate that different evolutionary processes might be associated with morphological and genetic differentiation. If spatial auto-correlation is present in either dataset, Mantel tests are biased towards inflated type-I error, whereby the null hypothesis of no relationship between distance matrices is rejected too often (e.g., Guillot and Rousset 2013). Consequently, significant correlations between distance matrices that are suspected to have some spatial auto-correlation should be interpreted cautiously.

In contrasts to Procrustes ANOVA that partitioned variation among covariates associated with marten harvest locations (nodes), subsequent analyses allowed us to describe how variation in skull shape was associated with landscape features between marten harvest locations (edges). Just as neutral genetic variation can be structured by limited dispersal, phenotypic variation can be associated with shared ancestry, where dispersal is limited by geographic distance (isolation by distance; Wright 1943) between individuals or dispersion of landscape features (isolation by landscape resistance; McRae 2006). We calculated Euclidean distance among individuals to test for associations between morphological distance and geographic distance. A positive correlation indicates support for the hypothesis of isolation by distance. To assess the hypothesis that inter-individual morphometric variability is associated with degree of landscape resistance between individual harvest locales, we calculated ‘least-cost’ distances between all pairs of individuals. Previous research at the scale of the entire Upper Peninsula for all harvested individuals regardless of genetic cluster affiliation indicated that roads are the landscape feature most strongly associated with genetic differentiation (Chapter 1). A positive correlation between least cost distance and morphological distance would indicate support for the hypothesis of isolation by landscape resistance. Least cost distances were calculated using the Least Cost Path (LCP) tool in the Landscape Genetics extension for ArcGIS9.3 (Etherington 2011). The LCP method calculates a single path of least resistance between individual marten based on underlying movement surfaces, input as coded grids. Grid cells are coded based on hypothesized relationships between specific landscape features within a given grid cell and the ability of an organism to move through landscape features. Using the Michigan Geographic Framework All Roads dataset and Michigan Department of Transportation National Functional Classification codes (NFC), we classified grid cells at a 100m resolution with higher cost (higher grid cell value) assigned to roads versus grid cells without roads (see roads delineated in Figure 2.3 A and 2.3B).

RESULTS

Comparisons of Skull Morphology Between Source and Descendent Individuals

Based on a principal component analysis of shape, descendent and source individuals differed along the second principal component (19% variance explained in the ventral cranium view (Figure 2.4), 18% variance explained in lateral cranium view, Figure 2.5).

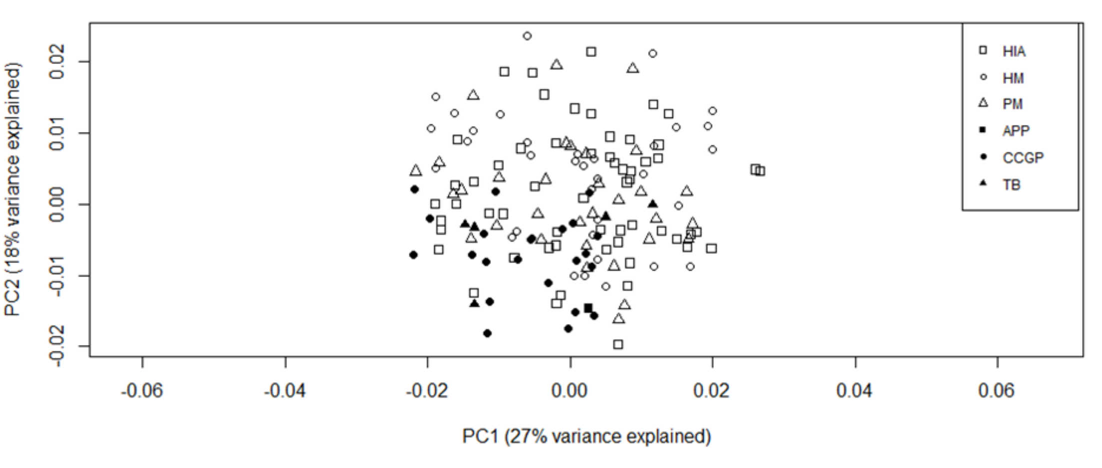


Figure 2.4. Summary of inter-individual variation in skull shape based on principal component analysis for individual American marten based on landmarks for the ventral cranium view. Percent variance explained by each principal component is shown. Distribution of individuals demonstrates separation in morphological space between source (APP – Algonquin Provincial Park, CCGP – Crown Chalpeau Game Preserve, TB – Thunder Bay) and descendent (HIA - Hiawatha, HM – Huron Mountain, PM – Porcupine Mountain) individuals along the second principal component.

