DISSECTING THE GEOGRAPHICAL EXPANSION OF *PEROMYSCUS LEUCOPUS* IN THE NORTHERN GREAT LAKES: INSIGHTS FROM GENETICS, MORPHOMETRICS AND ECOLOGY

By

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ABSTRACT

DISSECTING THE GEOGRAPHICAL EXPANSION OF *PEROMYSCUS LEUCOPUS* IN THE NORTHERN GREAT LAKES: INSIGHTS FROM GENETICS, MORPHOMETRICS AND ECOLOGY

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Geographical and ecological distributions of biological species are primarily limited by prevailing climate conditions, and thus it is hardly surprising that recent rapid climate change has triggered evolutionary processes that are deeply affecting the biology of many organisms. One consequence of rapid climate change has been the occurrence of shifts in geographical ranges of many species, such as the white-footed mouse (*Peromyscus leucopus*), which has experienced a northern range expansion in Michigan and Wisconsin over the last few decades and as a consequence is now common in localities where it was absent until recently. In this study, I sought to understand some of the historical causes and consequences of the expansion process undergone by this rodent species. I analyzed complete D-loop sequences from 595 mice collected throughout the Great Lakes region to investigate the origin of the newly established populations. Phylogeographic analyses strongly support two lineages, spanning populations from Wisconsin through the western Upper Peninsula (UP), and from the Lower Peninsula (LP) to the eastern Upper Peninsula (UP) of Michigan, respectively. With few exceptions, western populations in the UP originate from Wisconsin while the eastern UP populations originate from LP. Both lineages display a genetic signature of spatial expansion, whereas demographical expansion is apparent only for the western UP-Wisconsin lineage. I also investigated whether the rapid geographic expansion of populations of this species in the northern Great Lakes region has been accompanied by rapid morphological divergence, and whether the pattern of divergence suggests a neutral process or reflects the influence of non-random factors. Using morphometric analyses of mandible shape, I found significant differences between recently expanded and long established populations not only in the magnitude of shape variation but also in the dimensionality of variation, which suggest that the expansion process has been accompanied by rapid morphological change along directions of the phenotype space that are absent in ancestral populations. Furthermore, phenotypic and phylogeographic patterns are incongruent in these populations, suggesting that changes in morphology have not been caused by random phenotypic drift. To further explore possible causes for the phenotypic divergence observed between old and recent populations, I examined the association between geographical and environmental variables and patterns of genetic and morphometric variation. Results from these analyses suggest that even though most factors analyzed contribute to the global variation in the mandible, cold temperatures and snowfall magnitudes appear to be the best predictors for the differentiation between old and new populations.

Copyright by ROSA ANNA MOSCARELLA 2011 To my parents, Emilia y Gaetano, because all I am is because of them, literally To my best friend, my soul mate, and the love of my life: Eladio To my daughter Veronica

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CHAPTER I

INTRODUCTION

Overview

Even though most living species have been exposed to climatic shifts throughout their history, the current scenario of change has been deemed unique inasmuch as temperature is rising unusually quickly. Only during the last century it has been registered an increase of 0.6° C on average temperatures, from which 0.2° C have been gained during the last 30 years (Stott *et al.* 2000, Walther *et al.* 2002). The environmental changes associated to this phenomenon are expected to trigger responses in organisms, including migration, adaptation, speciation, and extinction (Lynch and Lande 1993). Indeed, the effects of this accelerated climate change are already noticeable in a vast number of organisms, affecting their physiology, phenology, and distribution, which has led to local extinctions in some cases and adaptation to novel local conditions in others, presumably affecting species interactions, and hence community structure and composition (Hughes 2000, McCarty 2001, Walther *et al.* 2002, Parmesan and Yohe 2003, Root *et al.* 2003, Parmesan 2006, Thomas *et al.* 2006, Salamin *et al.* 2010, and references therein).

This study focuses on some of the consequences of rapid climatic change, specifically changes in geographical distribution of land populations undergoing range expansion. Despite the fact that changes in distribution do not necessarily imply evolutionary changes (Thomas *et al.* 2001), geographic expansion might create conditions for differentiation and divergence to occur. Moreover, such expansions can have important effects on genetic diversity and spatial structure

of populations, leaving characteristic footprints on the genome (Stone *et al.* 2003, Zheng *et al.* 2003). Only in the last decades, we have witnessed the invasion of boreal forests in Northern Michigan and Wisconsin by temperate species that were previously absent in those areas, often accompanied by the declining of the ecologically similar boreal populations (Long, 1996; Myers *et al* 2009). A clear example of this phenomenon is the effect that the expansion of *Peromyscus leucopus* in the northern Great Lakes seems to have on populations of the dwarf deer mouse *P. maniculatus gracilis* (Pmg), where the numbers of the last species are declining noticeably while the former are flourishing (Myers *et al.* 2009). These two species, however, have coexisted in northern Michigan despite their similarity in sympatry (Baker 1983, Myers *et al.* 2005) with no evidence of hybridization (Dice 1933). Their coexistence has been attributed to their dissimilar adaptation to winter, which favors survivorship in *P. maniculatus,* balanced by a higher reproductive rate: in *P. leucopus* (Wolff 1996). Therefore, it is not surprising that the onset of warmer climates gives *P. leucopus* a competitive advantage over Pmg.

One of the greatest concerns about rapid global warming is to understand how fast species can respond to these changes and how entire communities may be affected. The remarkable range expansion that Pln has experienced in northern Michigan and Wisconsin makes it an ideal model to study the short term consequences of global warming and the dynamics of range expansion. Although the effects of climate change are currently not very dramatic in this area, it is predicted that they will have a major impact on the Great Lakes region, through drastic temperature increase and changes in the water level of the lakes in the next decades (Dempsey 2004). These predicted changes will likely have major effects on the biological composition of entire communities.

Because the processes associated to current climate change are expected to cause not only changes in the genetic structure of populations but also on their morphological attributes (Root *et al.* 2003), this study examines the genetic and morphological consequences of the geographical expansion of Pln in the northern Great Lakes region, as well as the ecogeographical factors related to this phenomenon. Similar studies found in the literature usually analyze either the genetic (e.g., Excoffier *et al.* 2009) or morphological (e.g., Goheen *et al.* 2003) consequences of range expansion, but few offer a comprehensive evaluation of the effects of range expansion taking into account not only genetics and morphometrics, but also ecological factors. In this study I have focused on the newly established populations of this mouse in Michigan's Upper Peninsula (UP) and their putative progenitor populations in northern Michigan's Lower Peninsula (LP) and Wisconsin.

Geographical expansion of *Peromyscus leucopus* in the northern Great Lakes region

The white footed mouse *Peromyscus leucopus* is one of the most common mammals in the northeastern United States. It is a generalist rodent, inhabiting habitats as diverse as woodlands, brushlands, and farmlands (Baker 1983). It is an unusually tractable species because of its abundance across its range, and relative ease with which they to catch. Within its range, 17 subspecies have been proposed by different authors (Fig. I.1; Hall 1981, Lackey *et al.* 1985).

In the Great Lakes region, *P. leucopus* is represented by the subspecies *P. l. noveboracensis* (Pln), which is widely distributed in deciduous forests throughout the central and northeastern United States (Lackey *et al.* 1985). It is of small size, similar to a house mouse (weighing less than 30 g); it has dark dorsal pelage, usually brown or caramel, and white venter;

its tail is shorter than its head-body length, and its dental formula is (Baker 1983). It is omnivorous, incorporating seeds and nuts, fruits, grasses, and insects in its diet (Holman 2001).

In Michigan, Pln is at the northern limit of its range, and in this area it seems to prefer woodlands and brushlands, although it is also found in open fields (Baker 1983). The population abundance of this mouse can show dramatic seasonal fluctuations. Individuals normally have a short life span of less than one year (Baker 1983). Studies of natural populations in southern Michigan carried out in the 1940s suggest that the reproductive season spans early March to late October, with females producing on average 4.3 young per litter and four litters per year (Burt 1940). Nests are made of mostly organic material and are built above ground, usually under rocks or logs or in holes at the base of trees (Holman 2001).

In a study that combined 100 years of museum records with 14 years of intensive collecting, Myers and co-workers (2005) showed the remarkable expansion of Pln in Michigan. Historical records indicate that the northern limit of Pln in the early 1900s barely reached 45° N (Osgood 1909), which corresponds to approximately three fourth of the LP in latitude; museum records, however, suggest that this mouse might have been already present in the northern LP at this time (Myers *et al.* 2009). In the late 1930s, an isolated population was reported from Menominee County in the UP (Baker 1983). No further populations in the UP outside of Menominee were found until the early 1990s and since then, Pln has expanded eastward about 225 Km across the UP. It has become the commonest small mammal at some localities in the UP from which it was absent only 30 years ago (Fig. I.2; Myers *et al.* 2005, Myers *et al.* 2009). Its range has extended into the central and eastern areas of the UP (Myers *et al.* 2009) and northward in Wisconsin (Long 1996) and Minnesota (Jannett *et al.* 2007). The geographic

expansion of Pln is correlated with a trend toward milder winters, suggesting that climate change may play a causal role in this process (Myers *et al.* 2005).

Range expansions of populations of Pln in the Great Lakes region, however, are not a recent phenomenon; the margins of the range of this species have extended northwards since the late Pleistocene, following the retreat of ice after the Last Glacial Maximum (LGM). During the LGM, when the Laurentide Ice Sheet covered the Great Lakes region (18,000 YBP; Pielou 1991), many temperate species are believed to have survived in southern refugia that remained ice-free (Pielou 1991, Rowe *et al.* 2004). As the ice receded, species like Pln expanded northward, occupying the newly available habitat (Pielou 1991). In Michigan, the ice sheet began to retreat about 14,800 YBP. Wisconsin and northern Michigan, including the UP, were ice-free by about 10,000 YBP (Pielou 1991). By that time, southern Michigan was already covered by diverse forests similar to those found today (Holman 2001). Presumably, Pln would have inhabited those forests, and subsequently would have colonized the northern LP and WI as conditions became increasingly more favorable. Although northward expansion after LGM is a common process for most northern species who survived the last ice age, those expansions did not take place at the pace they have been occurring over the last few decades.

Northern Great Lakes Ecoregions

The Great Lakes region is the final product of glacial erosion during the Pleistocene, although its actual formation started in the Paleozoic era (Holman 2001). The Laurentide Ice Sheet covered the Great Lakes regions during the Last Glacial Maximum, ca. 18,000 YBP; in Michigan, the ice sheet began to retreat about 14,800 YBP. By 10,000 YBP, southern Michigan was covered by

diverse forests similar to those in the present, while northern Michigan was still too unstable to sustain viable communities (Holman 2001).

The northern Great Lakes is the point of encounter of two ecological regions: the northern forests and the eastern temperate forests (CEC 1997). The transition between these two ecoregions represents the northern or southern distributional limit of many North American species (e.g. Hall 1981). Current climate change seems to have diffused this barrier, and some temperate species that had their northern limit at those margins are invading the northern forest (Myers *et al* 2009). The northern forests are typified by extensive boreal forests of conifers, with mixed hardwoods toward the south; the climate of this ecoregion is characterized by long, cold winters and short, warm summers (CEC 1997). These forests are found in northern Wisconsin, the UP, and some areas of the northern LP, Pennsylvania, and New York. The eastern temperate forests occupy a vast area of the eastern United States and consist mostly of beech-maple and maple-basswood forests, although mixed-oak associations are more frequent in the northern Midwest; climate varies over a latitudinal gradient, from cool to subtropical; this area has a very rich biodiversity (CEC 1997).

Objectives and Outline

The main objectives of this study are to investigate the phylogeographic and genetic structure, and morphological differentiation of populations of the white-footed mouse in the northern Great Lakes region, where the species is undergoing geographical expansion. Additionally, ecogeographical factors are analyzed to investigate their association with genetic and morphological changes observed in expanding populations of Pln. Among of the unknowns addressed in this Dissertation are the origin of the newly founded populations in the UP, the

phenotypic changes that have accompanied the rapid geographical expansion of these mice, and the geographical and environmental factors that account for morphological variation and the differentiation between old and new populations. These questions are developed in three research chapters:

- Chapter II: this chapter focuses on the genetic structure and phylogeographic pattern of
 Pln in northern Michigan and Wisconsin, which were inferred based on mtDNA sequence
 polymorphism. The analysis of 595 sequences from 21 populations in Wisconsin, 9
 populations in the UP, and 9 populations in the LP shows two well-differentiated
 lineages. One of the lineages includes all populations from western UP and Wisconsin
 (WUP-WI), and the other consists of all populations in the LP and eastern UP (LP-EUP).
 A clear signature of spatial expansion is evident for both lineages, although demographic
 expansion is only supported for WUP-WI. Contemporary expansion is evidenced by the
 geographic proximity of divergent haplotypes. These results show that the UP is a zone
 of secondary contact of these two very differentiated lineages, whose divergence
 occurred over 30,000 YBP, which might have important consequences for the evolution
 of the resulting admixed populations.
- Chapter III: In this chapter, the morphological consequences of range expansion are
 investigated based on the analysis of the shape of the mandible of Pln. The main
 objective was to assess whether geographic expansion has been accompanied by
 morphological divergence, and whether such divergence is attributable to random factors.
 Shape analysis was based on geometric morphometric techniques. Results suggest that
 new populations are significantly different from the old established ones, not only in
 magnitude but also in the dimensionality of variation. Because the patterns of genetic and

morphometric distances are incongruent, morphological differentiation of new populations is likely caused by non-random factors. Regardless of whether phenotypic changes have been caused by plasticity and/or adaptation, it is clear that the rapid geographic expansion that Pln has experienced in the northern Great Lakes has already noticeable phenotypic consequences.

• Chapter IV: This last chapter seeks to find the geographical and environmental factors associated to patterns of genetic and morphometric variation observed in the expanding populations of Pln in the northern Great lakes. Those factors are expected to explain not only general variation in shape among populations, but also the divergence between old and new populations in the two lineages. To this end, several statistical analyses were carried out to study the association between genetic and morphometric variables with several geographical and environmental variables factors, including ecological and geographical distances, climate, geographical coordinates, and land cover/land use. For LP-EUP, all factors analyzed show a similar effect on the shape of the mandible, i.e., contraction of mandibular processes. For WUP-WI, the different factors affect different regions of the mandible. The examination of these geographical and environmental factors shows that climate variables, in particular those associated to cold climate (e.g., low temperatures and snowfall) are the best predictors that explain the differentiation between old and new populations in both lineages.

Figures



Figure I.1. Distribution of *Peromyscus leucopus*, according to Lackey *et al.* (1985). The numbers indicate the distribution of the 17 sub-species proposed for this mouse in North America. The grey area with the number 14 shows the range of the subspecies *P. l. noveboracensis*. Note that in this map this subspecies is not reported for the Upper Peninsula of Michigan. Reproduced with permission of the authors and the American Society of Mammalogists, Allen Press, Inc.



Figure I.2. Distribution of the white-footed mouse, *Peromyscus leucopus*, in Michigan during a) 1883-1980 and b) 1981-2006 (Myers *et al.* 2009).

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CHAPTER II

PHYLOGEOGRAPHY, GENETIC STRUCTURE AND HISTORICAL DEMOGRAPHY OF EXPANDING POPULATIONS OF *PEROMYSCUS LEUCOPUS* IN THE NORTHERN GREAT LAKES

Abstract

Rapid climate change affects the biology of many organisms, which is not surprising given the key role that climate plays in determining the limits under which an organism can live. One consequence of rapid climate change is shifts in geographical ranges. The white-footed mouse (Peromyscus leucopus) has experienced a northern range expansion in Michigan and Wisconsin over the last decades and is now common in localities where it was recently absent. Complete Dloop sequences were analyzed from 595 white-footed mice collected throughout the Great Lakes region to investigate the origin of the newly established populations. It was found that mice from Wisconsin and the western Upper Peninsula (UP) of Michigan make up a well-differentiated lineage, while the eastern UP and the Lower Peninsula (LP) of Michigan form another. With few exceptions, western populations in the UP originate from Wisconsin while the eastern UP populations originate from LP. Both lineages display a genetic signature of spatial expansion, while demographical expansion is apparent only for the western UP-Wisconsin lineage. These results suggest that the UP is a zone of secondary contact for these two well differentiated lineages, which requires further investigation of the potential consequences of admixture, e.g., outbreeding depression.

Introduction

Organisms naturally move across geographical regions; they may migrate seasonally or disperse over their lifetimes. However, their distribution is limited by the suite of environmental conditions to which they are adapted (Gaston 1991), which basically constitutes the species' ecological niche (Sexton *et al.* 2009). Understand the factors that determine a species' range and how its boundaries are maintained over time are among the most challenging issues in biogeography and evolutionary biology.

Climate is a key factor in determining the suitable living environment of organisms and the shape of their distribution (Pigot *et al.* 2010). Substantial evidence suggests that the present distribution of most organisms in North America was influenced significantly by the last glaciation and its associated climate changes (Comes & Kadereit 1998, Taberlet et al. 1998, Hewitt 2000, Zheng et al. 2003, Rowe et al. 2004, Rowe et al. 2006, Taylor & Keller 2007, Centeno-Cuadros et al. 2009, Kerdelhue et al. 2009). Similarly, accelerated climatic change documented in recent decades (Stott et al. 2000) is transforming environments and triggering shifts in distribution. The effects of climate change on the geographic range of some species are already noticeable, with populations sometimes declining as their habitats shrink (e.g., Beever et al. 2003, Parmesan 2006, Stirling & Parkinson 2006, Moritz et al. 2008 Myers et al. 2009), or in other cases expanding their range (e.g., Parmesan et al. 1999, Thomas & Lennon 1999, Thomas et al. 2001, Parmesan 2006, Jannett et al. 2007, Moritz et al. 2008, Myers et al. 2009). Additionally, recent climate change is affecting the physiology and phenology of a wide variety of organisms and has caused local extinctions and promoted adaptation to novel local conditions, presumably affecting species interactions and hence community structure and composition (e.g., Brown et al. 1997, Visser et al. 1998, Brown et al. 1999, Hughes 2000, Bradshaw & Holzapfel

2001, McCarty 2001, Parmesan & Yohe 2003, Parmesan 2006, Root *et al.* 2003, Thomas *et al.* 2006, Salamin *et al.* 2010).

This study examines the genetic consequences of range expansion in the white-footed mouse, Peromyscus leucopus noveboracensis (Pln), in the Great Lakes region. This rodent species is one of the most common mammals in the northeastern United States. In northern Michigan, it is at the northern limit of its range, and in that region it has experienced a very rapid geographic expansion over the last century (Myers et al. 2009). Its range has extended into the central and eastern parts of the Upper Peninsula of Michigan (Myers et al. 2009) and northward in Wisconsin (WI) and Minnesota (Long 1996, Jannett et al. 2007). Myers and colleagues (2005) hypothesized that this expansion is related to a rapid increase in local temperatures and an observed trend toward shorter winters in the northern Great Lakes region. Indeed, according to data from the National Oceanic and Atmospheric Administration (http://www.noaa.gov), the mean winter temperature in the midwestern United States has increased by 4.2 °C (about 7.6 °F) since 1970. In addition, the day of ice breakup for the Grand Traverse Bay, in the northwestern LP, now takes place almost 50 days earlier than it did at the beginning of the 20th century, (Myers et al. 2005). This shows a remarkable advance in the 2000s, in comparison with the 11.4 days reported for this same area by Magnuson and colleagues (2000) between 1850 and 1995. In both cases, the data show clear evidence of earlier onset of spring conditions. If the northern limit of Pln's range is influenced by its tolerance to winter (Wolff 1996, Myers et al. 2005), shorter winters and earlier springs may explain the northward expansion of this species.

Little information about the phylogeography and post-glacial expansion of Pln is available, despite it being one of the most studied rodent species in North America. Only one study (Rowe *et al.* 2006) has investigated the phylogeographic patterns of this mouse, . These

authors used mtDNA sequence data to test the hypothesis that Pln and another species, the eastern chipmunk, both associated with deciduous forests, share a history of expansion into regions previously covered by the Laurentide Ice Sheet. They found support for three mutually monophyletic clades in both species, with phylogenetic topologies suggesting a biogeographic pattern that could not be explained by the contemporary landscape. These observations led the authors to suggest that modern populations arose from different glacial refugia. Moreover, the phylogeographic pattern found for these two species is congruent with that of a subset of deciduous trees, suggesting a shared pattern of association and expansion since the Last Glacial Maximum (LGM).

In this chapter, gene genealogies and population genetic methods based on coalescent theory are used to investigate the phylogeography, genetic structure, and historical demography of newly founded populations of Pln in northern WI and the UP, as well as their putative progenitor populations in the LP and WI, with the general objective of understanding the genetic consequences of historical and recent range expansion. Both demographic explosions and spatial expansion leave characteristic footprints on the genome (Cann *et al.* 1987, Rogers & Harpending 1992, Hewitt 2000, Lessa *et al.* 2003, Zheng *et al.* 2003, Rowe *et al.* 2004, Rowe *et al.* 2006, Taylor & Keller 2007, Centeno-Cuadros *et al.* 2009, Kerdelhue *et al.* 2009). For any population, the genetic consequences of contemporary range expansions are strongly affected by the evolutionary history previous to the expansion, because past population processes determine the phylogenetic and genetic diversity of the colonizers (Taylor & Keller 2007). Additionally, contemporary events affecting the number and diversity of sources of emigrants contributing to the newly established populations, e.g., whether dispersers come from a single or multiple populations (Slatkin 1977), will also impact their genetic make-up. Consequently, knowledge of

both the phylogeographic and genetic pattern of post-Pleistocene expansion, as well as the life history and population dynamics of Pln are crucial for understanding the genetic consequences of current range expansion.