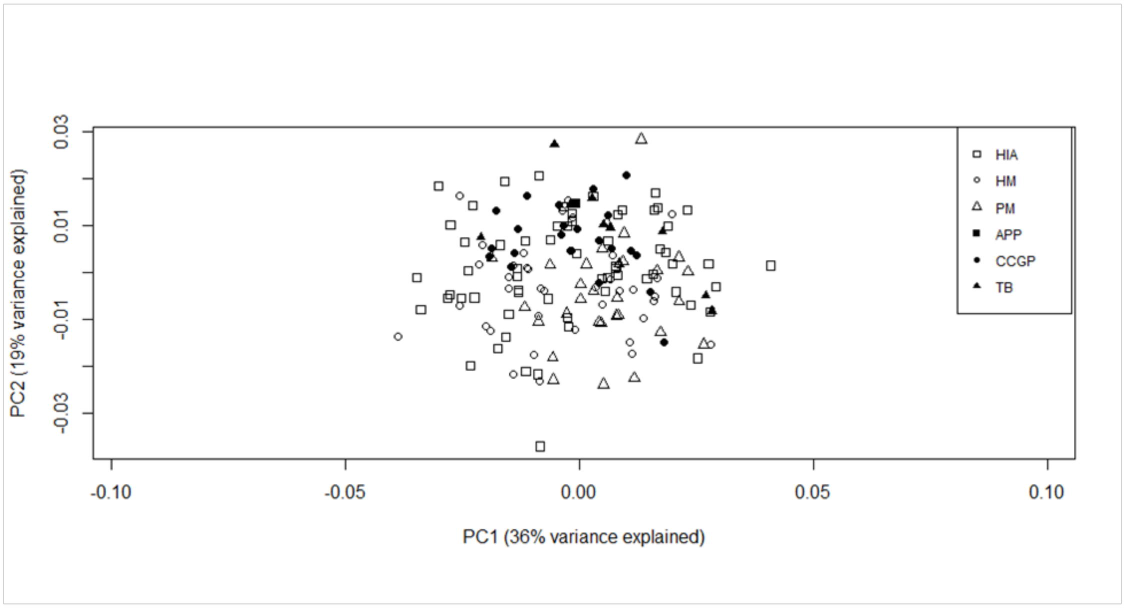


Figure 2.5. Summary of inter-individual variation in skull shape based on principal component analysis for individual American marten based on landmarks for the lateral cranium view. Percent variance explained by each principal component is shown. Distribution of individuals demonstrates separation in morphological space between source (APP – Algonquin Provincial Park, CCGP – Crown Chalpeau Game Preserve, TB – Thunder Bay) and descendent (HIA - Hiawatha, HM – Huron Mountain, PM – Porcupine Mountain) individuals along the second principal component.

Skull Shape Comparisons Among Descendent Individuals

Analysis of the ventral cranium view showed that skull centroid size, age, and genetic cluster were the factors most strongly associated with skull shape (Table 2.3). Most of the variation was attributed to centroid size (Table 2.3). Plots of the ventral cranium centroid size versus shape scores from the regression of shape on size indicated a strong relationship between shape and size (Figure 2.6). In adults of the same sex and age, an increase in size is accompanied by a lengthening and broadening in the skull near the ventral region of the palate, contraction of the posterior portion of the skull, and slight broadening of the zygomatic arch (Figure 2.7). For individuals of the same sex and skull size, differences in skull shape between juveniles and adults is primarily due to elongation of the posterior half of the skull, a small amount of expansion near the mid-palate, and slight broadening of the zygomatic arch (Figure 2.8).

In the lateral cranium view, skull shape was most related to age, skull centroid size, and genetic cluster (Table 2.4). Local fisher harvest density and landscape cover were not associated with variance in marten skull shape (Table 2.4). Plots of the lateral cranium centroid size versus shape scores from the regression of shape on size indicate a moderately strong relationship between shape and size ($R^2 = 0.21$, $P=0.01$, Figure 2.9). For adults of the same sex, differences in skull shape between individuals with small and large skulls is primarily explained by skulls becoming more streamlined, nuchal region expansion and movement upward of the zygomatic arch (Figure 2.10). Among individuals of similar skull size, differences in skull shape between juveniles and adults can primarily be explained by skulls becoming more streamlined (including the sagittal crest), nuchal region expansion, and expansion of postorbital relative to preorbital (Figure 2.11).

Table 2.3**.** Procrustes analysis of variance showing contribution of each factor (main effects) on ventral cranium shape of American marten. Log(CS) = log of centroid skull size. SS = Sum of squares, MS = mean squares, df=degrees of freedom associated with each categorical (age, sex, genetic cluster, dominant cover type) or continuous (log of centroid skull size, localized fisher harvest density) covariate. F= F statistic associated with each covariate, Rsq = amount of variation in shape attributed to each covariate and the significance (P) evaluated through permutation of the shape data (Procrustes aligned landmark coordinates).

Effect	SS	MS	MSx1000	df	F	P	Rsq
Log (CS)	0.011	0.0114	11.4	1	35.14	0.01	0.21
Age Class	0.004	0.004	4	1	10.98	0.01	0.06
Sex	0.001	0.001	1	1	2.87	0.08	0.02
Genetic Cluster	0.001	0.001	1	2	2.32	0.03	0.03
Dominant Cover Type	0.0002	0.0002	0.2	1	0.86	0.86	0.005
Localized fisher harvest density	0.0002	0.0002	0.2	1	0.95	0.95	0.003
Total	0.055	0.0004	0.4	123			

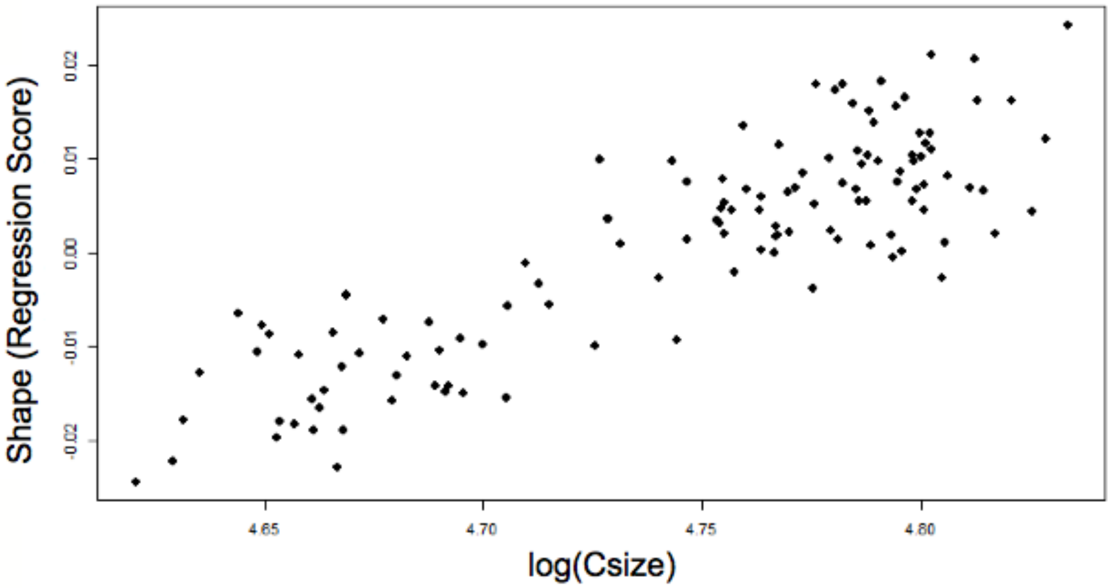


Figure 2.6. Plot of log(centroid size) against shape scores for the regression of ventral cranium shape on size. Lack of a scatter demonstrates a strong relationship between centroid size and shape for American marten; $R^2 = 0.21$, $P=0.01$ for skull centroid size.

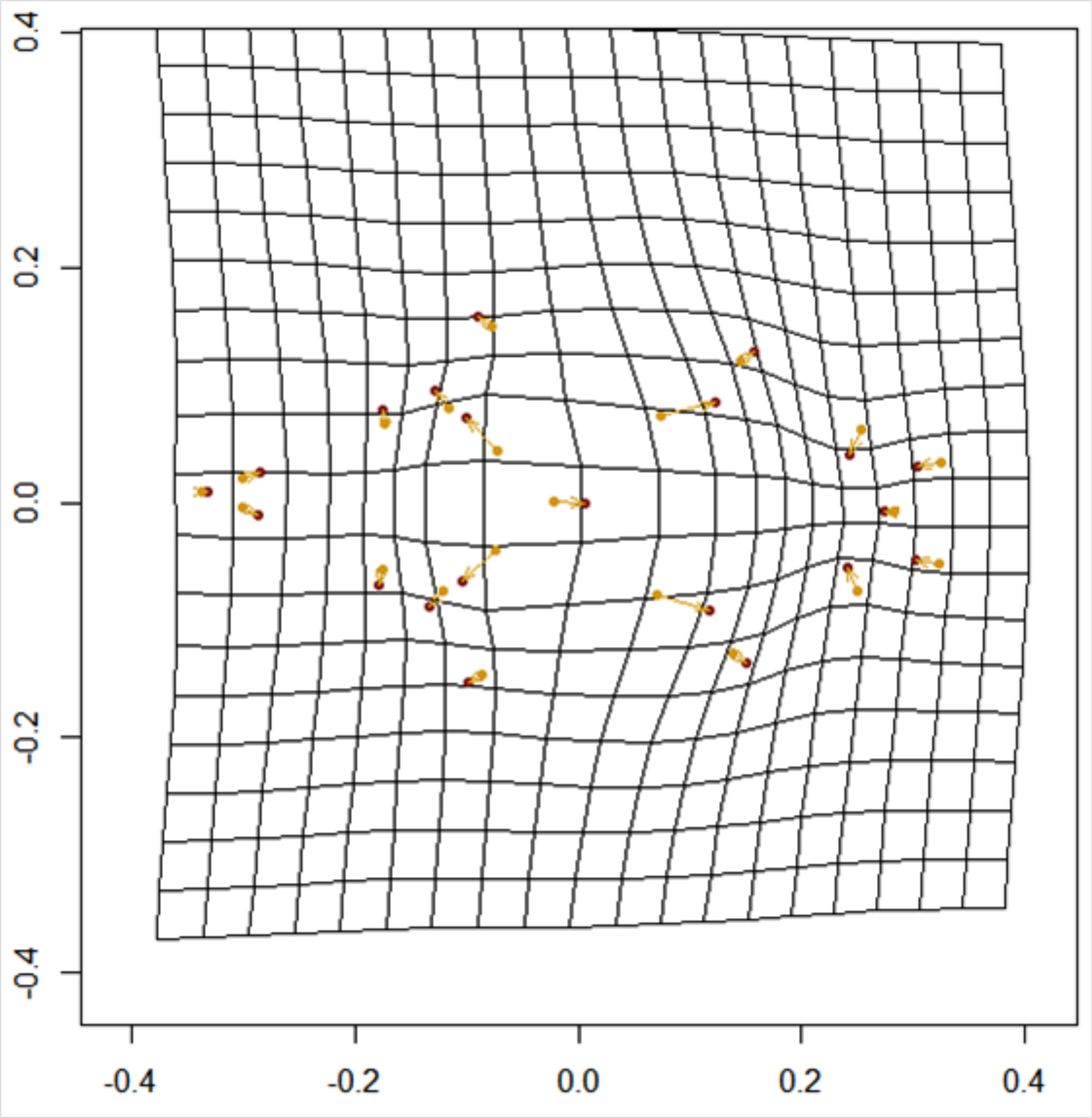


Figure 2.7. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis. Deformation grid displaying areas of the ventral cranium where most change occurs in shape from small (yellow) to large (red) adult American marten (3x magnification).