We evaluate the following three hypotheses regarding the genetic diversity, differentiation, and connectivity of Pln populations in the LP, UP and WI: (1) mouse populations came to occupy these regions after the retreat of the Laurentide ice sheet and the concomitant expansion of deciduous forests; therefore, a genomic signature of range expansion should be evident in all populations, characterized by high haplotype diversity, low nucleotide diversity, and unimodal mismatch distributions; (2) because WI and the UP are geographically continuous, mouse populations in those regions are likely exchanging genes and should have relatively high genetic similarity; and (3) LP lineages will be equally differentiated from WI and UP populations, because the Great Lakes should be an effective geographical barrier.

Materials and Methods

Sampling

Five-hundred and eighty-four tissue samples were obtained from mice collected in 39 counties in Michigan and Wisconsin, encompassing the current range of Pln including newly invaded localities (Fig. II.1, Table II.1). Multiple trapping sites within 25 km of each other and in the same general environment were considered to represent a single population, and the geographical midpoint of these combined sites was determined (http://www.geomidpoint.com) and used to calculate geographic distances among populations. In the text that follows, each population is given the name of the county of origin, and LP (140 samples from 9 counties), UP (166 samples from 9 counties), and WI (278 samples from 21 counties) are referred to as "regions."
Collecting sites were in forested areas above 44° North in both Michigan and WI and were chosen based on previous knowledge of the local mouse populations, type of vegetation, and accessibility. Roughly 200,000 km² was covered by this sample design. Adjacent populations were separated by 50 km on average. Additionally, Pln tissue samples from Butler Co., Ohio (2); Johnson Co., Illinois (5); and Livingston Co., Michigan (4) were used to represent potential southern refuge sources. Three samples of *Peromyscus maniculatus bardii* (Pmb) from the LP (1), WI (1), and OH (1), and 14 *P. m. gracilis* (Pmg) from the LP (4), UP (5), and WI (5) were used as outgroups in these analyses.

The majority of samples (389) were from mice that were live-trapped between 2003 and 2007, using both small (5 x 6.4 x 16.5 cm) and large (7.6 x 8.9 x 22.9 cm) Sherman folding traps. Captured mice were weighed to the nearest 0.1 g and the following information was recorded: length of right ear and hind foot (in mm), age class (juvenile, sub-adult, adult), and reproductive status (descended testicles for males; pregnancy and visible nipples for females). A piece of tissue was clipped from the right ear and placed in a 1.6 ml centrifuge tube with SET buffer (Brinster et al. 1985). Tissues were stored in a cooler while in the field and later moved to a -20° C laboratory freezer. All field-captured mice were released at the site of capture. Additional samples (195) were obtained from tissue collections of the Michigan State University Museum, the University of Michigan Museum of Zoology, and the Museum of Natural History of the University of Wisconsin at Stevens Point, or from frozen carcasses provided by the Wisconsin Department of Natural Resources. Animals were handled according Michigan State University Institutional Animal Care and Use Committee protocol # 08/04-108-00, in compliance with the American Society of Mammalogists guidelines for the use of wild mammals in research (Gannon et al. 2007).

Laboratory Procedures

DNA was extracted from mouse ear tissue using Qiagen DNeasy Tissue Kits (Qiagen Inc.), following the manufacturer's protocol. The complete D-loop and some nucleotides of the adjacent tRNAs were amplified using universal primers, L15926 5'

TACACTGGTCTTGTAAACC 3' and H00651 5' AAGGCTAGGACCAAACCT 3', located on the Pro and Phe tRNAs, respectively (Kocher et al. 1989). Polymerase chain reactions (PCR) were carried out in a volume of 25 µl, containing 1.75 mM of MgCl, 0.2 mM of each dNTP, 1 µM of each primer, 1 U of Platinum Taq (Invitrogen Co.), and 50-100 ng of DNA template, on an Eppendorf Mastercycle thermocycler (Eppendorf Co.). After 2 min of denaturation at 95° C, 35 cycles were run under the following conditions: denaturation at 95° C for 45 s, annealing at 56° C for 60 s, and expansion at 72° C for 75 s, were run. DNA products were checked on a 1.5% agarose gel (Invitrogen Co.) and cleaned using QIAquick Gel Extraction Kits (Qiagen Inc.) in preparation for direct automated sequencing. DNA was sequenced at the University of Michigan DNA Sequencing Core, in an Applied Biosystems DNA Sequencer, Model 3730 XL (Applied Biosystems Inc.). The same primers as used in PCR reactions were used for sequencing; however, reverse sequencing was incomplete because the reaction terminated at approximate 200 bp. This phenomenon was also reported by Rowe and collaborators (2006), while sequencing the control region of *Peromyscus leucopus* and *P. maniculatus*. According to these authors, sequencing from the Phe tRNA stopped after 180 bp due to a palindromic sequence (GGGGGGAAGGGGGG) interfering with the reaction. Sequences obtained were between 1000 and 1050 bp in length. A representative of each haplotype has been submitted to GenBank (accession numbers GU810187- GU810356).

Gene Genealogy

Sequences were visually checked and edited using Chromas Pro Version 1.49 (Technelysium Pty Ltd.), aligned using ClustalX (Thompson *et al.* 1997), and reduced to haplotypes using Collapse 2.1 (http://darwin.uvigo.es). A gene genealogy, based on haplotypes only, was estimated through a Neighbor Joining analysis (implemented by Mega 4,Tamura *et al.* 2007), with gaps and missing data completely excluded from the analysis. Pairwise distances were estimated by the Maximum Composite Likelihood method (Tamura *et al.* 2004). The model of nucleotide substitution used by Mega 4 with this method is that of Tamura & Nei (1993). Support for each node was evaluated with 1000 bootstraps repetitions (Felsenstein 1985).

Gene Diversity and Population Structure

Genetic diversity was measured at both nucleotide and haplotype levels. The most common measure of nucleotide diversity is \prod (Nei 1987), which is calculated as $\sum_{ij} x_i x_j \pi_{ij}$ where x_i and x_j are the frequencies of the *i*th and *j*th DNA sequences in the sample and π_{ij} is the proportion of nucleotide differences between them. For haplotypes, *H* is the most appropriate measure of genetic diversity. This parameter is defined in terms of haplotype frequencies; equivalent to the expected heterozygosity for diploid data, it measures the probability that two randomly chosen haplotypes in the sample are different. It is estimated as

$$H = \frac{n}{n-1} \left(1 - \sum_{i=1}^{k} p_i^2 \right)$$

where *n* is the number of gene copies, *k* is the number of haplotypes, and p_i is the frequency of

the *i*th haplotype in the sample (Nei 1987). Both \prod and *H* were estimated using Arlequin 3.11 (Excoffier *et al.* 2005).

Spatial population structure and potential barriers to gene flow were investigated using Spatial Analysis of Molecular Variance (SAMOVA) v.1 (Dupanloup *et al.* 2002). This program performs a simulated annealing procedure that assigns populations to *K* groups defined *a priori*. The composition of these groups takes into account the geographic proximity and genetic homogeneity of populations, so that the proportion of total variance due to differences between groups (F_{ct}) is maximized. Spatial population structure was evaluated for K = 2, 3, and 4 groups, with 100 starting conditions; statistical significance was assessed with 1000 permutations.

Population genetic structure among groups obtained with SAMOVA was inferred by analysis of molecular variance (AMOVA, Excoffier *et al.* 1992). AMOVA was performed using Arlequin 3.11 (Excoffier *et al.* 2005). The significance of the estimations was assessed using 1000 permutations. Because the power of statistical inferences depends on sample size, only samples with 8 individuals or more were included in the analyses unless otherwise noted.

Migration and Divergence Time

Migration rates and the timing of population splits were estimated for the two groups obtained from SAMOVA, using the isolation with migration model, as implemented by the IMa program (Hey & Nielsen 2007). The full model has six parameters: the population mutation rates of the descendent (θ_1 , θ_2) and ancestral populations (θ_a); the number of migrants/1000 generations entering population 1 from population 2 (*m*1), and the number of migrants/1000 generations entering population 2 from population 1 (*m*2); and time since divergence (*t*). The program estimates the posterior probability distributions for each parameter through MCMC simulations

of gene genealogies. Simulations were run assuming the HKY mutation model (Hasegawa *et al.* 1985). Several preliminary simulations were run to find appropriate priors for each parameter. Once priors that produced bell-shaped curves with low dispersion of points were found for each parameter, 1×10^6 cycles for the burn-in time and 10×10^6 cycles for the Markov chain were run. The burn-in time is the number of initial cycles that were discarded; burn-in provides a way to "dememorize" the process and thus avoid dependence on initial conditions. To assess consistency of the parameters estimated across runs, each simulation was repeated 3 times, each time using a different random seed and on a different machine. The simulation that yielded the highest effective sample size was chosen for further analysis. Additionally, nested models were examined and compared to the full model using a likelihood ratio test (2LLR) to identify the simplest model that fit the data.

The estimate of model parameters is given by the mode of their posterior probability distributions, and those estimates can be converted to demographical parameters. The effective population size of females, $N_{ef^{+}}$ can be obtained from the equation $N_{ef} = \theta/\text{ug}$, where u is the mutation rate per year per locus and g is the number of years per generation; the migration rate between populations per generation is equal to m = mu; and the divergence time, in generations, is t = t/u (Nielsen & Wakeley 2001, Hey & Nielsen 2004, 2007). The mutation rate was calculated based on the genetic divergence observed between Pln and Pmg in mtDNA D-loop sequences, assuming a molecular clock. These two species appeared in the fossil record about 500,000 years before present (Hibbard 1968). Haplotypes for 43 Pln and 37 Pm were compared; of the 913 aligned sites (after excluding gaps and missing data), there were 82 substitutions (~13%), which yields a mutation rate, μ , of 1.588*10⁻⁷ mutations/year/site/lineage. This estimate is roughly the same as the one obtained by Rowe *et al.* (2006) and Taylor & Hoffman (2010) for

the same mitochondrial region, but using shorter sequences. However, any estimation based on this rate have to be interpreted with caution because it is uncertain the actual date when Pl and Pm diverged. The mutation rate for the whole D-loop was obtained by multiplying μ by the total length of the sequence (934 bp), which yielded a rate of 1.2202*10⁻⁴ mutations/year/locus/lineage. Obtaining a precise estimate of the generation length for this species is very challenging, because reproductive rates vary depending on when females are born during the season, which is also influenced by geography, and because of overlapping generations. In this study, 2 generations/year was assumed (Miller 1989, Rowe *et al.* 2006, Centeno-Cuadros *et al.* 2009, Taylor & Hoffman 2010).

Isolation by Distance

To investigate whether the pattern of genetic structure observed in Pln might be explained by limited dispersal and isolation by distance, a Mantel test was performed to assess the correlation between genetic and geographic distances. Pairwise geographical distances between the geographical midpoints of each pair of populations were calculated as the shortest distance over the earth's surface. For the matrix of genetic distances, pairwise Φ_{st} were obtained with Arlequin 3.11 (Excoffier *et al.* 2005). For the correlation, linearized Φ_{st} (i.e. Φ_{st} /(1- Φ_{st}) and the log of genetic distance were used (Rousset 1997). The significance of the correlation was evaluated using 1000 permutations. All calculations for this test, and obtaining values for the geographic distance matrix, were done with Matlab R2006a (The MathWorks, Inc.). This analysis was carried out for the whole sample and for each group obtained with SAMOVA.

Demographical and Spatial Expansions

Sudden (demographical) expansion (Rogers & Harpending 1992) was modeled using a method implemented by Arlequin 3.01 (Excoffier et al. 2005), based on the distribution of the frequency of mistmatches among all sequences. The demographical parameters estimated are τ , the time to expansion in generations, and θ_0 and θ_1 , the neutral population mutation rates before and after the expansion. Given that $\tau = 2ut$, where u is the neutral mutation rate per locus per year (Sherry *et al.* 1994), $\theta = 2\mu N_0$ and $\theta = 2\mu N_1$, the time to the expansion (in generations) and change in population size were calculated. To convert t to YBP, t was multiplied by the number of years in one generation (i.e., 0.5). Confidence intervals for these parameters were calculated using a parametric bootstrap that compares the observed mismatch distribution to a simulated distribution under the sudden expansion model. The sum of square differences (SSD) between the observed and the simulated mismatches was used to calculate a P-value, which served as the test statistic. This P-value takes on large values if the observed distribution fits a model of sudden expansion, and small values if the data correspond to a stationary population. The Harpending's raggedness index, r, was also calculated; this index takes on large values in stationary populations and small values in populations that have undergone demographical expansion.

Spatial expansion was examined using a continent-island model implemented in Arlequin 3.01 (Excoffier *et al.* 2005), where the island exchanges *m* migrants with a continent that has a population of infinite size. Three parameters were estimated: τ , θ (= $\theta_0 = \theta_1$), and *M* (= 2*Nm*), from the distribution of the frequency of mistmatches among all sequences. The statistical test and the fit of the observed mismatch to a model of spatial expansion were performed following the same procedure as for the model of sudden expansion described above. The mismatch

distributions for the whole sample, and the groups identified by SAMOVA, were obtained and compared to models of sudden and spatial expansion.

In addition, Fu's Fs values (Fu 1997) for each group were calculated using DnaSp 5.1 (Librado & Rozas 2009). Fs takes on negative values when there are many new mutations (i.e. rare alleles), which is usually the case in expanding populations; large negative values of Fs indicate departure from neutrality and therefore is an indicative of demographic expansion.

Results

Sequencing and Gene Genealogy

Aligning, editing, and fitting all sequences to the same length resulted in a 934 bp segment that included the complete D-loop and a few nucleotides from each of the flanking tRNAs. The 595 total sequences included 170 unique haplotypes. The majority of haplotypes were found in 1-3 copies, but a handful were present in more than 10 and the maximum number of copies observed for a haplotype was 38. The LP and WI regions shared no haplotypes, the UP and WI shared 16, and the LP and UP shared 4. The 4 haplotypes shared between LP and UP were apparently of LP origin: Mackinac (UP) shared one haplotype with Alpena, Cheboygan, Presque Isle, and Crawford counties (all in the LP); Chippewa (UP) shared one haplotype with Cheboygan Co. and one with Alpena Co. (both in the LP); and two haplotypes from Ontonagon Co. (UP) were also found in Otsego Co. (LP).

A Neighbor Joining tree based on 187 sequences (i.e., the total number of observed Pln haplotypes) plus 17 Pm (outgroup) sequences (GenBank accession numbers GU810357-GU810373), revealed two well-supported (>90% bootstrap) clades: Pln and Pm (Fig. II.2). The Pln clade has two main sub-branches, one clustering the haplotypes from the Western UP (WUP)

and WI (72% bootstrap) and the other grouping the haplotypes from the LP and Eastern UP (EUP, 40% bootstrap). With the exception of those from two counties in the eastern UP (Chippewa and Mackinac), mice from the UP clustered with those from WI. Chippewa and Mackinac mice were genetically more similar to LP than WI mice, with only 2 haplotypes from Chippewa and 1 from Mackinac found in the WUP-WI clade ("+" in Fig. II.2). While most other haplotypes were in the expected clade based on geography, there were a few exceptions ("*" in Fig. II.2): one haplotype from the LP (Benzie Co.) clustered with the WUP-WI haplotypes, and one haplotype from WI (Jackson Co.) and another from the UP (Ontonagon Co.) were found in the LP-EUP clade. All of the haplotypes from Illinois and Ohio were contained in the LP-EUP clades, the internal clades are shallow, with very short branches suggesting few nucleotide differences.

Gene Diversity and Differentiation

Haplotype diversity across populations was relatively high, between 0.69 and 0.96, while nucleotide diversity was rather low, from 0.04 to 0.18 (Table II.1). Marathon Co., in central WI, showed the highest haplotype diversity, while Mackinac Co., in Michigan's eastern UP, had the lowest. The highest and lowest nucleotide diversities were found in the eastern UP (Chippewa and Delta Counties, respectively). Regional differences for both parameters were small, with the highest haplotype diversity observed in WI and the lowest haplotype diversity in Michigan's UP.

The SAMOVA separates the Pln populations into 2 groups (Table II.2), in agreement with the results of the gene genealogy. This analysis suggests that the populations from WUP and WI make up one group and LP and EUP populations define a second. Increasing the value of *K* creates groups each comprising only one population, while the variance due to inter-group

differences (Φ ct) does not change, thus not adding information. Differences among all populations (Φ st) explain over 70% of the genetic variance observed, while differences between the two groups defined by SAMOVA account for over 65% of this variance; differentiation among populations within groups is moderate (Φ sc = 14%).

Migration and Divergence Time

The three simulations that were run after appropriate priors had been identified produced very similar results. For each parameter of the model, the posterior probability distributions were smooth and bell-shaped. The results suggest that the population that gave rise to those currently in the LP-EUP and WUP-WI split in the late Pleistocene, about 34,000 YBP (Table II.3). The descendent populations probably experienced demographical expansion, since their female effective population sizes are larger than the one estimated for the ancestral population. Current migration between the two descendent populations is near zero and historical migration between them has been asymmetrical. The comparison of nested models to the full-parameter model was run twice. In one of the runs, only one model was accepted (Table II.4), the model in which the female effective population sizes of the ancestral population and LP-EUP were the same. However, the value of 2LLR was very close to the X^2 critical value for 1 degree of freedom and $\alpha = 0.05$. In an independent run, none of the nested models was accepted, suggesting that the full-parameter model best explains the data.

Isolation by Distance

The Mantel test indicated a high and significant positive correlation between genetic and geographic distance among all populations (r = 0.41, P < 0.001). Because this test can be

sensitive to extreme values and does not take into account geographic barriers like the Great Lakes, the test was performed separately for each partition obtained with SAMOVA (Fig. II.3, a and b). The positive correlation between genetic and geographical distances remained significant in each of the groups (LP-EUP: r = 0.58, P < 0.01; WUP-WI: r = 0.33, P < 0.01), even after adjusting for multiple comparisons using a Bonferroni's correction. To better visualize the relationship between genetic and geographic distances, I mapped populations and connected them with color-coded lines based on their levels of genetic similarity, i.e., colder colors for more similar genetically and warmer colors for more differentiated. Two maps were generated, one connecting populations within lineages with up to 35% of genetic differences, and another connecting populations across lineages with differences over 35% (Fig. II.4). At those levels of genetic differentiation, all WI and western UP populations are connected, and the eastern UP is connected to the entire LP; however, UP populations are the most different in both lineages. The greatest genetic distances are between populations from the LP-EUP and those from the WUP-WI, with the exception of one locality in the eastern UP (i.e. Chippewa), which stands out as the least different from populations in WUP-WI group.

Demographical and Spatial Expansions

The analysis of pairwise differences (mismatch distributions) showed that for the two groups of Pln defined by SAMOVA, and for all populations pooled together, the data fit a model of spatial expansion (Table II.5, Figs. II.5 a, c, and e). However, the distribution of LP-EUP is ragged (Fig. II.5a), and the distribution of all populations considered at the same time is bi-modal (Fig. II.5e). It appears that LP-EUP started to expand first, as early as 26,000 YBP, followed later by WUP-WI populations, about 18,000 YBP (Table II.5). Grouping all populations suggests that the

spatial expansion process might have begun around 60,000 YBP. Estimated levels of gene flow are high within each group, with an average 13 migrants per generation in LP-EUP, almost 35 in WUP-WI, and over 50 when all populations are considered.

The comparison of the mismatch distributions to Roger and Harpending's model of sudden expansion (Rogers & Harpending 1992) is significant for the WUP-WI, and when all populations are considered together, but not for the LP-EUP (Table II.5), indicating that the latter group has not experienced recent demographical growth. The mismatch distribution of LP-EUP is ragged (Fig. II.5b), and the distribution of all data is bi-modal (Fig. II.5f). This result is not congruent with Fu's *Fs*, which is significant for the two groups and all populations, indicating a departure from neutrality, usually caused by changes in population size (Table II.5). The estimated times since demographical expansion for each group are similar to those for spatial expansion: LP-EUP about 29,000 YBP, WUP-WI roughly 19,000 YBP, and all populations approximately 45,000 YBP.

Discussion

This study used variation in mtDNA control region sequences to investigate the phylogeography, genetic structure, and historical demography of populations of Pln in the northern Great Lakes region. The results showed that populations of Pln sort into two well-differentiated groups, one comprising populations from the WUP-WI, and the other populations from the LP-EUP. This grouping was based on genetic similarity and was concordant with geographic proximity. The two groups appeared to be reciprocally monophyletic, and in both, a signature of spatial expansion was identified. However, demographical expansion is apparent only for WUP-WI. The two groups diverged from each other about 34,000 YBP, and shortly afterward (26,000

YBP), the LP-EUP population started to expand, followed by the WUP-WI population about 6,000 years later.

Gene genealogy

The tree topology has the characteristics typical of populations that have gone through recent demographic/spatial expansion, with most haplotypes represented either by a single or few copies, and only a small number of haplotypes widespread. This produces a shallow tree, which is more evident for the WUP-WI clade than for LP-EUP one. With the exception of a few haplotypes, these two clades appear clearly differentiated. However, because of some unresolved nodes in each clade, the bootstrap support for LP-EUP is only 40% and for WUP-WI, 72%. A few haplotypes were not found in the cluster expected given their collection locality; this might be explained by ancestral polymorphism, natural dispersal (across ice or by rafting), and/or human-mediated dispersal. Given that WI and LP have no haplotypes in common and are reciprocally monophyletic, ancestral polymorphism is an unlikely explanation for most of these exceptional haplotypes. Natural dispersal, although possible, is also unlikely because it would require that mice cross Lake Michigan. Instead, human-mediated dispersal is the most plausible explanation. The haplotype from Benzie (LP) found in the WUP-WI clade, and the haplotype from Jackson (WI) found in the LP-EUP clade, might reflect transport of mice across the lake by ferry. Two vehicle and passenger ferries cross Lake Michigan, one between Muskegon (MI) and Milwaukee (WI), and another between Ludington (MI) and Manitowoc (WI). These piers are not necessarily close to the localities were mice used in this study were collected, but they provide a potential mechanism for these mice to cross the lake. These mice are frequently found occupying human dwellings and even nesting in vehicles; they undoubtedly are carried by people on

occasion. Similarly, the mice of likely LP origin found in UP counties (Chippewa, Mackinac, and Ontonagon) might have crossed the Straits of Mackinac with humans via the Mackinac Bridge. The other two haplotypes found in Chippewa were most similar to haplotypes from Illinois and not found in either the LP or WI. Because it is unlikely that the similarity between those haplotypes would be caused by gene flow between Illinois and the UP, ancestral polymorphism would be a better explanation.

The gene genealogy found in this study is consistent with the findings of Rowe *et al.* (2006), who analyzed 575 partial sequences from the mtDNA D-loop of *Peromyscus leucopus* from 50 sites in the eastern United States, comprising almost the entire distribution of the species. Those authors found 3 clades: a western clade, grouping haplotypes from Wisconsin, Minnesota, Illinois, Indiana, Iowa, and Missouri; a central clade, consisting of haplotypes from southern Illinois, southern Indiana, Kentucky, Ohio, Tennessee, and Pennsylvania; and an eastern clade, with haplotypes from Michigan, southern Illinois, Indiana, Ohio, Tennessee, West Virginia, North Carolina, Pennsylvania, New York, Rhode Island, Massachusetts, New Jersey, Nova Scotia, Maine, and New Hampshire. Although their sampling did not include extensive representation of northern WI and northern Michigan, the WUP-WI lineage found in this study fell in their western clade, and the LP-EUP clade in their eastern clade.

Gene Diversity and Differentiation

Further evidence of a recent demographic/spatial expansion is provided by gene diversity indices. The high haplotype diversity and low nucleotide diversity are the result of a high frequency of derived haplotypes differentiated by only a few mutations. Spatial and demographical expansion are usually accompanied by an initial reduction in haplotype diversity,

and the consequent loss of information about deep coalescent events, due to a reduction in population size associated with bottlenecks or founder effects (Excoffier *et al.* 2009).

The SAMOVA yielded two interesting results: (1) Lake Michigan is (not surprisingly) an effective barrier to gene flow between the LP and WI; and (2) the newly founded mouse populations in the UP have two different origins, in the LP and in WI. Genetic differences between mice from these two regions explain more than 65% of the total genetic variation observed. The partition supported by this analysis is consistent with the LP-WUP and WUP-WI clades found in the gene genealogy.

Isolation by Distance

White-footed mice have a home range of about 1,000 m² (Burt 1940) and may disperse up to 1km in an unfragmented landscape (Krohne *et al.* 1984); although Maier (2002) reported exceptional long-distance movements of two female in New England, which were recaptured at almost 15 and 8 km from the original capture site. Given these short dispersion distances, it is not surprising that populations of these mice display a pattern of isolation by distance. Moreover, it has been shown that dispersal in this species is male biased (Jacquot and Vessey 1995), and because genetic distances were estimated from polymorphism in mtDNA, which is maternally inherited, this pattern reflects female philopatry. Although significant for both regions, the pattern of isolation by distance was more evident for LP-EUP. The largest genetic distance was between LP and WI (Fig. II.4), supporting the idea that Lake Michigan has been an effective barrier for preventing gene flow.

Spatial and Demographical Expansion

Depending on the levels of historical and current gene flow among subpopulations, both demographical and spatial expansions can have similar genetic consequences, characterized by a smooth, unimodal mismatch distribution (Harpending 1994, Excoffier 2004, Excoffier *et al.* 2009). In light of this, goodness of fit of models of sudden (pure demographical) expansion and stepwise (spatial) expansion were fitted to the data. As expected, both regions showed a significant signature of spatial expansion. Independently of where populations survived during the glacial maximum, they moved northward after the ice sheet started to retreat. In both regions, gene flow among populations is high; the estimated number of migrants/generation is higher for WUP-WI than for LP-EUP, probably reflecting differences in the landscape permeability of these two regions. Habitat fragmentation does not limit the dispersal capability of Pln (Mossman and Waser 2001), but urban areas do, as reported by Munshi-South and Kharchenko (2010) in New York City, where populations of this mouse were fragmented and genetically differentiated.

Range expansion has occurred repeatedly in the history of terrestrial organisms (Excoffier *et al.* 2009) and is perhaps the most remarkable feature of the Quaternary Period (Hewitt 2000). I found evidence of Pln range expansion from different sources. As mentioned above, the shallow depth of the gene genealogy and the characteristics of the gene diversity indices are typical of populations that have undergone recent range expansion (Hewitt 2000, Taylor & Keller 2007). Moreover, the analysis of pairwise differences suggests a major role of post-glacial range expansions in generating the pattern of polymorphism observed in sequences of the mtDNA control region of mice in these populations. This genetic signature of post-glacial expansion is a common trait of many organisms in the northern hemisphere (Lessa *et al.* 2003). Its effect was so pervasive here that when all samples were analyzed together, it was not possible to distinguish the effects of contemporary expansions, presumably because populations have not

yet recovered from the loss of genetic structure caused by post-Pleistocene range expansion (Pinho *et al.* 2007, Taylor & Keller 2007, Kerdelhue *et al.* 2009). Qualitative evidence of contemporary range expansion is only apparent when the pattern of isolation by distance is examined. Several populations in WUP-WI (Fig. II.4), share genetically similar haplotypes while they are geographically distant from one another, which suggests movement of those haplotypes as populations expanded.

During spatial expansion, population size is usually reduced due to founder effects; if the population is successfully established, population size should increase and a demographical expansion footprint should be evident in the genome. Contrary to this expectation, a model of demographical expansion did not fit the LP-EUP data, which displayed a ragged mismatch distribution (Fig. 5a). Additionally, the only nested model tested with IMa that fit the data significantly (in only one of the two simulations carried out) was the model in which θ_a (ancestral population mutation rate) and θ_1 (LP-EUP population mutation rate) were equal (Table II.4), also suggesting a stationary population. A plausible explanation for this incongruent pattern could be that the signature of geographical expansion has been obscured by admixture. The pattern of mismatch distribution found here shows a number of pairwise differences > 20and in low frequency (Fig. II.5b), indicative of an old expansion (Rogers & Harpending 1992). Rowe et al. (2004) found a similar pattern for the eastern chipmunk, Tamias striatus, and argued that this is the signature of two independent expansion episodes. Evidence that admixture can erase (or overwhelm) the signature of demographical expansion is found in the North American populations of the invasive plant *Silene latifolia* (Taylor & Keller 2007), which does not show a pattern of demographical expansion despite the fact that its ancestors did experience post-glacial expansion. This weed is original from Europe and was introduced into North America around

200 YBP. While European populations show a clear footprint of post-glacial demographical expansion, North America populations display very low genetic diversity and evidence of admixture caused by introductions from two differentiated lineages, a history that is reflected in the bimodal mismatch distribution of haplotypes from this region.

Similarly, LP-EUP mice should have experienced a demographical expansion, as suggested by the significantly negative Fu's *Fs*, but the genetic signature of that expansion might have been erased by at least two waves of expansion in this region. The agreement of the estimated time ranges of expansion for the study populations (26,000 to 14,000 YBP for LP-EUP, and 18,000 to 9,000 YBP for WUP-WI) with the actual date that the Laurentide ice sheet began to retreat after the LGM is striking. Southern Michigan was ice free about 14,800 YPB, while WI and northern Michigan were ice free around 10,000 YBP (Pielou 1991). Moreover, these expansion times fall within the range obtained by Rowe *et al.* (2006) for the expansion of Pln and *Tamias striatus*, and by Taylor & Hoffman (2010) for Pmg, from this same region. Although these results should be interpreted with caution, because they are affected by the estimated mutation rate and the assumed generation time, such consistency among three different species strongly and coherence with known geological events suggest a common demographical history for small terrestrial mammals in the region.

Differentiation between LP and WI, and Origin of UP Populations

These data suggest that the LP and WI populations began to diverge several thousand years before the formation of Lake Michigan, about 45,000 YBP (Table II.3). At that time, they may have occupied a common refugium or several different refugia south of the glaciated region (Rowe *et al.* 2006); subsequently, as they expanded to newly open northern lands, populations

would have become isolated by cooling cycles. Later, the formation of Lake Michigan would have reinforced this isolation. The estimation of demographical parameters from a divergence with migration model implemented by IMa suggests that gene flow between the LP-EUP and WUP-WI is extremely low (Table II.3). Mice from these two regions are now genetically distinct, and thus the hypothesis that Lake Michigan has been an effective barrier to gene flow between these two regions is well supported. However, this Lake has been a permeable barrier at its northern end, where there is evidence of gene flow between populations of Pmg in the UP, northern LP and Beaver islands (an island group in Lake Michigan; Taylor & Hoffman 2010). Taylor & Hoffman (2010) found a similar date (59,000 YBP) for the divergence between eastern and western clades of Pmg in this same region, suggesting a common history of expansion.

I hypothesized that recently established populations in the UP originated in WI, but results showed that there have been two expansion pathways: one west to east, from WI to the WUP, and another south to north from the LP to the EUP. A similar pair of pathways was found for UP populations of Pmg, except that for that (boreal) species, the source of EUP populations is to the north in Canada (Taylor & Hoffman 2010). In addition, Pmg is an old resident of the UP that has inhabited this region for thousands of years, while Pln is a recent invader that has been found over most of this area only over the last two decades (Burt 1957; Myers *et al.* 2009). This is an intriguing pattern of faunal sorting, and I wonder if other species show a similar pattern of distribution. Michigan's Upper Peninsula is known to have a very complex climate, with conditions changing dramatically from shore to inland and from the mainland to the peninsula. The WUP has a strongly continental climate, with very cold winters and vegetation characterized by mostly northern hardwoods (Albert 1995). The EUP climate is greatly influenced by the Great Lakes, with warmer winters and cooler summers relative to other areas at the same latitude in the

WUP and WI, and with vegetation characterized by northern hardwoods, pine forests, hardwoodconifer and conifer swamps (Anderson 1986). Such differences in climate and vegetation surely should play a key role as selective agents on the distribution of organisms in the UP.

Evolutionary Implications and Future Directions

This study suggests different expansion histories for Pln in the LP-EUP vs. WUP-WI. It is likely that the populations that ultimately gave rise to these two clades not only survived in different refugia during the last episode of glaciation, but also that the WUP-WI populations originated from a single source (as suggested by the unimodal mismatch distribution), while the LP-EUP populations were likely founded by mice from more than one source (as shown by the ragged mismatch distribution). Because the sampling here did not include populations from the southern limit of the range of Pln, and because the expansion process has likely resulted in the loss of deep coalescence events, it is not clear where the LP populations originated. The WI population, however, may have arisen from animals that survived the last glaciation in a more northern refugium, as suggested by Rowe et al. (2004, 2006) for T. striatus and Pln currently found in WI and northern Illinois. Alternatively, if WI populations originated in some southern refugium, the observed pattern could have been caused by the loss of all ancestral haplotypes, either because of a selective sweep on the mtDNA or as a consequence of the expansion process. The fact that two different studies show a similar genetic pattern for two different species (T. striatus and Pln) in the same region, suggests a common demographical history that affected their genomes in comparable ways. This alternative hypothesis can be assessed by broadly sampling Pln populations from unglaciated locations to identify possible refugial origins. Additionally, the

examination of other molecular markers would allow a test of whether a selective sweep might account for the lack of deep coalescent events in our samples.

Spatial expansion of Pln has had an impact on the genetic diversity of northern populations of this mouse. Michigan's Upper Peninsula is a potential zone of secondary contact for two differentiated lineages of Pln. Further research, with nuclear DNA, needs to be carried out to evaluate the extent of gene flow between eastern and western UP populations, and the possible phenotypic effects of admixture. Additionally, the expansion of this mouse has affected the composition of northern Great Lakes communities, where it is outcompeting Pmg (Myers *et al.* 2005). As discussed by Myers *et al.* (2009), although the consequences of this change are unpredictable, it can be anticipated that it will have a major impact given the ecological role of small mammals in these communities. Also of great concern is the fact that *Peromyscus leucopus* is a natural reservoir of *Borrelia burgdoferi*, the causative agent of Lyme disease in this region, and the expansion of Pln may influence the spread of that disease.

Tables

Table II.1. Origin of the samples analyzed in this study, where n is the sample size, h is the number of haplotypes observed, *H* is the haplotype diversity, *S* is the number of segregating sites over the complete sequence, Π is the nucleotide diversity, and Lat and Long are the decimal geographic coordinates of the geographic midpoint of each locality. NA indicates that these parameters were not calculated for those populations because of small sample size.

Michigan's Lower Peninsula (LP)										
County	Code	n	h	Н	H S II		Lat (north)	Long (west)		
Alpena	AP	17	7	0.8676 (+/- 0.0503)	24	0.009069 (+/- 0.004946)	44.9997	83.5471		
Benzie	BZ	15	9	0.9333 (+/- 0.0397)	41	0.013877 (+/- 0.007434)	44.6067	85.1494		
Charlevoix	СХ	17	7	0.8603 (+/- 0.0475)	22	0.008063 (+/- 0.004438)	45.2614	84.8037		
Cheboygan	СН	19	7	0.8480 (+/- 0.0515)	35	0.010896 (+/- 0.005825)	45.5634	84.6637		
Crawford	CR	20	8	0.8684 (+/- 0.0475)	27	0.008566 (+/- 0.004645)	44.7888	84.7340		
Emmet	EM	9	4	0.7500 (+/- 0.1121)	19	0.007540 (+/- 0.004442)	45.5431	85.0478		
Grand Traverse	GD	8	4	0.7500 (+/- 0.1391)	19	0.007988 (+/- 0.004765)	44.6448	85.4962		
Otsego	ОТ	17	12	0.9485 (+/- 0.0368)	29	0.009213 (+/- 0.005018)	45.0549	84.5688		
Presque Isle	PI	18	7	0.8562 (+/- 0.0466)	21	0.008857 (+/- 0.004820)	45.4372	84.0489		
Total LP		140	65	0.9856	56	0.010087				

M: 1: $\frac{1}{2}$ $\frac{1}{2}$ $\frac{1}{2}$ $\frac{1}{2}$

				(+/- 0.0023)	(+/- 0.005162)			
Michigan's Up	oper Pen	insula (l	UP)					
County		n	h	Н	S	П	Lat (north)	Long (west)
Alger	AL	12	8	0.9242 (+/- 0.0575)	18	0.005281 (+/- 0.003118)	46.3412	86.7884
Chippewa	CW	17	8	0.8529 (+/- 0.0663)	48	0.018187 (+/- 0.009534)	46.4320	84.7266
Delta	DE	20	7	0.7684 (+/- 0.0689)	15	0.004057 (+/- 0.002386)	46.0788	86.8345
Gogebic	GO	17	11	0.9412 (+/- 0.0364)	24	0.006490 (+/- 0.003644)	46.3437	89.3678
Mackinac	MW	20	7	0.6895 (+/- 0.1053)	37	0.010401 (+/- 0.005560)	46.0025	84.8929
Menominee	MN	20	б	0.7579 (+/- 0.0645)	13	0.004875 (+/- 0.002798)	45.1580	87.7000
Marquette	MA	20	7	0.8105 (+/- 0.0689)	18	0.005543 (+/- 0.003134)	46.8736	87.8958
Ontonagon	ON	22	10	0.8918 (+/- 0.0419)	41	0.010757 (+/- 0.005709)	46.7364	89.7394
Schoolcraft	SC	18	б	0.7582 (+/- 0.0701)	11	0.004353 (+/- 0.002552)	46.0886	86.2278
Total UP		166	71	0.9797 (+/- 0.0032)	75	0.013992 (+/- 0.007011)		
Wisconsin (W	I)							
County		n	h	Н	S	П	Lat (north)	Long (west)
Adams	AD	20	13	0.9421 (+/- 0.0340)	32	0.007680 (+/- 0.004203)	43.9012	89.8676

Table II.1. Continued

Ashland	AS	10	8	0.9556 (+/- 0.0594)	22	0.007552 (+/- 0.004388)	46.1568	90.4755
Barron	BN	18	11	0.9412 (+/- 0.0328)	27	0.007005 (+/- 0.003890)	45.6839	92.0936
Bayfield	BY	20	13	0.9421 (+/- 0.0340)	26	0.006675 (+/- 0.003700)	46.3705	91.3376
Buffalo	BF	20	12	0.9421 (+/- 0.0318)	26	0.007313 (+/- 0.004020)	44.5892	91.8022
Burnett	BU	15	9	0.8762 (+/- 0.0697)	29	0.007001 (+/- 0.003937)	46.0418	92.1906
Douglas	DG	11	6	0.8364 (+/- 0.0887)	19	0.005567 (+/- 0.003297)	46.2722	91.6982
Forest	FO	1	NA	NA	NA	NA	45.9640	88.9674
Iron	IR	13	7	0.8718 (+/- 0.0670)	19	0.004738 (+/- 0.002814)	46.3579	90.3774
Jackson	JK	20	11	0.8684 (+/- 0.0640)	44	0.008678 (+/- 0.004702)	44.2055	90.4484
Marathon	MH	20	14	0.9632 (+/- 0.0255)	29	0.007333 (+/- 0.004030)	44.8175	89.7509
Marinette	MR	18	9	0.8889 (+/- 0.0488)	19	0.006571 (+/- 0.003672)	45.5900	87.9464
Oneida	ON	11	6	0.8000 (+/- 0.1138)	16	0.005830 (+/- 0.003436)	45.8709	89.6527
Outagamie	OU	16	9	0.8583 (+/- 0.0772)	26	0.006456 (+/- 0.003643)	44.3961	88.6663
Ozaukee	OZ	10	6	0.8444 (+/- 0.1029)	12	0.004589 (+/- 0.002812)	43.4039	87.9906

Table II.1. Continued

Total WI		278/ 268*	159	0.9941 (+/- 0.0010)	110	0.007639 (+/- 0.003982)		
Wood	WO	16	8	0.8833 (+/- 0.0522)	18	0.005679 (+/- 0.003247)	44.3785	90.1090
Taylor	ТА	12	8	0.8939 (+/- 0.0777)	29	0.007995 (+/- 0.004531)	45.3127	90.6268
Shawano	SH	2	NA	NA	NA	NA	44.7173	88.5953
Rusk	RU	3	NA	NA	NA	NA	45.3686	90.5151
Price	PR	4	NA	NA	NA	NA	45.6751	90.1751
Portage	РО	18	9	0.8039 (+/- 0.0907)	29	0.007666 (+/- 0.004222)	44.5412	89.5654

Table II.1. Continued

*The number above indicates the total number of samples from the region; the number below, which omits small samples, was used for the calculation of h, H, S, and Π .

Source of Variation	Fixation Index†	Percentage of Variation§
2 groups		
Among all populations	$\Phi st = 0.70653$	70.66
Among groups	$\Phi c t = 0.65897$	65.90
Among populations within groups	$\Phi_{sc} = 0.13945$	4.76
Within populations		29.35
3 groups		
Among all populations	$\Phi st = 0.70271$	70.27
Among groups	$\Phi ct = 0.66452$	66.45
Among populations within groups	$\Phi_{sc} = 0.11383$	3.82
Within populations		29.73
4 groups		
Among all populations	$\Phi st = 0.69927$	69.93
Among groups	$\Phi_{ct} = 0.66127$	66.13
Among populations within groups	$\Phi_{sc} = 0.11219$	3.80
Within populations		30.07

Table II.2. Spatial population structure of Pln in the Northern Great Lakes, as suggested by spatial analysis of molecular variation (SAMOVA). This analysis was run assuming K = 2, 3 and 4 groups.

†All *p*-values were < 0.001

\$Note that the variance explained by differences among all populations is equal to the variance explained by differences among groups plus the variance explained by differences among populations within groups.

Table II.3. Demographic parameters estimated using the isolation with migration model implemented in IMa. θ_1 , θ_2 , and θ_a are the mutation rates of population 1 (LP-EUP), population 2 (WUP-WI), and the ancestral population, respectively; N1, N2, and N3 are the effective population sizes of females (number of individuals) of LP-EUP, WUP-WI, and the ancestral population, respectively; m1 and m2 are the migration rates per generation into population 1 from population 2, and into population 2 from population 1, respectively; and t is the time since the two groups split, in years. Conversions of model parameters to demographic parameters were done assuming 0.5 years/generation and a mutation rate of $1.2202*10^{-4}$ substitutions/ locus/year. HiPt is the value with the highest frequency (i.e. mode), and HPD90Lo and HPD90Hi are the lower and upper bounds of the estimated 90% Highest Posterior Density Interval.