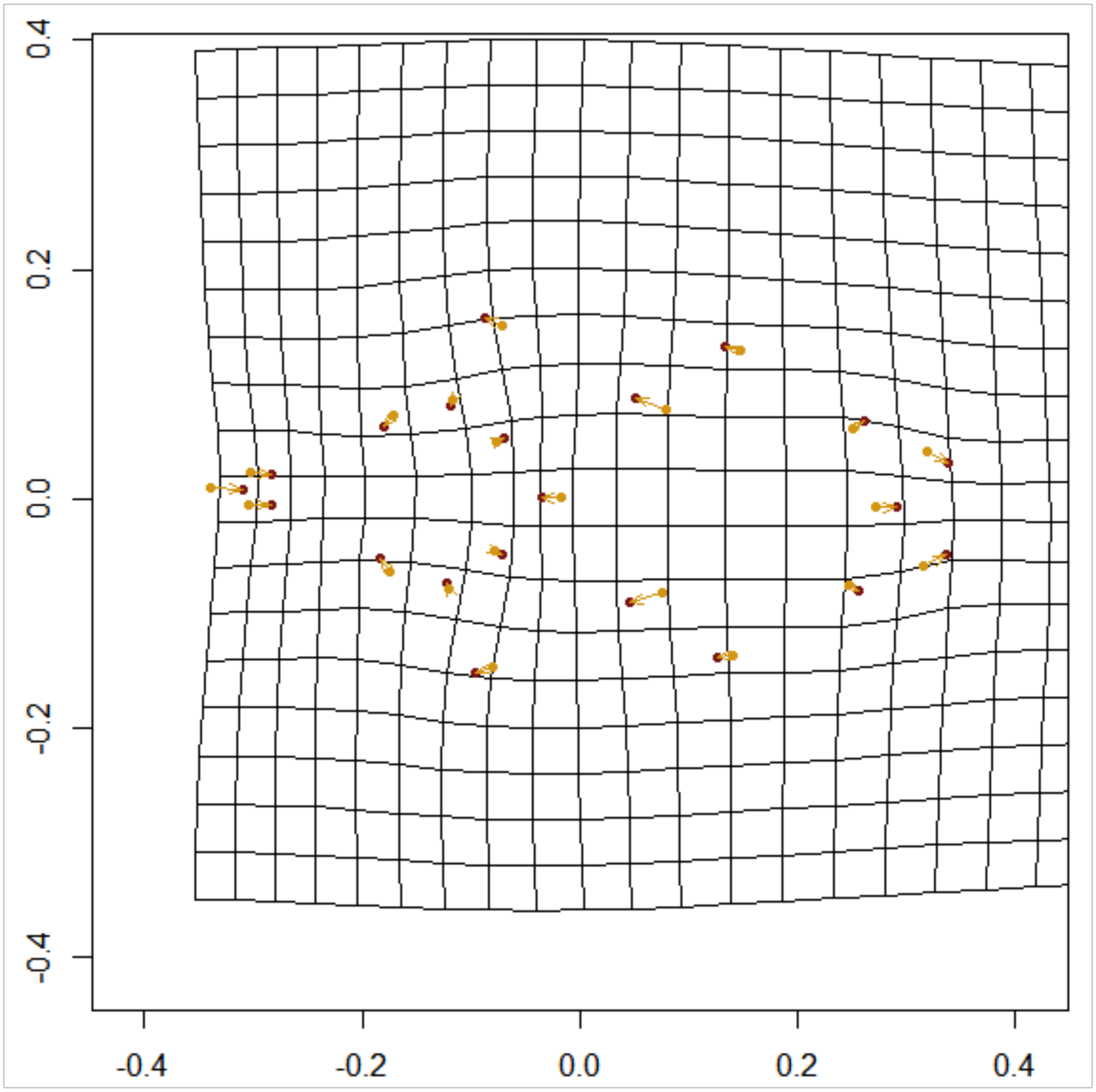


Figure 2.8. Deformation grid displaying areas of the ventral cranium change in shape from juvenile (yellow) to adult (red) American marten of similar centroid size (3x magnification).

Table 2.4. Procrustes analysis of variance showing contribution of each factor (main effects) on lateral cranium shape of American marten. Log(CS) = log of centroid skull size. SS = Sum of squares, MS = mean squares, df=degrees of freedom associated with each categorical (age, sex, genetic cluster, dominant cover type) or continuous (log of centroid skull size, localized fisher harvest density) covariate. F= F statistic associated with each covariate, Rsq = amount of variation in shape attributed to each covariate and the significance (P) evaluated through permutation of the shape data (Procrustes aligned landmark coordinates).

Effect	SS	MS	MSx1000	df	F	P	Rsq
Log (CS)	0.008	0.008	8	1	15.87	0.01	0.09
Age	0.014	0.014	14	1	28.76	0.01	0.16
Sex	0.001	0.001	1	1	1.24	0.5	0.01
Genetic Cluster	0.003	0.001	1	2	3.39	0.03	0.04
Dominant Cover Type	0.001	0.001	1	1	1.06	0.5	0.01
Localized fisher harvest density	0.001	0.001	1	1	1.72	0.34	0.01
Total	0.089	0.001	1	131			

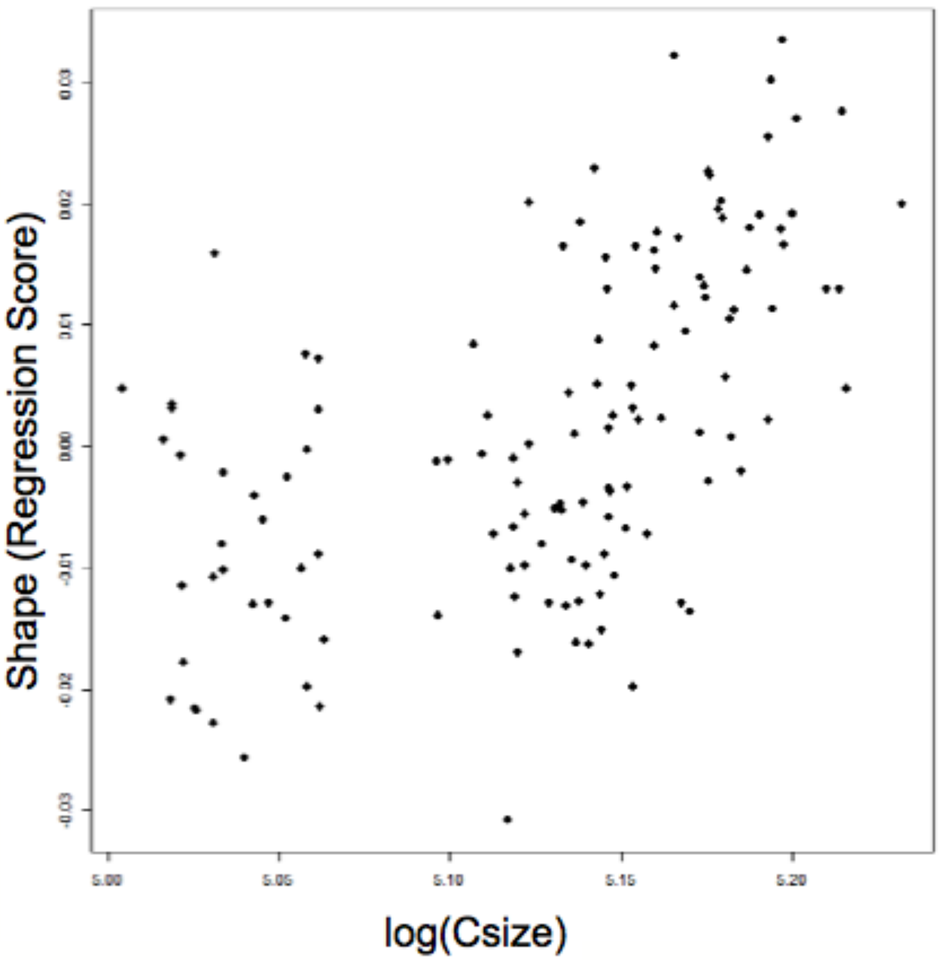


Figure 2.9. Plot of log(centroid size) against shape scores for the regression of lateral cranium shape on size. Some scatter demonstrates a moderate relationship between centroid size and shape for American marten; $R^2 = 0.09$, P=0.01 for skull centroid size.