Value -	Population mutation rate			Females ef	ffective pop	ulation size	migration rat	Time	
	θ1	θ2	θa	N 1	N2	Na	m1	m2	t
HiPt	87.1341	237.2285	39.508	1428194	3888354	647566	4.54525*10 ⁻⁰⁶	6.7111*10 ⁻⁰⁷	34469.76
HPD90Lo	66.5683	199.7049	20.3854	1091105	3273314	334132.1	1.7998*10 ⁻⁰⁶	1.28121*10 ⁻⁰⁷	28274.05
HPD90Hi	113.4728	282.329	79.918	1859905	4627586	1309916	9.60908*10 ⁻⁰⁶	1.92792*10 ⁻⁰⁶	45090.97

Table II.4. Comparison of nested models to the full-parameter model of isolation with
migration, using IMa. θ_1 , θ_2 , and θ_a are the mutation rates for population 1 (LP-EUP),
population 2 (WUP-WI), and the ancestral population, respectively; m1 and m2 are the migration
rates per generation into population 1 from population 2, and into population 2 from population
1, respectively.

Model	Log (P)	2LLR	d.f.
$\theta_1 \ \theta_2 \ \theta_a \ m_1 \ m_2$	-5.2036	-	-
$\theta_1 \ \theta_2 \ \theta_a \ m_1 = m_2$	-7.9641	5.521	1
$\theta_1 \ \theta_2 \ \theta_a \ m_1 \ m_2 = 0$	-9.2249	8.0425	1*
$\theta_1 \ \theta_2 \ \theta_a \ m_1 = 0 \ m_2$	-29.5687	48.7303	1*
$\theta_1 \ \theta_2 \ \theta_a \ m_1 = m_2 = 0$	-38.1349	65.8627	2*
$\theta 1 = \theta 2 \ \theta a \ m 1 \ m 2$	-16.9191	23.4311	1
$\theta 1 = \theta 2 = \theta a m 1 m 2$	-23.4555	36.5038	2
$\theta 1 = \theta 2 \ \theta a \ m 1 = m 2$	-18.0838	25.7604	2
$\theta 1 = \theta 2 \ \theta a \ m 1 = m 2 = 0$	-56.8541	103.301	3*
$\theta 1 = \theta 2 = \theta a m 1 = m 2$	-24.9274	39.4476	3
$\theta 1 = \theta 2 = \theta a m 1 = m 2 = 0$	-64.6224	118.8376	4*
$\theta 1 = \theta a \ \theta 2 \ m 1 \ m 2$	-7.0983	3.7894 §	1
$\theta_1 = \theta_a \ \theta_2 \ m_1 = m_2$	-9.9595	9.5118	2
$\theta_1 = \theta_a \ \theta_2 \ m_1 = m_2 = 0$	-40.3996	70.3921	3*
$\theta 1 \ \theta 2 = \theta a \ m 1 \ m 2$	-11.722	13.0369	1
$\theta_1 \ \theta_2 = \theta_a \ m_1 = m_2$	-14.0896	17.772	2
$\theta_1 \ \theta_2 = \theta_a \ m_1 = m_2 = 0$	-45.6183	80.8294	3*

§The only model that was not rejected; marginally significant (X^{2} 1, 0.5 = 3.841). In an independent run, this model was not accepted (2LLR = 3.9149)

*When one or more parameters are set to zero, the expected distribution of 2LLR is a mixture. In the case when only one parameter is fixed, 2LLR will distribute as a random variable that would take both the value of zero and the value of X^{2} 1 with probability 0.05 (see Hey and Nielsen 2007)

Table II.5. Estimated parameters of the Spatial and Sudden Expansion models, and results of tests of goodness of fit of observed data to each model for each group suggested by SAMOVA, and for the whole sample. *M* is the number of migrants per generation, τ is the time to expansion, in generations; $\theta 0$ and $\theta 1$ are the population mutation rates before and after expansion, respectively; *r* is Harpending's raggedness index, and P is the goodness of fit to each model.

		Spatial Expansion						Sudden Expansion					_
Regions	N	М (95% СІ)	τ (95% CI)	Estimated expansion time (YBP)	r	Р	θ0 (95% CI)	θ1 (95% CI)	τ (95% CI)	Estimated expansion time (YBP)	r	Р	Fu's Fs
LP- EUP	177	12.77 (6.45- 27.9)	10.31 (6.87- 12.93)	14,080- 26,500	0.02	0.10*	0 (0- 2.96)	37.02 (23.43- 419.76)	10.94 (6.73- 14.15)	13,797- 29,000	0.02*	0.01	-4.40*
WUP- WI	397	34.44 (21.71- 82.69)	6.27 (4.47- 8.91)	9,163- 18,262	0.01	0.77*	0.446 (0- 2.41)	57.93 (32.55- 1184.81)	7.09 (4.65- 9.57)	9,533- 19,614	0.51	0.43*	- 97.32*
All	574	52.97 (0.49- 137.31)	3.734 (1.88- 31.43)	3,852- 64,396	0.002	0.49*	13.869 (0- 31.80)	173.57 (31.10- 2824.82)	3.84 (1.86- 22.20)	3,809- 45,493	0.002	0.24*	- 76.69*

**p*-values ≤ 0.01

Figures



Figure II.1. Geographic location of *Peromyscus leucopus* populations analyzed in this study. The code for each population is as in Table II.1. LI = Livingstone, Michigan; IL = Johnson, Illinois; OH = Butler, Ohio. Black circles indicate populations in the former northern limit of the species in the region. Grey circles indicate populations in newly invaded localities.



Figure II.2. Neighbor Joining tree showing the haplotype genealogy of *Peromyscus leucopus* in Wisconsin (WI), and Michigan's Lower Peninsula (LP) and Upper Peninsula (UP). *Peromyscus maniculatus* (Pm) is the outgroup. Some internal clades have been magnified for better visualization. "+" indicate haplotypes from Chippewa County (CW) that cluster with the WUP-WI clade and "*" haplotypes that are not in the expected clade given collection locality. Bootstrap support values are given for the main nodes. Acronyms as in Table II.1; LI = Livingstone, Michigan; IL = Johnson, Illinois; OH = Butler, Ohio.



Figure II.3. Results from Mantel's tests evaluating the correlation between genetic (Φ st/1- Φ st) and geographical (km) distances between populations. a) Population comparisons within the LP-EUP group. c) Population comparisons within the WUP-WI. Correlation coefficients and *P* values are indicated in the text.



Figure II.4. Interconnectivity among populations based on pairwise genetic distances. The color of the line indicates genetic distance between population pairs, based on pairwise $\Phi_{st}/1-\Phi_{st}$ values, with dark blue indicating the most similar genetically and dark red the most different. On the left are represented populations with genetic differences up to 35%, and on the right populations with genetic differences > 35%. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.



Figure II.5. Distribution of pairwise differences of the observed data (dark grey) and the simulated data (light grey) under a model of stepwise (spatial) expansion (left) and sudden (demographic) expansion (right) for a and b (LP-EUP), c and d (WUP-WI), and e and f (all data). Results of tests of goodness of fit of the data to each model are shown in Table II.5.

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CHAPTER II

MORPHOMETRIC DIFFERENTIATION OF EXPANDING POPULATIONS OF PEROMYSCUS LEUCOPUS IN THE NORTHERN GREAT LAKES: HISTORICAL AND GENETIC FACTORS

Abstract

The white-footed mouse, *Peromyscus leucopus*, has experienced a remarkable northward range expansion in the Great Lakes region over the last decades. In this study, I investigate whether the rapid geographic expansion of populations of this species in the northern Great Lakes region has been accompanied by rapid morphological divergence, and whether the pattern of divergence suggests a neutral process or reflects the influence of non-random factors. Shape was measured and analyzed using geometric morphometric techniques for statistical inference and graphical interpretation. Significant differences were found between recently expanded and long established populations not only in the magnitude of shape variation but also in the dimensionality of variation, which suggest that the expansion process has been accompanied by rapid phenotypic change. Additionally, the pattern of shape variation is incongruent with the phylogeographic pattern observed in these populations, ruling out that changes in morphology are caused by random phenotypic drift. These phenotypic changes might have been promoted by phenotypic plasticity and/or adaptation, and provide clear evidence that although the geographic expansion of Peromyscus leucopus in the northern Great Lakes is relatively recent, the phenotypic consequences are already noticeable.

Introduction

Overview

A rapid geographical expansion of the white-footed mouse, *Peromyscus leucopus*, in the northern Great Lakes region has been taking place over the last 30 years. This expansion is particularly remarkable in Michigan's Upper Peninsula (UP), where this species was almost completely absent just a few decades ago (with the exception of populations found in the southernmost county, Menominee), and is now common throughout most of the peninsula (Myers *et al.* 2009). Phylogeographic analyses of white-footed mouse populations in the northern Great Lakes, including northern Wisconsin (WI), the UP, and Michigan's northern Lower Peninsula (LP), show a clear genetic footprint of expansion and reveal an admixture of newly founded populations in the UP with western populations originating in WI and eastern populations from the LP (see Chapter II). The short time frame of this demographic expansion raises questions about the evolutionary processes that might have allowed the rapid invasion of novel environments at higher latitudes. These processes might be reflected in geographic patterns of phenotypic variation in ecologically relevant phenotypic traits, such as foraging structures (e.g., the mammalian mandible).

Rapid morphological divergence is not uncommon among populations of small mammals. For example, Pergams and colleagues (Pergams & Ashley 1999; Pergams & Lacy 2008; Pergams & Lawler 2009) found rapid morphological changes (over *circa* 100 years) in several species of rodents inhabiting both mainland and island environments. Morphological changes in island mammals, especially changes in size, are usually attributed to island effect, e.g. limited resources and reduced diversity, predation and competition (Millien 2006). In contrast, the rapid morphological changes observed in mainland mammals are more often associated with the impact of human activities and climate alterations.

Evidence of rapid morphological change has been found in expanding populations of North American red squirrels in the central United States (Goheen *et al.* 2003). These squirrels are experiencing a geographic expansion similar to that of *Peromyscus leucopus*. Since the late 1800s, they have expanded their range from central hardwoods to conifer-dominated forests. Differences in diet between animals inhabiting these habitats are reflected in cranial morphologies, which have diverged significantly in accord with changes in mandibular force.

Another example of rapid morphological change is found in house mouse populations on the Mediterranean islands of Corsica and Sardinia and the small islet of Piana, near France (Renaud & Auffray 2010). Populations on the two larger islands are thought to have arrived with the first human settlers, while the population on the islet was first documented about 200 years ago. The shape of the mandible of animals from the mainland is different from that on the islands, and mandible shape on the small islet differs from that on both the mainland and the two larger islands. The authors argue that because multiple colonizations of the larger islands would counteract the effect of drift, morphological differences between those mice and mainland mice are likely related to ecological causes, in particularly widening of the dietary niche of the island mice. In contrast, differentiation of mice on the islet might reflect founder effect and drift, a consequence of reduced population size and lack of gene flow with the adjacent island populations.

As the preeminent anatomical element of masticatory function, and given its correlation with dietary breadth (Dixon *et al.* 1997; Kantomaa & Rönning 1997), the mandible is an ideal structure for studies of evolutionary change in the phenotype. It is reasonable to suspect that the mandible would also be among the first structures to respond to novel environmental conditions, as has been recently demonstrated in avian systems, where a combination of environmentally-

induced variation and modular development have favored a rapid adaptive divergence in size and shape of the house finch beak, following expansion into novel environments (Badyaev 2009, 2010).

The mammalian mandible as an evolutionary model system

In addition to its ecological relevance, the mandible has become a popular model system for the study of the origin of phenotypic variation in general (Atchley & Hall 1991; Hall & Miyake 2000; Klingenberg et al. 2003), which is likely a reflection of the relative simplicity of its largely modular early development (Hall & Miyake 2000) and its amenability to theoretical and experimental functional research (Herring 1993). To understand the link between the modular developmental origin of the mandible and its evolutionary potential, Atchley & Hall (1991) proposed a quantitative genetic model that describes the sources of variation in the mammalian mandible. In that model, selection acts on variation caused by genetic and epigenetic factors, and maternal and environmental effects refine and reshape the different regions of the mandible during ontogeny, producing a functionally integrated structure that is not only adapted to the organism's immediate environment, but that is also partly shaped by it. In this context, the mandible is seen as a complex structure, i.e., comprising an array of functionally and developmentally integrated components. In the model's most basic form, a total of six major partitions have been postulated for the mandible (Atchley & Hall 1991; Atchley 1993; Hall & Miyake 2000), which corresponds to the mesenchymal condensations that give rise to the coronoid, condyloid, and angular processes, the ramus, and the incisor and molar alveoli in the developed dentary bone (Fig. III.1). According to this model, these basic units of the mandible may vary independently or become integrated by function and development (Zelditch et al.

2009). The mandible thus provides a theoretical framework that can take into account potential biases introduced by function and development in analyses of patterns of variation and diversification due to ecological pressures.

Because the development of the mammalian mandible has an important epigenetic component, the interpretation of mandible variation can be very challenging; plasticity, adaptation, and chance are all potential causes of phenotypic divergence. The mandible is often regarded as highly plastic, because variation in its form has a large environmental component (Hanken & Hall 1993). In mice and rats, for instance, a large proportion of the phenotypic variability observed in some traits of the adult mandible (e.g., over 70% in the mouse coronoid area, and over 50% in the rat coronoid and condyloid areas) has been shown to be caused by residual environmental variance, likely associated with diet and other ecological factors (Atchley 1993). Also, it is possible to alter the shape of the mandible experimentally by raising animals under different diets (Corruccini & Beecher 1982; Bouvier & Hylander 1984; Ulgen *et al.* 1997; Liu *et al.* 1998; Kiliaridis *et al.* 1999; Maki *et al.* 2002; Renaud *et al.* 2010), inducing functional alterations of masticatory muscles (Ghafari & Heeley 1982; Bresin & Kiliaridis 2002; Renaud *et al.* 2010), or allowing experimental subjects to develop under colder than normal temperatures (Steegman.At & Platner 1968).

At a macroevolutionary level, adaptation, especially to different diets, as suggested by the diversity of mandible shapes, has been postulated as one of the main causes for the radiation of platyrrhine primates (Anapol & Lee 1994), bats (Nogueira *et al.* 2009; Monteiro & Nogueira 2010), and rodents (Michaux *et al.* 2007; Samuels 2009). In contrast, random factors like founder effects and subsequent genetic drift due to reduced population size have also been causally linked to the emergence of large phenotypic differences in the shape of the mandible among

populations inhabiting small islands or otherwise isolated habitats (LeBoulenge *et al.* 1996; Renaud & Auffray 2010).

Even though adaptation and plasticity are often treated as alternatives to explain phenotypic differences across environments, they do not constitute mutually exclusive explanations for the origin of such divergence. On the contrary, developmental plasticity is currently broadly seen as a possible link between adaptation and evolutionary diversification (West-Eberhard 2003; Angers *et al.* 2010; Pfennig *et al.* 2010; Young & Badyaev 2010). Thus, adaptation of organisms to new habitats, especially in fluctuating environments, might be facilitated by the presence of highly plastic responses to changing conditions (Lande 2009; Canale & Henry 2010), much in the same way as it is facilitated by greater standing genetic variation.

The idea that plasticity plays an important role in diversification has found some opponents, who argue that although there is evidence for developmental plasticity, its central role in the evolution of phenotypic novelty lacks support (de Jong & Crozier 2003). Developmental plasticity has also been misinterpreted by some as neo-Lamarckism, as pointed out by Pfennig and collaborators (2010). Currently, several epigenetic mechanisms are known to regulate gene expression (Angers *et al.* 2010; Katsnelson 2010; Pfennig *et al.* 2010), and a role of phenotypic plasticity in morphological divergence has been suggested for house finches (Badyaev 2009) and shrews (Young & Badyaev 2010).

This study examines the pattern of variation in size and shape of the mandible of *Peromyscus leucopus* in relation to lineage divergence and geographical range expansion. The central aim is to address whether the rapid geographic expansion of populations of this species in the northern Great Lakes region has been accompanied by rapid morphological divergence, and

whether the pattern of divergence suggests a neutral process or reflects the influence of local eco-geographical factors. Shape was measured and analyzed using geometric morphometric techniques. Because shape variation is expected to reflect differences in diet, a nested MANOVA design was used to assess the potential role of non-dietary factors (i.e., history, genetics) on the morphological differentiation of these populations. In addition, a hypothesis of phenotypic drift was assessed by studying the strength of the association between matrices of morphometric and genetic distances, with the latter based on a neutral marker (the mtDNA control region). Results support the hypothesis that differences in the shape of the mandible between old and newly established populations are related to, and plausibly caused by, the expansion process. Additionally, the absence of evidence of phenotypic drift suggests that exposure to new environments might contribute to the emergence of novel phenotypes. These results further suggest that a plasticity-mediated process such as Baldwin effect and/or local adaptation might play a causal role in the geographic diversification of *Peromyscus leucopus* in the northern Great Lakes.

Materials and Methods

Samples

Mandibles were sampled from 24 of the 39 populations (Fig. III.2) included in the genetic analyses (Chapter II). Only mandibles from adult specimens (i.e., with the third molar erupted) and in excellent condition were included. Selection of populations was based on availability of specimens in museum collections. Populations analyzed comprised 12 localities from WI, 5 localities from the LP, and 7 localities from the UP. With few exceptions, 10 specimens were measured per locality, for a total of 227 specimens. Samples were obtained from the collections

of Michigan State University Museum, University of Michigan Museum of Zoology, and University of Wisconsin at Stevens Point Museum of Natural History (Table A.III.1).

Geographic characteristics of samples, such as region (i.e., WI, LP, UP) and geographic midpoint of collection sites, were defined using the same criteria as in previous genetic analyses (Chapter II). Samples were grouped by lineage according to the partitions obtained from SAMOVA (Chapter II), which are also the monophyletic groups observed in the haplotype genealogy (e.g., Fig. II.4 in Chapter II). Finally, sampling localities were classified as "old" or "new" with respect to the timing of establishment of their corresponding populations based on whether the population was known to be present at the site for more than 30 years, or was discovered within the last 30 years, respectively. All specimens included in this study were collected within the last 30 years.

Morphometric Data

Variation in morphological shape and size was analyzed using 2-D landmark coordinates placed on homologous sites of digital images of the mandible. For each specimen, hemi-mandibles were carefully separated prior to photography; only the lateral (buccal) surface of the right hemimandible was photographed. Hemi-mandibles were photographed with the dentary parallel to the focal plane and against a contrasting background. In those few cases where the right side was severely damaged, the left hemi-mandible was used instead. A reference ruler was included in all photographs. To minimize the effect of measurement error, all photographs were taken by the same person (B. Lundrigan), and each mandible was positioned and photographed twice; analyses were then based on the average of the landmarks extracted from the two images.

Photographs were acquired using a Fuji FinePix S5 Pro digital camera with a 70mm Sigma macro lens.

A total of 15 2-D landmarks and 5 curves (Fig. III.1) were obtained from the digital images using TPSDig2.1 (Rohlf 2006). Curves were sub-sampled to obtain 20 regularly spaced pseudo-landmarks (Bookstein 1997), using the software SemiThinner (Marquez 2006). Pseudo-landmarks were allowed to slide along their respective curves to minimize the Procrustes distance between each individual configuration and the mean (Zelditch *et al.* 2004), using the software SemiLand6 (Sheets 2009). After sliding, pseudo-landmarks were considered semilandmarks (Bookstein 1997). Semi-landmarks differ from ordinary landmarks in that their homology can only be established in relation to a smooth curve or edge, where characteristic homologous features are normally absent. For that reason, variation in the spacing of semilandmarks is arbitrary and thus minimized, resulting in semi-landmarks being constrained to vary only in the direction that is perpendicular to the margin (Zelditch *et al.* 2004).

Geometric Morphometric Methods

Shape is defined as the component of form that remains after removing information about location, scale, and orientation (Kendall 1977; Bookstein 1991; Zelditch *et al.* 2004). In landmark-based geometric morphometrics, these factors are removed by means of Generalized Least Square Procrustes superimposition (Rohlf & Slice 1990; Zelditch *et al.* 2004). In this procedure, each individual landmark (and semi-landmark) configuration is (1) translated so that it is centered at the Cartesian origin; (2) scaled so that its centroid size equals one, where centroid size is computed as the square root of the sum of square differences between each landmark and the centroid of the configuration (i.e., the average position of all its landmarks);

and (3) iteratively rotated so as to minimize the sum of squared differences between the landmarks of that specimen and those of the sample mean, which is also iteratively computed. Following Procrustes superimposition, each configuration is mapped as a single point in a multidimensional space, termed Kendall's shape space, which possesses a non-Euclidean geometry.

Following superimposition, and prior to their use in multivariate statistical analyses, shape-space data are customarily projected onto a Euclidean space that is tangent to the shape space at a predetermined reference point, which is usually the mean shape (Rohlf & Slice 1990; Zelditch *et al.* 2004). This projection can be accomplished by transforming each individual configuration of landmarks into a collection of residual values relative to the reference (i.e., mean) configuration after superimposition, so that for *k* landmarks, 2k of these Procrustes residuals are obtained (Dryden & Mardia 1998), which for most practical purposes can be treated as Euclidean shape variables. Procrustes residuals were calculated in Matlab® (The Mathworks 2006); implementations of the algorithms and equations are provided in Dryden & Mardia (1998).

The extraction of shape information from configuration data results in the loss of four degrees of freedom for 2-D landmarks: one from scaling by a scalar factor, two from translation along *x* and *y* axes, and one from rotation by a scalar angle. Additionally, each semi-landmark contributes only a single degree of freedom, since the other is lost during optimization of its position along a curve (Bookstein 1997). Consequently, the number of degrees of freedom of the set of Procrustes residuals is 2k + l - 4, where *k* and *l* denote the number of landmarks and semi-landmarks, respectively. This creates a discrepancy between the number of variables (2k + 2l) and the degrees of freedom of a data set, and results in covariance matrices of shape variables

that are not of full rank. These matrices are not usable in statistical analysis seeking to quantify the magnitude of group mean differences relative to within-group variation, because such analyses (e.g., MANOVA and CVA) require the inversion of the pooled within-group covariance (Krzanowski 2000). In morphometric applications, this discrepancy in degrees of freedom is dealt with by projecting landmark data onto a space of the correct dimensionality, such as the principal warps of the bending energy matrix (Bookstein 1991), or by using generalized inverse matrices in statistical analyses (Dryden & Mardia 1998). For this study, data were transformed by projecting them onto a space defined by the first 2k + l - 4 eigenvectors of the covariance matrix of the Procrustes residuals obtained from a superimposition of the combined set of all samples. Thus, the final number of degrees of freedom of samples used in this study, applying the formula above, was $(15 \times 2) + 20 - 4 = 46$.

To obtain a summary metric for the overall difference between the two lineages examined in this study, I used partial Procrustes distance, computed as the squared root of the minimized sum of square differences between homologous landmarks (Dryden & Mardia 1998). This distance represents a close approximation to the actual distance between each specimen and the sample mean in shape space (Zelditch *et al.* 2004).

Landmark data were also used to measure mandible size, estimated, as it is customary in geometric morphometric applications, as centroid size (CS, Dryden & Mardia 1998), defined above. Given that CS is a one-dimensional measure, statistical analyses of size variation were carried out using the univariate equivalents of the methods used to analyze shape.

Statistical Analyses

Statistical analyses were conceived as a hierarchy of geographical and genetic factors potentially able to explain the morphological differences observed between the mandibles of "old" and "new" populations. This hierarchical approach seeks to address three questions, two of which are primarily concerned with broad patterns of geographical and historical variation in mandible shape, and the other with more specific associations between genetic and morphological differentiation:

- 1. Are mandible phenotypes distinguishable between lineages, between old and new localities within lineages, and/or among populations within lineages?
- 2. Are differences in phenotypic means between old and new populations characterized by the emergence of novel directions of variation relative to the directions observed among old populations alone?
- 3. Are morphological differences among populations congruent with, and therefore explained by, their phylogeographic pattern based on a neutral genetic marker?

Prior to carrying out tests to address these questions, sexual dimorphism in shape and size was ruled out by testing for the effects of population, sex, and their interaction using MANOVA and ANOVA, respectively. Because the latter two effects were found to be non-significant (P > 0.05), males and females were pooled for subsequent statistical analyses.

Genealogical and Historical Effects

I used a nested MANOVA design to test whether there are significant differences in mean shape (multivariate dependent variable) between the LP-EUP and WUP-WI lineages, between old and new localities within lineages, and among populations within old and new localities (independent variables), corresponding to the model:

$$Y_{ijkr} = \mu + L_i + H_{ij} + P_{ijk} + \varepsilon_{ijkr}$$

where **L** denotes the effect of lineage (i.e., LP-EUP vs. WUP-WI); **H** denotes the historical effect (i.e., old vs. new populations), nested within lineage; **P** denotes the population effect, nested within lineage and historical groups; and μ and ϵ represent a mean vector and error matrix, respectively (Sokal & Rohlf 1995).

In order to apply MANOVA to test differences in shape among groups, two requirements need to be met: the measurement space must be Euclidean, and the intra- and inter-group covariance matrices must be non-singular (i.e., the number of variables included in analyses must equal the number of degrees of freedom of the variation). As described above, Procrustes superimposition and tangent space projection ensure that both of these conditions are met when using landmark data. To test the null hypothesis of no differences in mean shape among groups, I used the Hotelling-Lawley trace criterion and its associated F- and P- values, which is one of the multiple testing criteria often used in MANOVA. This statistic is computed as the sum of the eigenvalues of **BW**⁻¹, with **W** and **B** denoting the pooled within- and factor-specific betweengroup sums of squares and cross-products (SSCP) matrices, respectively. The Hotelling-Lawley trace thus estimates the total variance captured by a test factor after correcting for intra-group variation. The *F*-value is also a function of **W** and **B**, providing a measure of the magnitude of the variance of the dependent variables that is explained by the independent variables (Zelditch et al. 2004). The effects of lineage, expansion history, and population on centroid size (CS) variation were analyzed using a univariate equivalent to the nested MANOVA model used for shape data, i.e., nested ANOVA.

In addition to testing for statistical significance, the effect size of each factor of the model was estimated. Effect size for CS was estimated as the value of R^2 for each effect in the ANOVA

model. For shape, an effect size measurement analogous to R^2 was calculated using the following procedure (E. Marquez, personal communication). The least squares means corresponding to the predicted values for each treatment and factor in the nested MANOVA model were pooled, and the covariance matrix of these means was used to extract all eigenvectors with a corresponding non-zero eigenvalue. For the lineage effect, this decomposition yields one eigenvector, equivalent to the difference between the LP-EUP and WUP-WI mean vectors, whereas for the historical effect within lineage and the effect of population nested within lineage and historical groups, there are 3 and 23 non-zero eigenvectors, respectively. Next, the original shape data were projected onto these eigenvectors and the variance in each case was calculated. The estimated multivariate effect size is equal to $\rho_j^2 = \frac{\sum_i var(Xv_{ij})}{tr[cov(X)]}$, where the numerator is the accumulated variance explained by the eigenvectors v_i

of the values predicted for the j^{th} effect, and the denominator is the total shape variance, computed as the trace of their covariance matrix. The effect sizes in this analysis are not additive (i.e., they are not intended to add to 100).

Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA) were used for visual inspection of patterns of geographical and historical variation among populations. PCA finds linear combinations of the original variables that maximize the amount of information (i.e., variation) present in a homogeneous sample. In this application, PCA was used on population means to produce shape deformation grids (Bookstein 1991) depicting the main directions of mandibular diversity observed in the study. CVA uses group information to find linear combinations that optimally explain variation across means, maximizing the ratio of among- to pooled within-group variation. This is accomplished by computing the eigenvectors of the matrix $W^{-1}B$, where **B** and **W** are as defined above (Krzanowski 2000). Specimen scores on canonical axes were obtained by projecting Procrustes residuals onto these eigenvectors. CVA on shape data was used to visualize the distribution of differences among the 24 populations included in the study. These canonical scores were then grouped according to historical and geographical categories to visually assess the extent to which differences among populations are explicable in terms of lineage membership or history of population expansion.

To further investigate morphological differences between old and new populations, I addressed the question of whether variation in the dimensionality of population mean differences within each lineage is related to the timing of geographic expansion. This analysis complements results from MANOVA, which indicates whether at least one of the sampled groups differs from the rest in terms of magnitude, without considering the direction of such differences. There are two possible outcomes: (1) phenotypic differences appearing during or after geographic expansion might be accompanied by an increase in dimensionality; or (2) phenotypic differences might accumulate along the same dimensions of variation as already present among old populations. To assess these possibilities, I estimated and contrasted the dimensionality of mean population differences between two data sets, one containing only old populations and another containing both old and new populations combined. By treating old vs. (old + new) as two different blocks, I can address the question of whether new populations introduce morphological variation consistent with already existing variability, in which case no change in dimensionality would be expected, or whether the addition of new populations results in variation in new features, as indicated by the addition of new dimensions. The rationale for this comparison is that differences in dimensionality between the two blocks of populations would have to be accounted for by the new populations, wherein additional dimensions represent novel directions of

mandible variation. To estimate dimensionality, I used Bartlett's statistic (Krzanowski 2000), which tests for equality of the *q* smallest CVA eigenvalues, hence defining dimensionality as the maximum number of canonical axes that capture a distinct (i.e., non-error) proportion of the total shape variation (Anderson 1963). Bartlett's statistic is based on a likelihood ratio test and approximately follows a χ^2 distribution (Krzanowski 2000).

Nested MANOVA statistics were computed using SAS procedures. GML and IML (SAS Institute 2004), and principal components, canonical axes, and Bartlett's statistics were computed using Matlab® functions PCACOV and MANOVA1 (The Mathworks 2006).

Genetic Effects

The tests described above address geographic and historical variation in phenotypic means, asking whether differences found among populations can be explained by geographic and/or historical effects. However, even if a hypothesis invoking those effects is supported, a thorough examination of underlying patterns of genealogical associations is still necessary to fully account for possible sources of morphological variation. Because geographic, historical, and genealogical effects are not mutually exclusive, morphological similarities might merely mirror genealogical associations among populations, supporting a hypothesis of neutral divergence (Monteiro *et al.* 2005; Nogueira *et al.* 2005; Perez *et al.* 2009).

To assess the hypothesis that patterns of inter-population differentiation based on neutral genetic markers and on morphometric data are similar enough to be considered causally linked, I computed correlation coefficients between genetic and morphological distance matrices, and carried out Mantel tests to estimate the statistical significance of those correlations (Sokal & Rohlf 1995). In these tests, observed Pearson's correlation coefficients between the elements of

the two matrices are compared to a distribution of equivalent correlations computed after the rows and columns of one of the matrices have been randomly permuted. This procedure simulates the distribution of correlation values expected under the null hypothesis of no association between matrices; *P*-values for significance tests are computed as the proportion of correlations between randomized matrices that equals or exceeds the observed correlation (Dietz 1983; Manly 2007). I computed a total of 1,000 random permutations for each Mantel test; these were carried out using the CORRCOEF and RANDPERM Matlab® functions (The Mathworks 2006).

Mantel tests were based on two kinds of genetic metrics, pairwise Nei's distances (Nei's D, Nei 1987) and F_{st} estimates (Reynolds *et al.* 1983), which represent distinct but similarly informative genetic criteria. Nei's D assumes that a population is in mutation-drift equilibrium; it is calculated as $D_{Nei} = -\ln I$, where I is a measure of the identity of genes from two populations. Nei (1973) pointed out that this measure of genetic distance has some interesting properties, including that it is (a) related to the kinship coefficient, (b) linearly correlated with evolutionary time if the substitution rate is constant, and (c) linearly related to geographic distance under the assumptions of some migration models. In contrast, F_{st} can be used as an indicator of short-term genetic distance, where divergence between populations is assumed to have been caused by drift only. Assuming short divergence times, F_{st} -based pairwise distances were computed as $DF_{st} = -\log(1-F_{st}) \sim t/N$, where *t* is the number of generations since divergence and *N* is the size of haploid populations (Reynolds *et al.* 1983). Estimates of both distances were computed using Arlequin 3.11 (Excoffier *et al.* 2005), with the output formatted as distance matrices.

To estimate pairwise morphological distances between populations, I computed Euclidean distances between population means obtained from the shape variables projected onto a 46-dimensional planar tangent space as described above (Table A.III.4). These distances approximate the corresponding distances between mean shapes in shape space. A Euclidean metric was also used to compute distances among population means for centroid size.

Graphical visualization of the information in distance matrices was summarized using dendrograms created with the UPGMA method, which uses group averages to link clusters (Rencher 2002). These were produced using the Matlab® functions DENDROGRAM, LINKAGE, and COPHENET with the genetic and morphometric distance matrices described above.

Results

Effects of Genealogy and Expansion History on Morphological Variation

Mean Shape Variation Between Lineages and Among Populations

Results from the nested MANOVA on shape variables indicate that all tested factors are highly significant and have moderate to large effects (Table III.1). Effect size estimates indicate that the effect of lineage explains almost 9% of the overall variation in shape, whereas historical effect, which is nested within lineage, explains over 24% of the total shape variation. Differences among populations, which include the cumulative effect of lineage and history, explain over 85% of the differences in the shape of the mandible of *Peromyscus leucopus* in the geographical area covered by this study. The remaining 15% of the variation is not explained by any of the factors included in this nested model, and might be related to variation within populations. In contrast, the analysis based on CS indicates that none of the factors tested in the nested design have a significant effect on the pattern of mandible size variation (Table III.2).

The first four principal components computed from mean population shapes jointly explain 69% of the total variation among those means, suggesting a relatively high dimensionality of population variation, with no major trends. Plots of PC scores (Fig. III.3) suggest that the major axes of variation are unrelated to any of the factors of interest in this study; i.e. none of these axes provides unambiguous support for differentiation between the two lineages or between old and new populations.

Shape deformations implied by PCs 1 through 4 are shown in Figures 4a-d. PC1 (which accounts for 26% of the total variation) appears to be associated with contraction /expansion of the anterior margin of the coronoid process and caudoventral angular process, and indicates that these structures are highly variable with respect to mean shape. These changes are coupled with expansion /contraction of the caudal end of the masseteric ridge, which corresponds to the base of the incisor alveolus. Two UP populations belonging to the WUP-WI lineage, Marquette (MA) and Delta (DE), exhibit the most extreme shapes along this axis (Fig. III.3, top). PC2 (24%) captures a pattern of variation that simultaneously involves elevation /reduction of the condyloid process, expansion /contraction near the caudal margins of the coronoid and angular processes, displacement of the masseteric ridge, and expansion /contraction of the anterior incisor alveolus. Although the most contrasting populations along PC2 correspond to different lineages (Crawford -CR- and Schoolcraft -SC-), there is substantial overlap of the two lineages along this axis (Fig. III.3, top). Inspection of PC3 (11%) and PC4 (8%) suggest covariation between the coronoid and angular processes, and between these processes and the alveolar region, respectively (Figs. III.4 c and d), in neither case clearly separating the groups of interest (Fig. III.3, bottom).

Canonical axes are directions of maximal differentiation between population means relative to within-population variation and thus show different patterns of population distribution

than PCA axes. According to Bartlett's test, only four CVs capture a significant proportion of the shape variation among populations, and thus no additional axes were computed. These four canonical axes account for only 46% of the standardized variation among groups. In Figure III.5, CV scores are arranged by population (i.e., by county of collection) and region, making it possible to visually assess whether morphological differences among populations are structured by these biogeographical factors. The box plot of population scores on the first canonical axis (16.4% of the variation) suggests a contrast between old and new populations, with the most extreme values corresponding to newly invaded localities (e.g., Schoolcraft, Delta, Bayfield, and Chippewa). Subsequent canonical axes do not show clear patterns that can be related to the difference between old and new localities.

Visual inspection of deformation grids contrasting old and new populations shows that the patterns of morphological variation in the mandible differ between the two lineages (Fig. III.6). In the LP-EUP lineage, change from old to new involves moderate expansion of the ascending ramus, accompanied by contraction near the caudal end of the masseteric ridge and in the region of the angular process (Figs. III.6 a and III.7). In the WUP-WI lineage, change from old to new is characterized by contraction of the 1st molar alveolus and base of the coronoid process, increased curvature of the caudal margin connecting condyloid and angular processes, and expansion of the incisor alveolus (Fig. III.6b). Distances between the mean shapes of old and new populations equal 2.81×10^{-4} and 3.29×10^{-5} squared Procrustes units for the LP-EUP and WUP-WI lineages, respectively. Table III.3 shows proportional contributions of the major developmental blocks of the mandible to these distances, computed using the landmarks contained within each region (Fig. III.1). These contributions range from 10% (coronoid process) to 29% (masseteric ridge) in the LP-EUP and from 7% (incisor alveolus) to 28% (condyloid

process) in the WUP-WI, and suggest differences in the pattern of morphological divergence between these lineages. Shape change in the LP-EUP is focused primarily on the masseteric ridge and angular process, whereas in the WUP-WI, the condyloid process and 1st molar alveolus undergo the most change.

Changes in Shape Variation Dimensionality during Population Expansion

Comparison of estimates of dimensionality of shape variation in samples of means from old vs. (old + new) populations show a significant increase in dimensionality of the whole mandible only for WUP-WI. When the anterior and posterior regions of the mandible are analyzed separately, both lineages show an increase in dimensionality associated with geographical expansion ($\alpha = 0.0167$, after Bonferroni's correction; P < 0.01, for all comparisons).

In the WUP-WI lineage, Bartlett's test estimated an increase from 2 to 3 dimensions on addition of the new populations to the old ones, when the whole mandible was analyzed. Repeating this test by removing new populations one at a time does not change this result, suggesting that the gain in dimensionality is not caused by any single outlier population. In contrast, in the LP-EUP lineage Bartlett's test did not show a change in dimensionality for the whole mandible (3 for old and (old + new) populations). However, there is an evident shape change between old and new populations in the regions of the angular and coronoid processes and caudal masseteric ridge (Fig. III.6a). Separate Bartlett's tests of the posterior (angular, condyloid, and coronoid processes, and masseteric ridge) and anterior (incisor and molar alveoli) regions of the mandible found an increase of from 1 to 2 dimensions in the posterior mandible and unchanged dimensionality (=1) in the anterior mandible. Similarly, an analysis of dimensionality of anterior and posterior regions of the mandible in the WUP-WI lineage revealed

an increase of dimensionality in both partitions, with the posterior mandible displaying a more striking change (from 1 to 3 dimension) than the anterior mandible (from 1 to 2 dimensions). Together, these results support the hypothesis that geographic expansion in both lineages was accompanied by the emergence of novel directions of morphological change, a pattern involving the entire mandible in the WUP-WI lineage, but restricted to the posterior region in the LP-EUP lineage.

Genetic Effects

Correlations Between Genetic and Morphological Patterns

Mantel tests did not find a statistically significant association between genetic and morphological distances (Table III.4). Neither pairwise Nei's *D* nor pairwise *F*st's were significantly correlated with pairwise Euclidean distances between shapes (P > 0.0125), although for both genetic distance metrics, the correlation coefficients are of moderate magnitude. The incongruence between genetic and morphometric patterns is further evinced in the UPGMA dendograms based on these distance matrices (Figs. III.8-III.10). Together, these data suggest that the differences in mandible shape detected by MANOVA cannot be explained by genealogical relationships. Similarly, differentiation in mandible size (CS) is not significantly correlated with genetic distance (full dataset r = 0.0423, P = 0.5285; LP-EUP-only r = 0.0677, P = 0.4945; WUP-WI-only r = 0.1844, P = 0.2178).

Discussion

The questions addressed in this study have been organized as a hierarchy of potential explanations for observed patterns of geographic and historical variation in mandible

morphology. Thus, at a large scale, analyses focused on overall comparison of shape means among populations, whereas at a smaller scale, comparisons took into account patterns of differentiation along historical and genetic dimensions of variation, with the ultimate aim of explaining phenotypic differences between old and newly established populations. This hierarchical organization offers an innovative approach to search for possible causes of underlying patterns of morphological differentiation associated with the process of geographic expansion, by dissecting layers of information from broader to more specific potential causal factors.

The broadest level in the hierarchy addressed whether population means are different, within the framework of a nested MANOVA design. Results in this case suggest that not only are populations different overall, but there are also significant effects associated with lineage and expansion history. The effect of lineage accounts for only 9% of the shape variation observed among individuals; the effect of history, which takes into account the effect of lineage, explains over 24% of shape variation. In other words, over 24% of the shape variation among individuals is explained by membership in old or new populations that belong to the LP-EUP or to the WUP-WI lineage. Most of the shape variation explained by these factors involves the masseteric region (in the LP-EUP lineage), and condyloid process (in WUP-WI). While not the focus of this study, the largest effect on shape variation explained by this nested model was for differences among populations, which accounts for 85% of the total variation in shape among individuals. A large value is expected here, as this factor is the most inclusive source of variation in this nested MANOVA; it includes the cumulative effects of lineage and history among populations, in addition to other sources of geographical variation not examined in this study, such as local ecology.

Whereas a large population effect is expected in the absence of detailed information about local environments, the relative magnitude of the effect of lineage and expansion history within lineages suggests that the process of geographical expansion undergone by these populations in recent decades has resulted in morphological divergence comparable to that between the two lineages, which are estimated to have split *circa* 35,000 YBP (Chapter II). These results are consistent with a scenario in which abundant morphological diversification accumulated independently within each lineage, with variation accruing along multiple phenotypic dimensions until intra-lineage variation far exceeded inter-lineage divergence, a pattern that is clearly illustrated in the PCA and CVA results.

The next hierarchical level of explanation refined the previous question (whether old and new population means are different) by asking whether diversification has accrued along a single direction of divergence (as expected of a geographic or ecological cline, or where developmental biases restrict the direction of variation), or alternatively, whether the expansion process has produced novel directions of variation. Results from Bartlett's test comparing the dimensionality of shape variation between old vs. (old + new) populations indicate that the occupation of new environments during expansion has been accompanied by divergence along novel directions of variation, suggesting the possibility of adaptive processes brought about by exposure to novel selection pressures.

Graphical comparisons of mean shapes between old and new populations (Figs. III.6 and II.7) suggest that the expansion process has affected the mandible differently in the two lineages. In the LP-EUP, the angular process and masseteric region contribute the most to the divergence between old and new populations; both are points of insertion of masticatory muscles, and they jointly account for about 54% of the difference between group means. In contrast, in the WUP-

WI, the largest contribution to the divergence between old and new populations comes from the condyloid process, in the posterior mandible, and 1st molar alveolus, an anterior structure. Jointly, these regions account for about 51% of the difference between old and new populations (Table III.3, Fig. III.6).