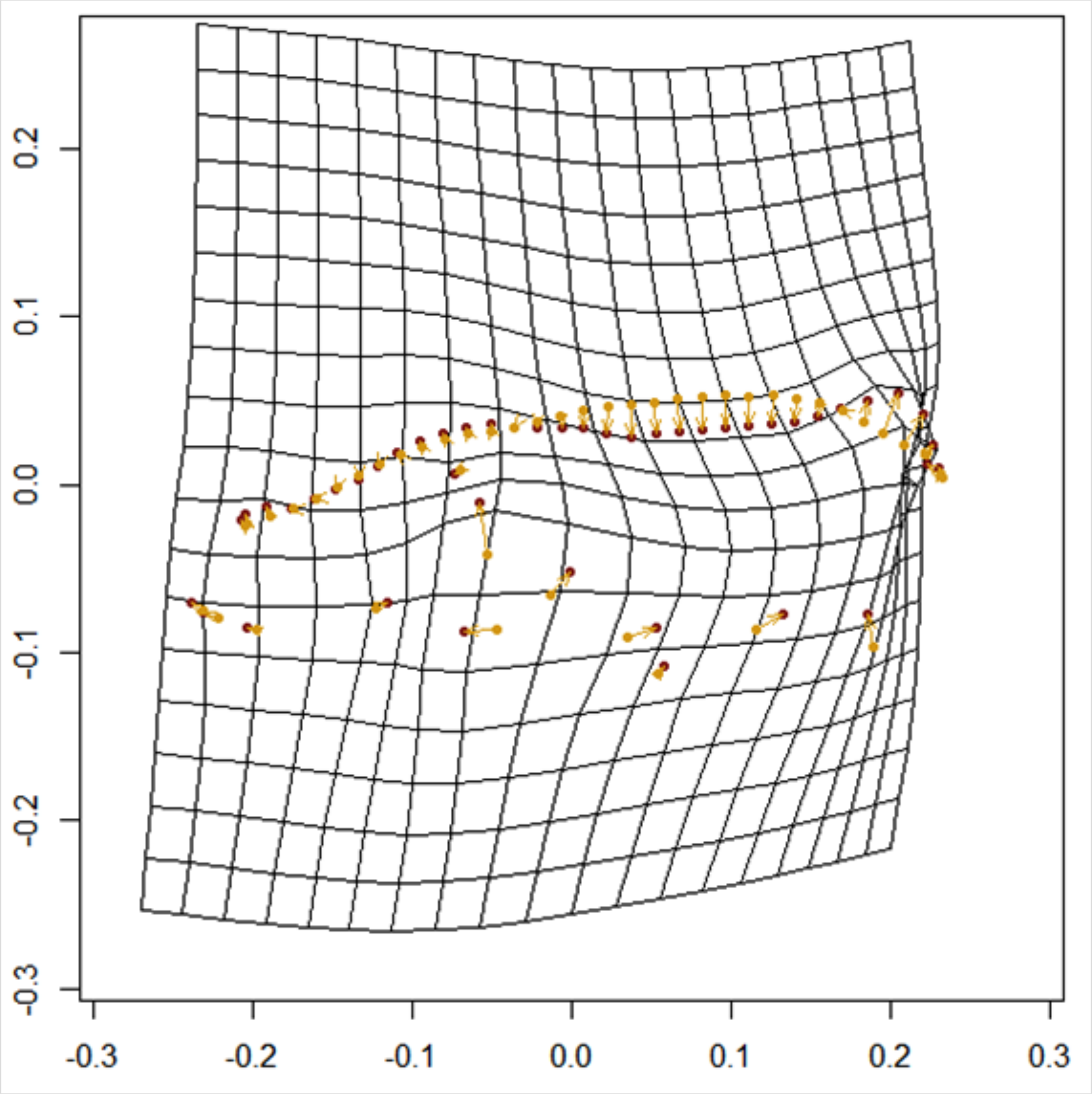


Figure 2.10. Deformation grid displaying areas of the lateral cranium change in shape from small (yellow) to large (red) adult American marten of the same age (3x magnification).