Despite this difference in the pattern of variation between old and new populations, a common feature of both lineages is the tendency for variation to be focused on the region of insertion of masticatory muscles, which includes the posterior end of the masseteric ridge, and the coronoid, condyloid, and angular processes (Atchley et al. 1992) (Table III.3). With few exceptions, most studies have also found that mandibular processes are the most variable region of the mandible. Some studies have found that a single mandibular process bears most of the variation (Cheverud et al. 1991); in other cases, a combination of processes shows most of the variation (Klingenberg et al. 2001a; Klingenberg et al. 2001b; Renaud & Michaux 2004; Perez et al. 2009); and in other instances, all threes processes combined concentrate most of the variation (Atchley et al. 1985; Duarte et al. 2000; Monteiro et al. 2005). However, other studies have also found a pattern of variation involving the anterior region of the mandible as well. For instance, Atchley et al. (1992) found that posterior structures like the coronoid and condyloid processes and the lower edge of the angular process show relatively large additive and phenotypic variance in random-bred house mouse, but also that the incisor alveolus (an anterior structures) shows similarly large variance. A contrasting pattern was reported by Cheverud et al. (1991), who found in a comparison of 10 inbred mouse strains that most of the variation in the mandible was restricted to the anterior mandible, and to a lesser extent, the coronoid process.

Several other studies have also reported that variation of the mandibular processes contributes significantly to shape variation associated with geographic differentiation. Duarte *et*

al. (2000) found that differences between spiny rats (*Thrichomys apereoides*) from Palmeiras and other populations are largely restricted to the coronoid and angular processes, and the alveoli. Monteiro *et al.* (2005) expanded the number of localities, skull views, and ecological variables in an analysis of the same group, and described similar changes in the mandible, which they attributed to a latitudinal ecological gradient. Similarly, Renaud (2005) found that the main axes of variation among populations of the European wood mouse (*Apodemus sylvaticus*) in Germany involve variation in the coronoid and angular processes. In a broader geographic study of this same species of mouse, which included mainland and insular populations over a latitudinal gradient, Renaud & Michaux (2007) found that differences among insular populations were mostly due to variation in the alveolar region in addition to the coronoid and angular processes. Because the pattern of variation of insular and mainland mice was different, these authors concluded that island ecology plays a key role in the morphological variation of the insular mice.

The prominence of the mandibular processes and masseteric ridge in overall shape variation is likely related to their functional role as insertion sites and loading regions for important masticatory muscles. These include the masseter superficialis and lateralis (attached to the angular process), masseter medialis and temporalis (coronoid process), masseter lateralis (masseteric ridge), and pterygoideus externus (condyloid process; Rinker 1954). The close interdependency between the development of bone and muscle tissue and the impact on morphology of mechanical forces generated during musculoskeletal (e.g., masticatory) function are well established (Herring 1993). Thus, the observation that most of the variation among populations is concentrated in the region of insertion of masticatory muscles is plausibly a consequence of dietary differences, in particular the physical properties of food, such as

hardness, size, and/or manageability. Several studies have measured the effects of dietary consistency on masticatory function and development of the mandible in rodents (Watt & Williams 1951; Bouvier & Hylander 1984; Myers et al. 1996; Ulgen et al. 1997; Kiliaridis et al. 1999; Maki et al. 2002), as well as humans and non-human primates (Bouvier & Hylander 1981; Taylor 2002; Varrela 2006). In most of these studies, differences in diet had a significant effect on mandible shape. However, in the few studies that actually measured that effect, the magnitude represented a small portion of the total variation. For example, Myers and colleagues (1996) found a significant effect of diet (i.e. hard versus soft dietary regimens) on cranial shape in prairie mice (Peromyscus maniculatus bairdii), but the magnitude of the effect was extremely small, suggesting that the effect of diet on mandible shape was almost negligible. Bouvier & Hylander (1984) did not find statistically significant differences in mandibular shape between rats fed soft versus hard diets, although animals on a soft diet had maxillary and mandibular measurements that were smaller on average than those on a hard diet regime. In a similar study by Watt & William (1951), differences between soft diet and hard diet groups were also small, accounting for only 1 to 3% of the observed differences in mandible morphology. Maki et al. (2002) reported a reduction of 6% of the posterior jaw height of laboratory mice fed a soft diet, which was likely manifest as a reduction in both coronoid and angular processes. In another study with laboratory mice, Renaud et al. (2010) investigated the effects of masticatory function on the shape of mandible. Although they did not measure the effect of diet on mandible shape variation directly, they reported that PC2, which accounted for most of the differences associated with food consistency, captured around 14% of the total variation.

Although a comparison across studies is made difficult by differences in methodological approaches, there is support for the hypothesis that diet affects the shape of the mandible, most

likely through masticatory activity, and that this effect is predominantly observed in the processes of the mandible. In my comparison of *P. leucopus* populations, change in the angular (LP-EUP) and condyloid (WUP-WI) processes was characterized by contraction (relative to surrounding morphological regions) in the newly established populations, suggesting a shift to a softer diet (Maki *et al.* 2002; Renaud *et al.* 2010). Such a shift would presumable require lower mastication forces, and therefore, smaller muscles. One possibility is that insects have become more prominent in the diet of UP mice than in mice from WI or LP, which probably rely more on hard-shell seeds. *Peromyscus leucopus* is an omnivorous rodent whose diet varies seasonally and geographically. Populations inhabiting deciduous forest in the Eastern United States feed on arthropods and insects, which may represent over 45% of their diet. However, the relative proportion of nuts and seeds increases when those become available, reaching about 30% in winter (Wolff *et al.* 1985). Another possibility is that the physical properties (e.g., hardness) of seeds and nuts differ between northern and southern localities.

None of these studies has explicitly measured changes in the dimensionality of mandible variation. However, results from the present study demonstrate that the dimensionality of shape variation can offer valuable insight in the study of phenotypic divergence, and should be included in comparison of populations, since regions showing a similar magnitude of variation can in fact diverge along different directions. This is a simple consequence of the multidimensional nature of morphological variation, and it illustrates the value of focusing on directions of variation, rather than the variation of anatomical regions of the mandible alone. As indicated by the nested MANOVA model used here, variation in mandible shape among populations within lineages is substantial, accounting for 85% of the total variation in shape, which is sufficient to obscure the differences between two long-isolated lineages, and old and

newly established populations within these, which account for 9% and 24% of the variation, respectively. Yet a pattern was elucidated, indicating the emergence of novel dimensions of divergence in an expansion process that is only a few decades old. Coupled with the fact that this invasion has resulted in the occupancy of novel habitats, i.e., boreal forests, with their own set of ecological challenges, it is plausible to hypothesize that ecological factors, more specifically diet, have had a significant role in the shape diversification of the mandible of expanding populations of *Peromyscus leucopus*, as they had on the radiation of old world rats and mice (Michaux *et al.* 2007; Samuels 2009).

The third level in the hierarchy of explanations explored in this study asks whether mean shape differences, which were previously shown to be significant in magnitude and direction, could be explained by genetic distances computed using neutral markers, in which case no role for local ecological processes has to be invoked. Comparisons between phylogeographic differentiation, based on mtDNA sequence data from the control region, and morphological disparity, based on geometric shape measurements, found a lack of congruence. This lack of association between genetic and morphometric distances is consistent with disparate causes underlying these patterns, and implies that morphological differentiation in these populations is not simply due to phenotypic drift.

Because patterns of morphometric variation observed in this study cannot be attributed to phenotypic drift, phenotypic plasticity and selection remain as plausible explanations. The only new population that was sampled from the LP-EUP lineage, namely Chippewa, is markedly different from other populations in the same lineage not only morphologically, as shown above, but also genetically (Chapter II). The specimens analyzed in this study came from an isolated patch of red oak forest in the Hiawatha Forest in the eastern UP, which has an unusual
abundance of oaks compared to most other localities in the UP (P. Myers, personal communication). It is thus possible that the divergence of this population has resulted from the combined effects of unique ecological conditions and genetic isolation. In contrast, new populations in the WUP-WI lineage do not show a sharp genetic differentiation with respect to other populations within that lineage (Chapter II), thus suggesting that morphological differentiation between old and new populations might predominantly reflect functional differences associated with local ecology, presumably due to of phenotypic plasticity or local adaptation.

Previous studies of wild populations of *P. maniculatus* (Holbrook 1982) and *P. leucopus* (Elrod & Kennedy 1995) have also found morphological differentiation at a microgeographic scale among populations inhabiting different habitats. Holbrook (1982) reported that mandibles of mice from woodlands and grasslands had significantly different morphologies, which the author attributed to diet, suggesting that high morphological plasticity in these generalist rodents might have enabled them to exploit a broad variety of microhabitats. Elrod & Kennedy (1995) compared cranial and mandibular measurements of mice from eight localities and discovered a pattern of morphological variation that was seemingly unrelated to geographic proximity, habitat type, or broad climatic variables. While authors in this case favored selection as the mechanism underlying differentiation, plasticity or a joint effect of plasticity and adaptation cannot be ruled out as explanations.

A large proportion of the studies of geographical variation in the mouse mandible attribute differences in morphology to dietary factors. The predominance of diet as an explanation reflects both the role of the mandible as the primary foraging structure, and the developmental plasticity of the mandible, a structure that continues to be influenced by

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masticatory function during postnatal life (Atchley 1993; Herring 1993; Renaud *et al.* 2010b). Thus, the developmental dynamics of the mandible favor the integration of environmental sources of variation on shape, which is a basis for plasticity (Badyaev & Foresman 2000; Young & Badyaev 2010) and could provide favorable conditions for rapid morphological divergence. Mammalian mandibles have been postulated to evolve rapidly, presumably because they have a single mechanical function, and are thus not generally subject to conflicting selective pressures (Caumul & Polly 2005). However, even though causal links between mandible morphology and masticatory function have often been hypothesized and assumed in studies of geographical variation, the necessary information to rigorously test these ideas is generally lacking, while critical research is still required in order to elucidate the complexity of the interaction between features of food items (e.g., hardness, nutritional content, mobility, toxicity) and their combined effect on mandible form.

In summary, results of this study show a pattern of rapid and abundant differentiation in the mandible, some of which appears to be the result of an expansion process into novel habitats. Rapid evolutionary rates in this structure are expected for a number of reasons, among the most important being that masticatory function, which is central for the morphogenesis of the mandible, continues to play a dynamic role in bone remodeling throughout ontogeny. The increased sensitivity to environmental variation that results might be translated into reaction norms with larger gradients, which could facilitate the invasion into new habitats as average plastic responses in the population would effectively shift approximately instantaneously into the adaptive zone characterizing the new environment (Pfennig *et al.* 2010).

An equivalent adaptive process relying on the spread of an advantageous mutation would take a greater number of generations, and might require the population to cross through adaptive

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valleys before reaching a new peak (Pfennig *et al.* 2010). However, a lack of functional constraints in the mandible (Caumul & Polly 2005) would presumably accelerate the rate of adaptation in this structure, suggesting that both selection and plasticity might simultaneously contribute to its rapid rate of evolution. These two processes are suitable explanations for the differences in the shape of the mandible found between old and new populations, within each lineage, but by no means are they mutually exclusive, as argued above.

Two distinct mechanisms have been proposed to explain phenotypic evolution mediated by plasticity, namely Baldwin's effect and genetic assimilation (Crispo 2007; Pfennig *et al.* 2010); in neither case is plasticity put forward as the direct cause of phenotypic evolution. Instead, it is the developmental processes underlying phenotypic responses to environmental variation that are considered potential targets of selection (Schlichting & Pigliucci 1998; Schlichting 2004; Crispo 2007; Angers *et al.* 2010; Pfennig *et al.* 2010). Both of these mechanisms assume the presence of environmentally induced phenotypic changes as a basis for the action of selection.

Results of this study suggest that even though the geographical expansion of *P. leucopus* in the northern Great Lakes is relatively recent, the phenotypic consequences are already noticeable. Therefore, it appears that in this case range expansion should not be regarded as an instance of habitat tracking. Even if diet is treated as the sole ecological factor playing a role in mandible form variation, it remains unclear which aspects of the diet are associated with particular directions of variation, or how distinct food properties might cancel or enhance each other's effect on the mandible, for all of which additional investigation is required in order to improve our understanding of the evolutionary and environmental forces shaping the mandible of these populations.

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Tables

Table III.1. Results from a nested MANOVA design measuring the effects of lineage (i.e., LP-EUP and WUP-WI), expansion history (i.e., old, established, vs. new, recently invaded populations) nested within lineage, and populations, nested within old and new localities, on shape of the mandible in *Peromyscus leucopus noveboracensis*. Note that Effect Size is not additive.

Effect	Hotelling-Lawley trace	F-value	<i>P</i> -value	Effect Size
Lineage, L _i	0.499	1.71	0.008	8.9%
Expansion history, $oldsymbol{H}_{ij}$	1.479	2.52	< 0.0001	24.29%
Population, P_{ijk}	8.166	1.39	< 0.0001	85.1%

Table III.2. Results from a nested ANOVA design measuring the effects of lineage, expansion history (i.e., old, established, vs. new, recently invaded populations) nested within lineage, and populations, nested within old and new localities, on centroid size of the mandible in *Peromyscus leucopus noveboracensis*. Note that Effect Size is not additive.

Effect	Mean Square	<i>F</i> -value	<i>P</i> -value	Effect Size
Lineage, l _i	441.89	3.02	0.084	1.3%
Expansion history, h_{ij}	211.72	1.45	0.238	1.3%
Population, p_{ijk}	146.57	1.00	0.462	8.8%

Table III.3. Contribution of mandible developmental blocks to the difference between old and new populations in each of the two lineages sampled in this study. Contributions are given in squared Procrustes units and as percentages of the total squared Procrustes distance between the mean shape of old and new populations (in parenthesis). Distances have been multiplied by 10⁵.

Lineage	Angular Process	Condyloid Process	Coronoid Process	Masseteric Region	Incisor Alveolus (anterior end)	Molar Alveolus
LP-	0.709	0.318	0.275	0.818	0.372	0.320
EUP	(25.22%)	(11.30%)	(9.77%)	(29.09%)	(13.24%)	(11.38%)
WUP-	0.065	0.092	0.026	0.046	0.023	0.076
WI	(19.78%)	(27.87%)	(7.96%)	(14.09%)	(7.00%)	(23.30%)

	Nei's	s D	Fst	t
	r	Р	r	Р
Full dataset	0.2645	0.05	0.3302	0.0260
LP-EUP	0.4989	0.1140	0.5106	0.1520
WUP-WI	0.3671	0.0930	0.4047	0.0730

Table III.4. Mantel test to assess the association between morphometric and genetic distances. Significance level corresponding to a 95% confidence interval after a Bonferroni's correction ($\alpha = 0.0125$).

Figures



Figure III.1. Configuration of landmarks (white circles) and semi-landmarks (black dots) sampled in this study. Shadowed areas indicate regions with developmental or functional significance. With the exception of the masseteric ridge, these regions are defined to roughly correspond to developmental units of the mandible (Atchley and Hall 1991).



Figure III.2. Geographic origin of the 24 populations examined in this research. The two-letter acronyms are as in Table A.III.1. Black circles represent old populations, while grey ones are new populations, according to the criterion explained in the text.



Figure III.3. Plots of scores of PC1 vs. PC2 (top) and PC3 vs. PC4 (bottom). Black circles are old populations in LP-EUP and white circle is the new population in this lineage. Black and white triangles are old and new populations, respectively, in WUP-WI. The two-letter acronyms are as in Table A.III.1.



Figure III.4. Shape deformations implied by first four PCs of population means: a) PC1 (26.32%), b) PC2 (23.57%), c) PC3 (11.17%), d) PC4 (7.78%). Colors represent continuous change in surface area of infinitesimally local regions of the mandible, as inferred via TPS interpolation, where colder colors (toward blue) are interpreted as local contraction and warmer ones (toward red) as expansion. Numbers in the scale represent percentage of local change (e.g., -0.1 means a local contraction of 10% with respect to the reference). Deformation vectors and grids (but not color patterns) have been magnified x5 to improve visualization.



Figure III.5. Box plots of canonical scores for all populations, sorted by lineage (i.e., LP-EUP, WUP-WI). Old populations are indicated by a shadowed background and new populations are on white (see text for details). The number of axes chosen for interpretation was based on Bartlett's test results, which estimated four significant dimensions for the differences among population means. Proportion of the variation explained by these canonical axes was CV1: 16.40%, CV2: 11.17%, CV3: 10.19%, and CV4: 8.67%.



Figure III.6. Differences between mean shapes of old and new populations within (a) LP-EUP and (b) WUP-WI lineages. Landmarks in mean shape reference of old samples are connected by a black line; landmarks in mean shape of new population are connected by a grey line. Note the difference in scale, showing more pronounced changes in (a). Colors represent proportional change throughout the mandible, where colder colors (toward blue) are interpreted as local contraction and warmer ones (toward red) as expansion. Numbers in the scale are percentage of local change (e.g., -0.10 means a local contraction of 10% with respect to the reference). To improve visibility of transformation grids, deformations have been multiplied by 4 in (a) and by 8 in (b).



Variation within old populations

Figure III.7. Trend of morphological variation within old populations and from old to new populations. The most extreme specimens are shown in each case to facilitate visualization of the changes already suggested by deformation grids (Fig. III.6).



Figure III.8. UPGMA dendrogram based on morphometric distances among populations. Distances were calculated as pairwise Euclidean distances between population means computed from the shape variables derived from projecting Procrustes residuals onto the first 46 eigenvectors of their covariance matrix.



Figure III.9. UPGMA dendrogram based on pairwise Nei's genetic distances among populations.



Figure III.10. UPGMA dendrogram based on pairwise Fst values among populations.

APPENDIX

Table A.III.1. List of specimens used in this study, including collection information (catalog number, museum), locality of origin (county and region), lineage, timing of population establishment, sex (0=males, 1= females), and coordinates of geographical midpoint of the locality of origin. LP=Michigan's Lower Peninsula, UP=Michigan's Upper Peninsula, WI=Wisconsin.

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Catalog number	Museum	County	ID	Region	Lineage	Timing	Sex	Lat	Log
37246	MSU	Adams	AD	WI	WUP-WI	Old	0	43.9012	-89.8676
37247	MSU	Adams	AD	WI	WUP-WI	Old	1	43.9012	-89.8676
37248	MSU	Adams	AD	WI	WUP-WI	Old	1	43.9012	-89.8676
37249	MSU	Adams	AD	WI	WUP-WI	Old	1	43.9012	-89.8676
37250	MSU	Adams	AD	WI	WUP-WI	Old	1	43.9012	-89.8676
37251	MSU	Adams	AD	WI	WUP-WI	Old	1	43.9012	-89.8676
37252	MSU	Adams	AD	WI	WUP-WI	Old	1	43.9012	-89.8676
37435	MSU	Adams	AD	WI	WUP-WI	Old	1	43.9012	-89.8676
37441	MSU	Adams	AD	WI	WUP-WI	Old	1	43.9012	-89.8676
37444	MSU	Adams	AD	WI	WUP-WI	Old	1	43.9012	-89.8676
37321	MSU	Ashland	AS	WI	WUP-WI	New	1	46.1568	-90.4755
37322	MSU	Ashland	AS	WI	WUP-WI	New	1	46.1568	-90.4755
37323	MSU	Ashland	AS	WI	WUP-WI	New	1	46.1568	-90.4755
37324	MSU	Ashland	AS	WI	WUP-WI	New	0	46.1568	-90.4755

Table A.III.1. Contin	nued.								
37325	MSU	Ashland	AS	WI	WUP-WI	New	1	46.1568	-90.4755
37327	MSU	Ashland	AS	WI	WUP-WI	New	1	46.1568	-90.4755
37328	MSU	Ashland	AS	WI	WUP-WI	New	0	46.1568	-90.4755
167367	UMMZ	Bayfield	BY	WI	WUP-WI	New	0	46.3705	-91.3376
167369	UMMZ	Bayfield	BY	WI	WUP-WI	New	1	46.3705	-91.3376
167372	UMMZ	Bayfield	BY	WI	WUP-WI	New	1	46.3705	-91.3376
167373	UMMZ	Bayfield	BY	WI	WUP-WI	New	1	46.3705	-91.3376
167375	UMMZ	Bayfield	BY	WI	WUP-WI	New	0	46.3705	-91.3376
167376	UMMZ	Bayfield	BY	WI	WUP-WI	New	0	46.3705	-91.3376
37319	MSU	Bayfield	BY	WI	WUP-WI	New	1	46.3705	-91.3376
37320	MSU	Bayfield	BY	WI	WUP-WI	New	0	46.3705	-91.3376
8204	UWSP	Bayfield	BY	WI	WUP-WI	New	1	46.3705	-91.3376
8205	UWSP	Bayfield	BY	WI	WUP-WI	New	1	46.3705	-91.3376
37255	MSU	Buffalo	BF	WI	WUP-WI	Old	1	44.5892	-91.8022
37260	MSU	Buffalo	BF	WI	WUP-WI	Old	1	44.5892	-91.8022
37262	MSU	Buffalo	BF	WI	WUP-WI	Old	0	44.5892	-91.8022
37263	MSU	Buffalo	BF	WI	WUP-WI	Old	0	44.5892	-91.8022

Table A.III.1. Contin	nued.								
37268	MSU	Buffalo	BF	WI	WUP-WI	Old	1	44.5892	-91.8022
37270	MSU	Buffalo	BF	WI	WUP-WI	Old	0	44.5892	-91.8022
37276	MSU	Buffalo	BF	WI	WUP-WI	Old	0	44.5892	-91.8022
37279	MSU	Buffalo	BF	WI	WUP-WI	Old	1	44.5892	-91.8022
37284	MSU	Buffalo	BF	WI	WUP-WI	Old	1	44.5892	-91.8022
37287	MSU	Buffalo	BF	WI	WUP-WI	Old	0	44.5892	-91.8022
37203	MSU	Charlevoix	СХ	LP	LP-EUP	Old	1	45.2615	-84.8037
37204	MSU	Charlevoix	СХ	LP	LP-EUP	Old	1	45.2615	-84.8037
37208	MSU	Charlevoix	СХ	LP	LP-EUP	Old	1	45.2615	-84.8037
37211	MSU	Charlevoix	CX	LP	LP-EUP	Old	1	45.2615	-84.8037
37216	MSU	Charlevoix	CX	LP	LP-EUP	Old	0	45.2615	-84.8037
37220	MSU	Charlevoix	CX	LP	LP-EUP	Old	1	45.2615	-84.8037
37221	MSU	Charlevoix	CX	LP	LP-EUP	Old	0	45.2615	-84.8037
37223	MSU	Charlevoix	CX	LP	LP-EUP	Old	0	45.2615	-84.8037
37225	MSU	Charlevoix	CX	LP	LP-EUP	Old	0	45.2615	-84.8037
37228	MSU	Charlevoix	CX	LP	LP-EUP	Old	0	45.2615	-84.8037
175151	UMMZ	Cheboygan	СН	LP	LP-EUP	Old	1	45.5634	-84.6637

Table A.III.1. Contin	nued.								
175214	UMMZ	Cheboygan	СН	LP	LP-EUP	Old	1	45.5634	-84.6637
175216	UMMZ	Cheboygan	СН	LP	LP-EUP	Old	1	45.5634	-84.6637
175217	UMMZ	Cheboygan	СН	LP	LP-EUP	Old	1	45.5634	-84.6637
175218	UMMZ	Cheboygan	СН	LP	LP-EUP	Old	0	45.5634	-84.6637
175220	UMMZ	Cheboygan	СН	LP	LP-EUP	Old	1	45.5634	-84.6637
175221	UMMZ	Cheboygan	СН	LP	LP-EUP	Old	0	45.5634	-84.6637
175226	UMMZ	Cheboygan	СН	LP	LP-EUP	Old	1	45.5634	-84.6637
175228	UMMZ	Cheboygan	СН	LP	LP-EUP	Old	0	45.5634	-84.6637
175230	UMMZ	Cheboygan	СН	LP	LP-EUP	Old	0	45.5634	-84.6637
177248	UMMZ	Chippewa	CW	UP	LP-EUP	New	1	46.4320	-84.7266
177249	UMMZ	Chippewa	CW	UP	LP-EUP	New	0	46.4320	-84.7266
177250	UMMZ	Chippewa	CW	UP	LP-EUP	New	0	46.4320	-84.7266
177253	UMMZ	Chippewa	CW	UP	LP-EUP	New	1	46.4320	-84.7266
177257	UMMZ	Chippewa	CW	UP	LP-EUP	New	1	46.4320	-84.7266
177259	UMMZ	Chippewa	CW	UP	LP-EUP	New	1	46.4320	-84.7266
177260	UMMZ	Chippewa	CW	UP	LP-EUP	New	0	46.4320	-84.7266
177261	UMMZ	Chippewa	CW	UP	LP-EUP	New	1	46.4320	-84.7266

Table A.III.1. Contin	nued.								
177267	UMMZ	Chippewa	CW	UP	LP-EUP	New	1	46.4320	-84.7266
177269	UMMZ	Chippewa	CW	UP	LP-EUP	New	0	46.4320	-84.7266
132046	UMMZ	Crawford	CR	LP	LP-EUP	Old	1	44.7888	-84.7340
132047	UMMZ	Crawford	CR	LP	LP-EUP	Old	0	44.7888	-84.7340
132049	UMMZ	Crawford	CR	LP	LP-EUP	Old	0	44.7888	-84.7340
132050	UMMZ	Crawford	CR	LP	LP-EUP	Old	1	44.7888	-84.7340
156424	UMMZ	Crawford	CR	LP	LP-EUP	Old	1	44.7888	-84.7340
156425	UMMZ	Crawford	CR	LP	LP-EUP	Old	0	44.7888	-84.7340
167287	UMMZ	Crawford	CR	LP	LP-EUP	Old	0	44.7888	-84.7340
167329	UMMZ	Crawford	CR	LP	LP-EUP	Old	1	44.7888	-84.7340
167330	UMMZ	Crawford	CR	LP	LP-EUP	Old	1	44.7888	-84.7340
167331	UMMZ	Crawford	CR	LP	LP-EUP	Old	1	44.7888	-84.7340
175993	UMMZ	Delta	DE	UP	WUP-WI	New	0	46.0788	-86.8345
175996	UMMZ	Delta	DE	UP	WUP-WI	New	0	46.0788	-86.8345
176004	UMMZ	Delta	DE	UP	WUP-WI	New	1	46.0788	-86.8345
176005	UMMZ	Delta	DE	UP	WUP-WI	New	0	46.0788	-86.8345
176006	UMMZ	Delta	DE	UP	WUP-WI	New	0	46.0788	-86.8345

Table A.III.1. Contin	nued.								
176007	UMMZ	Delta	DE	UP	WUP-WI	New	0	46.0788	-86.8345
176014	UMMZ	Delta	DE	UP	WUP-WI	New	1	46.0788	-86.8345
176015	UMMZ	Delta	DE	UP	WUP-WI	New	1	46.0788	-86.8345
176016	UMMZ	Delta	DE	UP	WUP-WI	New	1	46.0788	-86.8345
176046	UMMZ	Delta	DE	UP	WUP-WI	New	1	46.0788	-86.8345
165217	UMMZ	Emmet	EM	LP	LP-EUP	Old	1	45.5431	-85.0478
165265	UMMZ	Emmet	EM	LP	LP-EUP	Old	1	45.5431	-85.0478
165267	UMMZ	Emmet	EM	LP	LP-EUP	Old	0	45.5431	-85.0478
165270	UMMZ	Emmet	EM	LP	LP-EUP	Old	1	45.5431	-85.0478
165277	UMMZ	Emmet	EM	LP	LP-EUP	Old	1	45.5431	-85.0478
165278	UMMZ	Emmet	EM	LP	LP-EUP	Old	0	45.5431	-85.0478
165285	UMMZ	Emmet	EM	LP	LP-EUP	Old	1	45.5431	-85.0478
167388	UMMZ	Emmet	EM	LP	LP-EUP	Old	0	45.5431	-85.0478
167390	UMMZ	Emmet	EM	LP	LP-EUP	Old	0	45.5431	-85.0478
167394	UMMZ	Emmet	EM	LP	LP-EUP	Old	0	45.5431	-85.0478
177159	UMMZ	Gogebic	GO	UP	WUP-WI	New	1	46.3437	-89.3678
177175	UMMZ	Gogebic	GO	UP	WUP-WI	New	0	46.3437	-89.3678

Table A.III.1. Conti	nued.								
177388	UMMZ	Gogebic	GO	UP	WUP-WI	New	1	46.3437	-89.3678
177389	UMMZ	Gogebic	GO	UP	WUP-WI	New	0	46.3437	-89.3678
177390	UMMZ	Gogebic	GO	UP	WUP-WI	New	1	46.3437	-89.3678
177391	UMMZ	Gogebic	GO	UP	WUP-WI	New	1	46.3437	-89.3678
177392	UMMZ	Gogebic	GO	UP	WUP-WI	New	0	46.3437	-89.3678
177393	UMMZ	Gogebic	GO	UP	WUP-WI	New	0	46.3437	-89.3678
37331	MSU	Iron	IR	WI	WUP-WI	New	1	46.3579	-90.3774
37333	MSU	Iron	IR	WI	WUP-WI	New	1	46.3579	-90.3774
37334	MSU	Iron	IR	WI	WUP-WI	New	1	46.3579	-90.3774
37336	MSU	Iron	IR	WI	WUP-WI	New	0	46.3579	-90.3774
37337	MSU	Iron	IR	WI	WUP-WI	New	1	46.3579	-90.3774
37339	MSU	Iron	IR	WI	WUP-WI	New	1	46.3579	-90.3774
37342	MSU	Iron	IR	WI	WUP-WI	New	1	46.3579	-90.3774
37343	MSU	Iron	IR	WI	WUP-WI	New	0	46.3579	-90.3774
37344	MSU	Iron	IR	WI	WUP-WI	New	1	46.3579	-90.3774
37346	MSU	Iron	IR	WI	WUP-WI	New	0	46.3579	-90.3774
37373	MSU	Marathon	MH	WI	WUP-WI	Old	0	44.8175	-89.7509

Table A.III.1. Contin	ued.								
37375	MSU	Marathon	MH	WI	WUP-WI	Old	0	44.8175	-89.7509
37376	MSU	Marathon	MH	WI	WUP-WI	Old	0	44.8175	-89.7509
37377	MSU	Marathon	MH	WI	WUP-WI	Old	0	44.8175	-89.7509
37378	MSU	Marathon	MH	WI	WUP-WI	Old	0	44.8175	-89.7509
37380	MSU	Marathon	MH	WI	WUP-WI	Old	1	44.8175	-89.7509
37381	MSU	Marathon	MH	WI	WUP-WI	Old	1	44.8175	-89.7509
37382	MSU	Marathon	MH	WI	WUP-WI	Old	0	44.8175	-89.7509
37384	MSU	Marathon	MH	WI	WUP-WI	Old	0	44.8175	-89.7509
37391	MSU	Marathon	MH	WI	WUP-WI	Old	1	44.8175	-89.7509
37400	MSU	Marinette	MR	WI	WUP-WI	Old	0	45.5900	-87.9464
37401	MSU	Marinette	MR	WI	WUP-WI	Old	1	45.5900	-87.9464
37402	MSU	Marinette	MR	WI	WUP-WI	Old	1	45.5900	-87.9464
37406	MSU	Marinette	MR	WI	WUP-WI	Old	0	45.5900	-87.9464
37407	MSU	Marinette	MR	WI	WUP-WI	Old	0	45.5900	-87.9464
37409	MSU	Marinette	MR	WI	WUP-WI	Old	1	45.5900	-87.9464
37412	MSU	Marinette	MR	WI	WUP-WI	Old	0	45.5900	-87.9464
37417	MSU	Marinette	MR	WI	WUP-WI	Old	0	45.5900	-87.9464

Table A.III.1. Conti	nued.								
37427	MSU	Marinette	MR	WI	WUP-WI	Old	1	45.5900	-87.9464
37430	MSU	Marinette	MR	WI	WUP-WI	Old	1	45.5900	-87.9464
177394	UMMZ	Marquette	MA	UP	WUP-WI	New	1	46.8736	-87.8958
177395	UMMZ	Marquette	MA	UP	WUP-WI	New	1	46.8736	-87.8958
177396	UMMZ	Marquette	MA	UP	WUP-WI	New	1	46.8736	-87.8958
177397	UMMZ	Marquette	MA	UP	WUP-WI	New	0	46.8736	-87.8958
177398	UMMZ	Marquette	MA	UP	WUP-WI	New	0	46.8736	-87.8958
177399	UMMZ	Marquette	MA	UP	WUP-WI	New	0	46.8736	-87.8958
177400	UMMZ	Marquette	MA	UP	WUP-WI	New	1	46.8736	-87.8958
177401	UMMZ	Marquette	MA	UP	WUP-WI	New	0	46.8736	-87.8958
175318	UMMZ	Menominee	MN	UP	WUP-WI	Old	1	45.1580	-87.7000
176017	UMMZ	Menominee	MN	UP	WUP-WI	Old	0	45.1580	-87.7000
176022	UMMZ	Menominee	MN	UP	WUP-WI	Old	1	45.1580	-87.7000
176026	UMMZ	Menominee	MN	UP	WUP-WI	Old	0	45.1580	-87.7000
176027	UMMZ	Menominee	MN	UP	WUP-WI	Old	1	45.1580	-87.7000
176029	UMMZ	Menominee	MN	UP	WUP-WI	Old	1	45.1580	-87.7000
176047	UMMZ	Menominee	MN	UP	WUP-WI	Old	1	45.1580	-87.7000

Table A.III.1. Contin	nued.								
176181	UMMZ	Menominee	MN	UP	WUP-WI	Old	1	45.1580	-87.7000
176182	UMMZ	Menominee	MN	UP	WUP-WI	Old	1	45.1580	-87.7000
176191	UMMZ	Menominee	MN	UP	WUP-WI	Old	0	45.1580	-87.7000
37310	MSU	Oneida	OD	WI	WUP-WI	New	0	45.8709	-89.6527
37311	MSU	Oneida	OD	WI	WUP-WI	New	1	45.8709	-89.6527
37312	MSU	Oneida	OD	WI	WUP-WI	New	1	45.8709	-89.6527
37313	MSU	Oneida	OD	WI	WUP-WI	New	0	45.8709	-89.6527
37314	MSU	Oneida	OD	WI	WUP-WI	New	1	45.8709	-89.6527
37315	MSU	Oneida	OD	WI	WUP-WI	New	0	45.8709	-89.6527
37316	MSU	Oneida	OD	WI	WUP-WI	New	0	45.8709	-89.6527
37317	MSU	Oneida	OD	WI	WUP-WI	New	1	45.8709	-89.6527
37318	MSU	Oneida	OD	WI	WUP-WI	New	1	45.8709	-89.6527
177181	UMMZ	Ontonagon	ON	UP	WUP-WI	New	0	46.7364	-89.7394
177184	UMMZ	Ontonagon	ON	UP	WUP-WI	New	0	46.7364	-89.7394
177186	UMMZ	Ontonagon	ON	UP	WUP-WI	New	0	46.7364	-89.7394
177194	UMMZ	Ontonagon	ON	UP	WUP-WI	New	1	46.7364	-89.7394
177201	UMMZ	Ontonagon	ON	UP	WUP-WI	New	1	46.7364	-89.7394

Table A.III.1. Contin	nued.								
177211	UMMZ	Ontonagon	ON	UP	WUP-WI	New	0	46.7364	-89.7394
177212	UMMZ	Ontonagon	ON	UP	WUP-WI	New	0	46.7364	-89.7394
177214	UMMZ	Ontonagon	ON	UP	WUP-WI	New	1	46.7364	-89.7394
177216	UMMZ	Ontonagon	ON	UP	WUP-WI	New	1	46.7364	-89.7394
277222	UMMZ	Ontonagon	ON	UP	WUP-WI	New	1	46.7364	-89.7394
170135	UMMZ	Otsego	OT	LP	LP-EUP	Old	0	45.0549	-84.5688
170137	UMMZ	Otsego	ОТ	LP	LP-EUP	Old	0	45.0549	-84.5688
170316	UMMZ	Otsego	ОТ	LP	LP-EUP	Old	1	45.0549	-84.5688
170323	UMMZ	Otsego	ОТ	LP	LP-EUP	Old	1	45.0549	-84.5688
170454	UMMZ	Otsego	ОТ	LP	LP-EUP	Old	0	45.0549	-84.5688
175470	UMMZ	Otsego	ОТ	LP	LP-EUP	Old	1	45.0549	-84.5688
175471	UMMZ	Otsego	ОТ	LP	LP-EUP	Old	1	45.0549	-84.5688
175472	UMMZ	Otsego	ОТ	LP	LP-EUP	Old	0	45.0549	-84.5688
175473	UMMZ	Otsego	ОТ	LP	LP-EUP	Old	0	45.0549	-84.5688
175474	UMMZ	Otsego	ОТ	LP	LP-EUP	Old	1	45.0549	-84.5688
37230	MSU	Outagamie	OU	WI	WUP-WI	Old	1	44.3961	-88.6663
37233	MSU	Outagamie	OU	WI	WUP-WI	Old	1	44.3961	-88.6663

Table A.III.1. Contin	ued.								
37234	MSU	Outagamie	OU	WI	WUP-WI	Old	0	44.3961	-88.6663
37235	MSU	Outagamie	OU	WI	WUP-WI	Old	1	44.3961	-88.6663
37236	MSU	Outagamie	OU	WI	WUP-WI	Old	0	44.3961	-88.6663
37238	MSU	Outagamie	OU	WI	WUP-WI	Old	1	44.3961	-88.6663
37239	MSU	Outagamie	OU	WI	WUP-WI	Old	1	44.3961	-88.6663
37240	MSU	Outagamie	OU	WI	WUP-WI	Old	0	44.3961	-88.6663
37244	MSU	Outagamie	OU	WI	WUP-WI	Old	1	44.3961	-88.6663
37245	MSU	Outagamie	OU	WI	WUP-WI	Old	0	44.3961	-88.6663
8162	UWSP	Portage	РО	WI	WUP-WI	Old	0	44.5412	-89.5654
5385	UWSP	Portage	РО	WI	WUP-WI	Old	1	44.5412	-89.5654
5467	UWSP	Portage	РО	WI	WUP-WI	Old	1	44.5412	-89.5654
5468	UWSP	Portage	РО	WI	WUP-WI	Old	0	44.5412	-89.5654
5469	UWSP	Portage	РО	WI	WUP-WI	Old	1	44.5412	-89.5654
5535	UWSP	Portage	РО	WI	WUP-WI	Old	1	44.5412	-89.5654
5539	UWSP	Portage	РО	WI	WUP-WI	Old	1	44.5412	-89.5654
5671	UWSP	Portage	РО	WI	WUP-WI	Old	1	44.5412	-89.5654
6055	UWSP	Portage	РО	WI	WUP-WI	Old	1	44.5412	-89.5654

Table A.III.1. Contin	ued.								
8163	UWSP	Portage	РО	WI	WUP-WI	Old	0	44.5412	-89.5654
175526	UMMZ	Schoolcraft	SC	UP	WUP-WI	New	0	46.0886	-86.2278
175527	UMMZ	Schoolcraft	SC	UP	WUP-WI	New	1	46.0886	-86.2278
175528	UMMZ	Schoolcraft	SC	UP	WUP-WI	New	0	46.0886	-86.2278
175541	UMMZ	Schoolcraft	SC	UP	WUP-WI	New	0	46.0886	-86.2278
175542	UMMZ	Schoolcraft	SC	UP	WUP-WI	New	0	46.0886	-86.2278
37289	MSU	Taylor	TA	WI	WUP-WI	Old	1	45.3127	-90.6268
37291	MSU	Taylor	TA	WI	WUP-WI	Old	1	45.3127	-90.6268
37294	MSU	Taylor	TA	WI	WUP-WI	Old	1	45.3127	-90.6268
37295	MSU	Taylor	TA	WI	WUP-WI	Old	0	45.3127	-90.6268
37296	MSU	Taylor	TA	WI	WUP-WI	Old	0	45.3127	-90.6268
37298	MSU	Taylor	TA	WI	WUP-WI	Old	1	45.3127	-90.6268
37299	MSU	Taylor	TA	WI	WUP-WI	Old	1	45.3127	-90.6268
37304	MSU	Taylor	TA	WI	WUP-WI	Old	0	45.3127	-90.6268
37305	MSU	Taylor	TA	WI	WUP-WI	Old	0	45.3127	-90.6268
37308	MSU	Taylor	TA	WI	WUP-WI	Old	1	45.3127	-90.6268
37347	MSU	Wood	WO	WI	WUP-WI	Old	0	44.3785	-90.1090

Table A.III.1. Continued.												
37348	MSU	Wood	WO	WI	WUP-WI	Old	1	44.3785	-90.1090			
37349	MSU	Wood	WO	WI	WUP-WI	Old	1	44.3785	-90.1090			
37351	MSU	Wood	WO	WI	WUP-WI	Old	1	44.3785	-90.1090			
37352	MSU	Wood	WO	WI	WUP-WI	Old	0	44.3785	-90.1090			
37353	MSU	Wood	WO	WI	WUP-WI	Old	1	44.3785	-90.1090			
37356	MSU	Wood	WO	WI	WUP-WI	Old	1	44.3785	-90.1090			
37359	MSU	Wood	WO	WI	WUP-WI	Old	0	44.3785	-90.1090			
37361	MSU	Wood	WO	WI	WUP-WI	Old	1	44.3785	-90.1090			
37362	MSU	Wood	WO	WI	WUP-WI	Old	1	44.3785	-90.1090			

Table A.III.2. Matrix of pairwise genetic distances (Nei's *D*) between populations included in this study. Above diagonal: Average number of pairwise differences between populations (PiXY). Diagonal elements (in bold): Average number of pairwise differences within population (PiX). Below diagonal: Corrected average pairwise difference (PiXY-(PiX+PiY)/2).