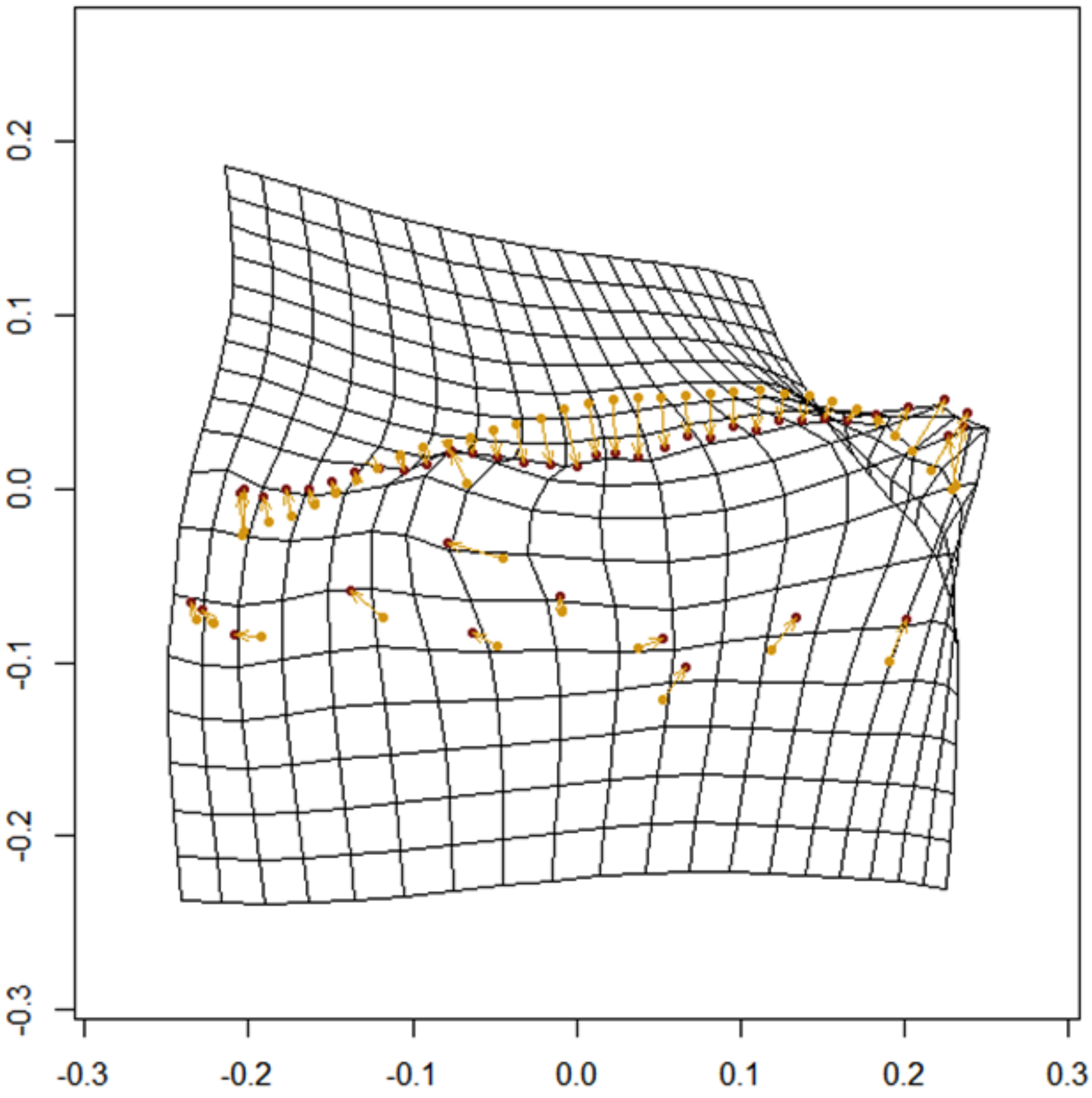


Figure 2.11. Deformation grid displaying areas of the lateral cranium change in shape from juvenile (yellow) to adult (red) American marten with similar centroid size (3x magnification).

Descendent Individuals – Genetic and Geographic Distance

Mantel tests did not detect a significant association between genetic and morphological inter-individual distances for all descendent individuals within the reintroduction range for either the lateral or ventral skull view (Mantel r=-0.02 and -0.06, P=0.32 and 0.07 for the ventral and lateral view, respectively). There was also no evidence for a pattern of isolation by distance for skull shape (Mantel r=-0.01 and 0.02, P=0.33 and 0.19 for the ventral and lateral view respectively). There was no evidence for a pattern of isolation by landscape resistance based on roads for skull shape (Mantel r=0.037 and 0.018 , p=0.09 and 0.26, respectively). This is in contrast to analyses based on neutral genetic markers at the scale of the reintroduction area that indicated roads are most closely associated with spatial genetic structure (Chapter 1).

DISCUSSION

Comparing skull features of source to descendent American marten revealed considerable divergence in skull shape in descendents after reintroduction and colonization of the Michigan Upper Peninsula (Figure 2. 4, 2.5). Individuals do not appear to cluster by lineage (Figure 2.4, 2.5), and so differences in skull shape between source and descendent individuals are most likely attributed to differences in habitat features between source and descendent regions rather than shared ancestry. Significant differences in skull shape among descendent individuals (Table 2.3, 2.4) is also therefore most likely associated with region differences in habitat features not measured in this study. This finding is further supported by a lack of association between descendent marten inter-individual morphological and genetic distances. In contrast to previous research of genetic distance based on neutral microsatellite loci, morphological distance among all descendent individuals was not correlated with Euclidean distance or least cost distance. Together, these findings suggest that different evolutionary processes are associated with morphological and genetic differentiation.

Comparisons of Skull Morphology Between Source and Descendent Marten

Analysis for both skull views based on a principal component analysis (PCA) revealed differences in skull morphology between source and descendent individuals. Results show that since reintroduction, skull shape of descendents of reintroduced marten have diversified across the UP relative to source populations (Figure 2.4, 2.5). However, it does not appear that marten phenotypes cluster by lineage whereby source and descendent individuals from the same lineage are readily distinguishable from other lineages (Figure 2.4, 2.5). The phenomenon of morphological diversification following reintroduction or invasion events has been documented in other taxa. Rapid morphological divergence has been shown to occur in a variety of organisms associated with climate change and subsequent range expansion into novel, unoccupied habitats (e.g., Hellberg *et al* 2001, Pease *et al.* 1989). For example, in 1977 and 1981, Anolis lizards (*Anolis sagrei*) were introduced from Staniel Cay island in the Bahamas onto 14 nearby islands (Losos *et al*. 1997). Following colonization, populations on each island where lizards were introduced exhibited morphological divergence relative to source populations (Losos *et al*. 1997). Additionally, the degree to which each population diverged was related to how different each island's vegetation was to the vegetation found on the source island (Losos *et al*. 1997).

Associations Between Skull Morphology and Genetic Clusters

We found a significant effect of genetic cluster on shape for both skull views (Table 2.3, 2.4). Genetic cluster subsumes variation associated with the effects of lineage (shared ancestry) and variation in habitats at and around locations of reintroduction events that differ from habitats associated with source populations (Williams *et al.* 2007). Reintroduction events began in 1955 in the Porcupine Mountain region (western UP) and continued until 1992 with translocations of individuals from within the west-central UP to the Keewenaw Peninsula in the north-central UP (Williams *et al.* 2007). Consequently, there is some regional variation in the number of years and generations between reintroduction/translocation events and when genetic/skull data were collected (2000-2004). Based on our PCA with source and descendent individuals, it does not appear that marten are clustering by lineage (Figure 2.4, 2.5). Thus, significant differences in skull shape associated with genetic cluster are more likely associated with differences in habitat features among regions within the reintroduction area compared to differences in ancestry. This result is further supported by a lack of association between genetic distance based on multilocus microsatellite markers and morphological distance for descendent individuals.

Phenotypic plasticity or selection in response to local habitat (and food resources) remain logical explanations for variation in skull morphology among genetic clusters within the reintroduction area. Studies of Carnivora indicate that bite force is an important adaptation to, and indicator of, different feeding ecologies among species (Christiansen and Wroe 2007). In studies of American mink (*Neovison vison*) morphology, authors found biogeographical variation in body mass associated with local environmental conditions and the percentage of large prey in their diet (Zalewski and Bartoszewicz 2012). In this cases, the relatively short time periods involved (1996-2004) are suggestive of plasticity dictating the environment-induced phenotypic changes.

The lack of an association between skull shape and landscape features that vary across the study area suggests that variation in skull shape may be more closely related to habitat features not measured in this study. Dominant cover type varies from the western to eastern Upper Peninsula, but was not found to significantly explain skull morphology (Table 2.3,2. 4). We assumed that cover type would be related to differences in prey type. However, the distribution of prey and diet of marten within different cover types was not measured directly. Other habitat variables (stand age, management regime, coarse woody debris that is associated with the distribution of small mammal prey including red-backed voles - Bowman *et al* 2000) may be better predictors of regional variation in prey distribution. Localized fisher harvest density was also not significantly related to variation in marten skull morphology. Previous research has found that when co-distributed, fisher and marten exhibit some partitioning of food resources with marten primarily feeding on smaller, subnivean prey (Raine 1987), although high dietary overlap has been observed in some sympatric populations (Zielinski and Duncan 2004). In areas of high fisher harvest density, there may be sufficient prey resources that even with some overlap in diet, marten are not limited or forced to switch prey. Consequently, marten may be eating similar food items in areas of both high and low fisher harvest density. Alternatively, marten or fisher may be responding to competition over food resources by first expanding habitat preferences or foraging behavior rather than dietary breadth (Hespenheide 1975). Thus, even in areas of high fisher harvest density, marten may be exploiting the same prey resources as in areas of low fisher harvest density by altering foraging strategies or habitat use. However, support for this hypothesis is difficult to quantify as we have resolution for marten harvest locations only to the level of a section (1mi^2) compared to estimates of space use based on core areas or home ranges.

Discordance Between Skull and Genetic Inter-individual Relationships

After accounting for the effect of size and demographic features on skull shape, morphological distance among descendent individuals was not correlated with genetic distance among individuals across the reintroduction area. This incongruence may indicate disparate causes underlying phenotypic and spatial genetic patterns. When considering all individuals, regardless of affiliation to a genetic subpopulation, genetic distance among individuals is most closely associated with the cost of movement associated with roads (Chapter 1; isolation by landscape resistance – McRae 2006). However, morphological distance is not related to least cost distance based on the distribution of roads in the UP or Euclidean distance among individuals (isolation by distance, Wright 1943). Although spatial genetic structure is associated with limited dispersal, variation in marten skull morphology does not appear to be consistent with a scenario of isolation by distance or landscape resistance. Rather, differences based on the relatedness among descendent individuals may primarily be related to regional differences in local habitat features.

Skull Allometry and Demographic Effects

Among marten descendents, variation in skull shape associated with the covariates used in this analysis was related to skull size and age of individuals (Table 2.3, 2.4). For both skull views, there was a significant relationship between shape and centroid size, although this relationship was more pronounced in the ventral than the lateral view (Figure 2.6, 2.9). The significant relationship between shape and size is not surprising as variation in shape among individuals is often attributed to variation in size (Zelditch *et al.* 2004). In the ventral view, larger adult individuals were characterized by a lengthening and broadening in the skull near the ventral region of the palate, contraction of the posterior portion of the skull, and slight broadening of the zygomatic arch (Figure 2.7).. Within Carnivora, skulls characterized by these features are more typical of an active predator as this shape has been linked to increased bite force relative to gape (Loy *et al.* 2004). In the lateral view, larger adult skulls are more streamlined, exhibit nuchal region expansion and movement upward of the zygomatic arch relative to smaller adults (Figure 2.10). Changes in the nuchal regiona are indicative of an increase in area for the temporal muscles and consequently an increased bite force.

Although more pronounced in the lateral than ventral view, skull shape was influenced by age (Table 2.3, 2.4). In the lateral view, differences among juveniles and adults were primarily related to skulls becoming more streamlined (including the sagittal crest), nuchal region expansion, and expansion of postorbital relative to preorbital (Figure 2.11). In the ventral view, differences in skull shape between juveniles and adults is primarily due to elongation of the posterior half of the skull, a small amount of expansion near the mid-palate, and slight broadening of the zygomatic arch (Figure 2.8). Skull elongation and expansion of the nuchal region are common ontogenetic changes in members of Order Carnivora. Juveniles are significantly handicapped relative to adults when it comes to feeding performance and efficiency (Tanner *et al.* 2010). The lengthening and broadening of the sagittal crest creates an increased area for the temporal muscles origin and consequently an increase in bite force and feeding performance in adults (LaCroix *et al*. 2011). Previous studies of intraspecific skull ontogeny in mammals have found evidence that adults differ in postnatal development likely related to adaptation to specific factors (e.g., food availability) of their habitats (Galatius and Gol’din, 2011). In contrast, ontogenetic changes in marten occur throughout the reintroduction area regardless of genetic cluster affiliation (no cluster x age interaction) and so appear to be consistent changes in skull morphology regardless of regional habitat differences. So, access to food items as juveniles is either a) similar throughout the UP, regardless of landscape heterogeneity, or b) differences in access to food items as juveniles is not sufficient to impact development of adult cranial morphology (in contrast to previous work on mammalian carnivores e.g., LaCroix Dissertation 2011).

In conclusion, variation in skull shape for reintroduced American marten in Michigan's Upper Peninsula was primarily related to skull size and age. Variation in skull morphology was also associated with genetic cluster and individuals did not appear to cluster by lineage (putative source with descendent marten). Rather, overall descendent individuals appeared to be distinct from source individuals, based on a principal component analysis. At a finer scale, we observed discordance between patterns of phenotypic variation and neutral genetic variation, suggesting disparate causes underlying phenotypic and spatial genetic patterns. Taken together this evidence supports the hypothesis that individuals differ in shape among regions based on habitat features that vary across the reintroduction area, but were not quantified in this analysis. Limited dispersal does affect spatial genetic structure (Chapter 1). However, phenotypic variation does not appear to be influenced by the geographic (Euclidean) distance or least cost distance among individuals. Future research including fine-scale habitat features, such as volume of coarse woody debris or population dynamics of primary prey species may improve our understanding of how variation in skull shape is partitioned.

APPENDIX

APPENDIX

Table A2.1 Landmarks. Description of Landmarks and Semilandmarks for each skull view

Lateral Cranium Landmarks

1	Intersection of anterior margin of I3 with premaxilla
2	Intersection of anterior margin of canine with maxilla
3	Intersection of posterior margin of canine with maxilla
4	Posterior end of molar row
5	Midpoint of the infraorbital foramen
6	Tip of the post-orbital process
7	Posterior point of the optic foramen on the orbitosphenoid
8	Tip of the most anterior of two ventral squamosal projections
9	Tip of the hamulus on the pterygoid process
10	Posterior point of the external auditory meatus
11	Tip of the jugular process
12	Posterior tip of the nuchal crest
13	Anterior extent of the nasal-premaxilla suture
14	Tip of the postmandibular process
15-16	Scale bar (10mm)
o	32 semi-landmarks along dorsal curve of the cranium and nasals (LM12 to LM13)

Ventral Cranium Landmarks

1	Juncture between incisors on the premaxilla
2,13	Posterior point of incisive foramen
3,14	Anterior edge of P4
4,15	Posterior edge of P4
5,16	Point of max external curvature of M1 at protocone
6	Posterior junction of left and right palatine
7,17	Tip of the most anterior of two ventral squamosal projections
8,18	Tip of ventral squamosal projection
9,19	Anterior point of external auditory meatus
10,20	Posterior edge of the jugular foramen
11,21	Tip of the occipital condyle
12	Anterior point of the foramen magnum
22,23	Scale bar (10mm)

FINAL CONCLUSIONS

Quantifying the relative influence of landscape features on morphological and genetic differentiation is a major goal in conservation biology. Landscapes can be thought of as influencing processes at sites (e.g., local adaptation) or between sites (e.g., movement and dispersal). In the context of movement through an environment, landscape features (e.g., roads, cover type) can either impede or facilitate dispersal and the exchange of migrants among groups of individuals. When landscape features disrupt successful dispersal, genetic discontinuities may arise whereby genetic clusters of individuals are spatially contiguous, but with little evidence for interbreeding (Williams and Scribner 2010). Quantifying the influence of specific landscape features on dispersal is important for predicting species-specific responses to changing landscapes and environments.

Similarly, morphological differences among groups of individuals can accumulate as a product of limited dispersal and drift whereby nearby individuals or those separated by fewer impediments to dispersal more closely resemble one another (Spurgin *et al* 2014). Environmental conditions can also act as agents of selection (at site), favoring locally adapted morphologies and limiting the likelihood of successful dispersal among populations (Clobert *et al.* 2009, Manel *et al* 2010). The relative importance of selection, isolation by distance, and isolation by landscape resistance to morphological variation are not well understood. Therefore, studies that simultaneously consider each of these features and their potential interactions are required in order to understand morphological divergence (Wang and Summers 2011).

In this thesis, I examined the factors influencing patterns of morphological and genetic variation in American marten (*Martes americana)* in the Upper Peninsula (UP) of Michigan (MI). Following extirpation, marten were reintroduced into the UP from multiple genetically distinct source populations. Details of the reintroduction history are known, providING a useful context for quantifying the influence of landscape features on contemporary spatial patterns of genetic and morphological differences among descendents. Following reintroduction events, three spatially distinct genetic clusters developed in regions proximal to release sites, suggesting founders from independent releases had colonized different regions with minimal overlap. Across the entire UP, regardless of genetic cluster affiliation, I found that genetic distance among individuals was most closely associated with landscape permeability based on the distribution of roads. Inter-individual comparisons within and between genetic clusters revealed that the proportion of forested area or a combination of the proportion forested area and percentage overhead cover were the landscape features most closely associated with genetic distance.

In contrast to spatial genetic differentiation, spatial morphological differentiation among American marten in the Upper Peninsula of Michigan appears to be a product of local adaptation (at site) to spatially varying habitat features. Descendent individuals' skull shape differed from that of the source population individuals, but not in relation to lineage. Within the reintroduction area, descendent skull shape was related to genetic cluster however, patterns of skull shape variation among descendents were distinct from spatial genetic structure based on neutral molecular markers. Morphological differences were not consistent with hypotheses of dispersal limitation (in contrast to spatial patterns of genetic differentiation), as evidenced by a lack of association between measures of inter-individual variation in skull morphology and by Euclidean distance or landscape resistance. This evidence suggests that skull shape is not related to shared ancestry and that there are different causes underlying spatial morphological and genetic patterns. In regions occupied by each genetic cluster, aside from variation in the initial source population used in reintroduction events, there is spatial variation in habitat features. For example, the Upper Peninsula (UP) of Michigan (MI) consists primarily of a mix of deciduous/coniferous forest in the central and western regions and broader diversity of cover types (e.g., forest as well as wetlands, agricultural area). Consequently, marten skull shape may differ depending on regional differences in land-cover associated with food resources across the UP.

My research contributes to our understanding of how landscape features can influence spatial patterns of variation by affecting landscape permeability (e.g., ability of an organism to move through a landscape) or by affecting processes occurring at a specific location. Consideration of both between and at site landscape effects is necessary for a comprehensive understanding of the factors contributing to the emergence and maintenance of spatial morphological and genetic variation. Reintroduction of American marten into the UP of MI was successful in that individuals from multiple, distinct genetic source populations colonized much of the reintroduction area. However, since colonization, genetic discontinuities have arisen and I have demonstrated that the genetic distance among individuals is associated with landscape permeability; specifically roads, the proportion of forested area, and percent canopy cover. Functional landscape connectivity is necessary to maintain interbreeding groups of individuals. Reduction in forested area and/or an increase in the landscape occupied by roads may further decrease genetic connectivity among American marten and exacerbate the potential for population extinction. In contrast to the factors influencing spatial patterns of genetic variation, morphological variation appears to be associated with habitat features that vary across the reintroduction area, but were not quantified in this analysis. Further research is required to correctly identify the specific landscape features contributing to spatial variation in skull morphology.

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LITERATURE CITED

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