	AD	AS	BY	BF	CX	CH	CW	CR	DE	EM	GO	IR
AD	7.1	7.2	7.0	7.5	26.3	28.0	22.2	25.3	6.4	27.9	7.2	6.4
AS	0.2	7.0	6.3	7.4	26.1	28.1	22.3	25.1	6.2	27.8	6.7	6.0
BY	0.4	-0.2	6.1	7.2	26.1	27.9	22.1	25.1	5.9	27.7	6.9	5.6
BF	0.4	0.3	0.6	7.1	24.9	26.6	21.0	23.9	5.8	26.4	7.0	6.4
CX	19.0	19.0	19.3	17.7	7.4	9.9	17.1	7.5	24.1	7.0	26.2	24.5
CH	19.4	19.6	19.8	18.0	1.2	10.0	17.7	9.9	25.4	10.2	28.0	26.2
CW	10.3	10.4	10.6	9.1	5.0	4.3	16.8	16.7	19.8	17.3	22.2	20.8
CR	17.8	17.7	18.1	16.4	-0.1	0.9	4.4	7.9	22.9	7.9	25.1	23.5
DE	1.0	0.9	1.0	0.4	18.5	18.6	9.6	17.1	3.7	25.8	5.9	5.0
EM	20.8	20.8	21.2	19.4	-0.2	1.7	5.4	0.5	20.5	6.9	27.7	26.1
GO	0.7	0.2	0.8	0.4	19.4	20.0	10.8	18.1	1.0	21.2	6.1	5.9
IR	0.6	0.2	0.2	0.6	18.5	18.9	10.2	17.3	0.9	20.4	0.6	4.5
MH	0.8	0.5	1.0	0.3	19.4	19.8	10.6	18.1	1.1	21.1	-0.1	0.9
MR	0.4	0.4	0.6	0.1	18.6	19.1	10.0	17.3	0.5	20.5	0.6	0.9
MA	0.6	0.4	0.8	0.9	19.0	19.4	10.3	17.8	1.2	20.5	0.7	0.8
MN	2.1	2.5	2.4	1.6	18.8	18.7	9.9	17.3	1.4	20.6	2.7	3.0
OD	0.2	0.1	0.8	0.7	19.6	20.1	10.9	18.4	1.3	21.4	0.6	0.9
ON	0.7	0.4	0.8	0.5	15.6	15.6	7.9	14.4	0.7	17.2	0.3	0.5
OT	19.1	19.1	19.5	17.8	0.3	0.1	5.1	0.0	18.3	1.1	19.5	18.6
OU	1.0	1.0	1.0	0.2	19.1	19.3	10.2	17.7	0.4	21.1	0.9	1.1
PO	1.4	0.7	1.2	0.9	17.7	18.2	9.6	16.4	1.6	19.5	0.8	1.7
SC	1.9	2.2	2.1	1.2	18.9	18.8	10.0	17.4	0.9	20.7	2.3	2.5
TA	0.5	0.2	0.4	0.3	18.0	18.6	9.5	16.8	0.8	19.6	0.4	0.6
WO	0.8	0.7	1.1	0.4	19.9	20.5	11.2	18.6	1.6	21.6	0.5	0.9

Table A.III.2. Continued.

	MH	MR	MA	MN	OD	ON	OT	OU	PO	SC	TA	WO
AD	8.1	7.0	6.8	7.9	6.6	9.3	26.9	7.6	8.5	7.5	7.7	7.2
AS	7.6	6.9	6.6	8.2	6.4	8.9	26.9	7.6	7.8	7.8	7.4	7.0
BY	7.8	6.8	6.5	7.7	6.7	8.9	26.8	7.2	7.9	7.3	7.1	7.0
BF	7.6	6.7	7.1	7.4	7.1	9.1	25.6	6.8	8.0	6.9	7.5	6.8
CX	26.8	25.4	25.4	24.7	26.2	24.3	8.3	25.9	25.0	24.7	25.4	26.4
СН	28.5	27.2	27.1	26.0	28.0	25.7	9.3	27.4	26.8	25.9	27.3	28.3
CW	22.6	21.4	21.4	20.5	22.1	21.3	17.7	21.6	21.5	20.4	21.6	22.4
CR	25.7	24.3	24.4	23.4	25.2	23.4	8.2	24.7	23.9	23.4	24.4	25.4
DE	6.6	5.4	5.7	5.5	6.0	7.6	24.4	5.4	7.0	4.9	6.4	6.3
EM	28.2	27.0	26.7	26.3	27.7	25.8	8.8	27.7	26.6	26.3	26.7	27.9
GO	6.7	6.7	6.5	8.0	6.4	8.4	26.8	7.1	7.5	7.4	7.1	6.3
IR	6.8	6.2	5.7	7.5	6.0	7.8	25.1	6.4	7.6	6.9	6.5	5.9
MH	7.4	7.4	7.6	8.6	7.4	9.3	27.3	7.6	8.1	8.0	8.0	6.8
MR	0.6	6.2	6.7	6.2	6.4	9.0	26.0	6.4	7.7	5.9	7.2	6.5
MA	1.2	0.9	5.4	8.1	6.1	8.3	26.1	7.3	8.5	7.6	6.5	6.6
MN	2.7	0.8	3.1	4.5	8.0	10.1	24.9	6.8	7.8	4.3	8.3	8.2
OD	0.8	0.5	0.5	2.9	5.7	8.5	26.8	7.4	8.0	7.6	7.3	6.6
ON	0.6	0.9	0.6	2.8	0.6	10.1	24.8	9.3	10.0	9.6	9.3	8.8
OT	19.4	18.6	19.2	18.4	19.7	15.4	8.5	26.3	25.6	24.9	26.1	27.1
OU	0.9	0.2	1.5	1.5	1.4	1.2	19.0	6.2	8.1	6.2	7.4	6.9
PO	0.9	1.0	2.3	2.0	1.6	1.3	17.8	1.5	7.2	7.6	8.5	8.1
SC	2.2	0.7	2.8	-0.1	2.6	2.4	18.5	1.0	1.9	4.2	7.8	7.7
TA	0.7	0.4	0.1	2.3	0.8	0.6	18.2	0.6	1.2	2.0	7.4	7.2
WO	0.3	0.6	1.1	3.1	0.9	0.9	20.0	0.9	1.7	2.8	0.7	5.6

	AD	AS	BY	BF	CX	CH	CW	CR	DE	EM	GO	IR
AD	0.0											
AS	0.0	0.0										
BY	0.1	0.0	0.0									
BF	0.1	0.0	0.1	0.0								
CX	0.7	0.7	0.7	0.7	0.0							
CH	0.7	0.7	0.7	0.7	0.1	0.0						
CW	0.5	0.4	0.5	0.4	0.3	0.2	0.0					
CR	0.7	0.7	0.7	0.7	0.0	0.1	0.3	0.0				
DE	0.2	0.2	0.2	0.1	0.8	0.7	0.5	0.7	0.0			
EM	0.7	0.7	0.8	0.7	0.0	0.1	0.3	0.1	0.8	0.0		
GO	0.1	0.0	0.1	0.1	0.7	0.7	0.5	0.7	0.2	0.8	0.0	
IR	0.1	0.0	0.0	0.1	0.7	0.7	0.5	0.7	0.2	0.8	0.1	0.0
MH	0.1	0.1	0.1	0.0	0.7	0.7	0.5	0.7	0.2	0.7	0.0	0.1
MR	0.1	0.1	0.1	0.0	0.7	0.7	0.5	0.7	0.1	0.8	0.1	0.1
MA	0.1	0.1	0.1	0.1	0.8	0.7	0.5	0.7	0.2	0.8	0.1	0.1
MN	0.3	0.3	0.3	0.2	0.8	0.7	0.5	0.7	0.3	0.8	0.3	0.4
OD	0.0	0.0	0.1	0.1	0.7	0.7	0.5	0.7	0.2	0.8	0.1	0.2
ON	0.1	0.0	0.1	0.0	0.6	0.6	0.4	0.6	0.1	0.6	0.0	0.0
OT	0.7	0.7	0.7	0.7	0.0	0.0	0.3	0.0	0.8	0.1	0.7	0.7
OU	0.1	0.1	0.1	0.0	0.7	0.7	0.5	0.7	0.1	0.8	0.1	0.2
PO	0.2	0.1	0.2	0.1	0.7	0.7	0.4	0.7	0.2	0.7	0.1	0.2
SC	0.2	0.3	0.3	0.2	0.8	0.7	0.5	0.7	0.2	0.8	0.3	0.4
TA	0.1	0.0	0.1	0.0	0.7	0.7	0.4	0.7	0.2	0.7	0.1	0.1
WO	0.1	0.1	0.2	0.1	0.8	0.7	0.5	0.7	0.3	0.8	0.1	0.1

Table A.III.3. Matrix of pairwise *F*st values between populations included in this study.

Table	A.III.3 .	Continued.
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	MH	MR	MA	MN	OD	ON	OT	OU	PO	SC	TA	WO
AD												
AS												
BY												
BF												
CX												
CH												
CW												
CR												
DE												
EM												
GO												
IR												
MH	0.0											
MR	0.1	0.0										
MA	0.2	0.1	0.0									
MN	0.3	0.1	0.4	0.0								
OD	0.1	0.1	0.1	0.4	0.0							
ON	0.1	0.1	0.1	0.3	0.1	0.0						
OT	0.7	0.7	0.7	0.7	0.7	0.6	0.0					
OU	0.1	0.0	0.2	0.2	0.2	0.1	0.7	0.0				
PO	0.1	0.1	0.3	0.3	0.2	0.1	0.7	0.2	0.0			
SC	0.3	0.1	0.4	0.0	0.4	0.2	0.7	0.2	0.2	0.0		
TA	0.1	0.1	0.0	0.3	0.1	0.1	0.7	0.1	0.1	0.3	0.0	
WO	0.0	0.1	0.2	0.4	0.1	0.1	0.7	0.1	0.2	0.4	0.1	0.0
Table A.III.4. Matrix of pairwise morphometric distances between populations included in this study. Distances were calculated as pairwise Euclidean distances between population means computed from the shape variables derived from projecting Procrustes residuals onto the first 46 eigenvectors of their covariance matrix. Values in each cell were multiply by 10³.

	AD	AS	BY	BF	CX	CH	CW	CR	DE	EM	GO	IR
AD	0											
AS	13	0										
BY	14	16	0									
BF	9	14	16	0								
CX	16	16	18	17	0							
CH	20	17	22	18	17	0						
CW	14	21	13	15	20	23	0					
CR	19	21	20	16	22	16	17	0				
DE	14	21	17	14	23	23	12	15	0			
EM	17	14	18	15	18	13	22	17	21	0		
GO	16	13	16	15	14	16	17	20	21	17	0	
IR	11	8	15	11	14	15	18	18	20	14	11	0
MH	11	15	14	13	14	20	16	20	17	20	14	12
MR	15	20	16	15	16	20	14	16	14	20	18	17
MA	24	19	23	25	17	16	28	23	28	19	20	19
MN	13	19	18	13	18	19	14	14	11	18	17	17
OD	10	14	14	11	17	18	14	17	15	18	12	11
ON	14	15	18	12	18	15	17	13	16	14	15	14
OT	15	15	17	17	13	15	18	16	19	19	13	13
OU	12	14	16	16	12	18	18	21	21	20	15	11
PO	14	15	17	17	11	16	20	21	23	20	13	12
SC	18	20	16	22	20	26	20	26	22	25	20	20
TA	12	13	15	17	15	21	20	24	21	19	16	14
WO	11	16	13	14	14	20	13	19	15	20	15	13

Table A.III.4. Continued.

	MH	MR	MA	MN	OD	ON	OT	OU	PO	SC	TA	WO
AD												
AS												
BY												
BF												
CX												
CH												
CW												
CR												
DE												
EM												
GO												
IR												
MH	0											
MR	16	0										
MA	22	22	0									
MN	17	11	23	0								
OD	10	15	23	14	0							
ON	17	16	21	12	13	0						
OT	14	14	18	15	14	16	0					
OU	11	17	20	18	13	18	11	0				
PO	11	17	17	18	13	18	9	8	0			
SC	16	23	27	24	19	25	18	17	18	0		
TA	12	18	21	19	14	20	15	11	13	14	0	
WO	8	13	21	14	11	16	14	12	13	17	13	0

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CHAPTER IV

MORPHOMETRIC DIFFERENTIATION OF EXPANDING POPULATIONS OF *PEROMYSCUS LEUCOPUS* IN THE NORTHERN GREAT LAKES: ENVIRONMENTAL AND GEOGRAPHICAL FACTORS

Abstract

Geographical range expansion associated with recent climate change is usually regarded as a simple process of habitat tracking. Yet, the invasion of new environments is also expected to produce phenotypic changes as populations adjust to the new local conditions. The identification of the factors causing phenotypic variations is very challenging because of the complexity of variables and interactions that mediate phenotypic change. Understanding the association between morphological variation and environmental factors is crucial to improve our ability to anticipate the evolutionary consequences of predicted climatic change scenarios. In this study, patterns of genetic and morphometric variation were examined in light of geographical and environmental variables, with the objective of identifying factors associated with both variation in mandible shape among populations, and differentiation between old and new populations. Distance-based methods are used to test for broad associations among morphological, genetic and environmental variables, and then PLS regression was used to find actual directions of variation responsible for these associations. The results of this study suggest that cold temperatures and snow fall are the main factors underneath the differentiation between old and new populations, while land cover contributes with variation that is not related to the expansion process.

Introduction

It is often assumed that the main effect of current trends in climate change on the geographic range of populations experiencing range expansion is to increase their available habitat, so that organisms simply track the habitat change (Hughes 2000; Thomas et al. 2001; Walther et al. 2002; Salamin *et al.* 2010). However, this might not be possible for populations in temperate zones, which are subject to seasonal changes and presumably adapted to multiple environmental factors that are not necessarily directly affected by climate (e.g., photoperiod). The decoupling between those environmental cues and the actual environmental conditions is, perhaps, one of the most devastating effects of climate change, because it leads to the wrong interpretation of seasonal cues, which may have detrimental effects on an organism's fitness (Bradshaw & Holzapfel 2006; Bronson 2009; Schlaepfer et al. 2002). Although climate is a key factor in determining species geographical range (Pigot et al. 2010), changes in biotic interactions may also play a crucial role in species range shifts (Van der Putten *et al.* 2010). In general, range expansion associated with current climate change should not be viewed as a passive process of tracking habitat, but as a complex process involving evolutionary mechanisms that would allow a population to establish successfully in a new locality.

It would expected that the invasion of new environments is accompanied by changes in phenotype, as populations readjust to new optima under the novel ecological circumstances, either by local adaptation or plasticity (Chevin *et al.* 2010). Those phenotypic changes are mediated by a number of factors, including genetic, developmental, and ecological, and the complex interactions among them, as well as deterministic and stochastic evolutionary forces (Monteiro *et al.* 2003; Poroshin *et al.* 2010; Straney & Patton 1980). In the case of small mammals, these phenotypic changes in response to novel environmental conditions can take place very rapidly (Goheen *et al.* 2003; Pergams & Ashley 1999; Pergams & Lacy 2008;

Pergams & Lawler 2009; Renaud & Auffray 2010), probably because of their short generation length and lifespan.

Identifying the key factors underlying observed phenotypic variation is very challenging. Most studies focus on the geographical variation in variables associated with climate (e.g., temperature, precipitation), topography (e.g., elevation), geographical distribution (e.g., latitude, longitude), and/or vegetation type (e.g., forest vs. grassland), aiming to find the basis of the morphological variation (Cardini *et al.* 2007; Monteiro *et al.* 2003; Poroshin *et al.* 2010; Rychlik *et al.* 2006). Because phenotype is the final product of the interaction of evolutionary forces, genes, and environment, each of those factors is just a piece of a very complex puzzle. A better understanding of the association between morphological variation and ecogeographical factors would be of value in climate change research, as it would improve our ability to anticipate some of the evolutionary consequences of predicted climatic change scenarios.

The white-footed mouse, *Peromyscus leucopus*, has experienced a remarkable northward range expansion in the Great Lakes region in recent decades. Expanding populations are not only facing photoperiodic changes, but also changes in their environment, the most notable reflected in the forest habitat, which transitions from deciduous in the south, to boreal in the north (Myers *et al.* 2009). Morphometric analyses suggest that there are significant differences in mandible shape between recently expanded populations (new populations) and long established ones (old populations). These differences involve not only the magnitude of shape variation, but also its dimensionality, indicating that rapid expansion has been accompanied by marked phenotypic change (Chapter III). The pattern of shape variation is incongruent with the phylogeographic pattern observed in these populations, suggesting a potential role of selection on the observed differences in morphology. Results also indicate that a large proportion of the variation in

mandible shape is related to differences among populations. Regardless of whether these phenotypic differences reflect plasticity or adaptation, or both, ecogeographical factors likely underlie both the variation in mandible shape among populations, and differentiation between old and new populations. In this study, I examine the association among morphometric, genetic, and ecogeographical factors in expanding populations of *Peromyscus leucopus* and their putative progenitors in the northern Great Lakes. My results clearly show that statements suggesting that expanding populations are merely "tracking" their habitat are not only oversimplifying a rather complex process but also ignoring the fact that, in a strict sense, no two habitats are the same.

Materials and Methods

Samples

Twenty-four populations comprising 12 localities from Wisconsin (WI), 5 localities from Michigan's Lower Peninsula (LP), and 7 localities from Michigan's Upper Peninsula (UP) were sampled (Fig II.1, Chapter III). These populations were sorted by lineage, i.e., LP-EUP and WUP-WI, according to results previously obtained from SAMOVA and haplotype genealogy analyses (Chapter II). Within each lineage, populations were classified as "old" or "new" depending on whether a population has been long or recently established, respectively (Table A.IV.1). Morphometric data were obtained from the mandibles of approximately 10 specimens per locality, for a total of 227 specimens (Table A.III.1). Genetic data were obtained from 9-20 individuals per locality (Table II.1, Chapter II). DNA sequences and landmark configurations were obtained as described elsewhere (Chapters II and III). Trapping sites within a 25 km radius and located within similar environments were considered to represent a single population. When more than one location satisfied these criteria, the geographical midpoint of the combined sites was used as their common geographical position; this point was computed using the web-based tool at <u>http://www.geomidpoint.com</u>. In the text, populations are also referred to as localities.

All animals trapped for this study were handled according to protocols approved by the Michigan State University Institutional Animal Care and Use Committee, application # 08/04-108-00, in compliance with the American Society of Mammalogists guidelines for the use of wild mammals in research (Gannon *et al.* 2007).

Ecological Data

Land Cover/Use

Using geographical midpoints as reference locations, custom-made land cover/ use maps for each locality were obtained from aerial photographs (Fig. IV.1). These maps were elaborated by a GIS/Remote Sensing Analyst (from Michigan State University Remote Sensing & Geographic Information Science, Research and Outreach Services) by overlaying a 454 m-radius (approximately 0.65 km²) circle on aerial images from the National Agriculture Imagery Program (NAIP). NAIP is administrated by the Farm Service Agency of the Department of Agriculture and charged with acquiring aerial imagery during agricultural growing seasons in the continental United States. The maps used for this study were based on photographs taken in 2007, and the radius of the focal circle was chosen to match the home range of the white-footed mouse (Burt 1940).

Land cover/use polygons were identified and drawn on aerial photographs for each locality, and then classified according to the Michigan Resource Information System (MIRIS) 2007 land cover/use classification system, which is based on the classification system by Anderson *et al.* (1976) for use with remote sensing data. In this way, the relative area covered by

each land cover category found in each locality was obtained. This system has four levels of classification depending on the resolution (i.e., altitude and scale) of the data: Level I contains satellite image data (i.e., LANDSAT) and offers the most comprehensive coverage; Level II consists of high-altitude data (12,400 m) with a scale below 1:80,000; Level III provides medium-altitude data (3,100 – 12,400 m), spanning a scale between 1:20,000 and 1:80,000; and Level IV contains low-altitude data (i.e., aerial photographs taken below 3,100 m) with a scale above 1:20,000, resulting in highly detailed information (Anderson *et al.* 1976). For this study, the land cover categories (herein also referred to as environments) Urban, Agricultural, Grass and Shrub, Water, and Barren were classified up to Level II, and the categories Forest and Wetland were classified up to Level III.

Climate Data

The closest climate station to each of the sample localities was located and historical climate data for the period of 1971 to 2000 were obtained from the Midwest Regional Climate Center (http://mrcc.isws.illinois.edu/index.jsp). The following climate variables were analyzed in this study: mean annual, winter, spring, summer and fall temperatures; annual snow precipitation, and annual rain precipitation (Table A.IV.1). Seasonal temperatures were calculated as the average of the mean monthly temperatures during the months of December, January, and February for Winter; March, April, and May for Spring; June, July, and August for Summer; and September, October, and November for Fall.

Index of Environmental Similarity

To compare localities in terms of their environmental compositions, Morisita's Index was

computed for each pair of localities (Brower *et al.* 1990). This index measures similarity among communities as the probability that two distinct environments sampled in two localities will be the same, equaling zero when two communities are totally dissimilar and one when they are completely similar. It is computed using

$$I_M = \frac{[(2)(\sum(x_i)(y_i))]}{[(l_1 + l_2)(N_1)(N_2)]}$$

where x_i is the area of environment *i* in locality 1; y_i is the area of environment *i* in locality 2; *N* is the total area of each locality; and l_1 and l_2 are Simpson's indices of dominance for localities 1 and 2, respectively. Simpson's index incorporates information about the relative abundance of environment types, and is computed using the following equation:

$$l = \frac{[\sum(x_i) (x_i - 1)]}{(N)(N - 1)}$$

where x_i is the area of environment *i* and *N* is the total area of each locality (Table A.IV.1). This index can be interpreted as the probability that two randomly selected environments at one locality will be the same. It varies from zero, indicating maximum diversity, to one, indicating absence of diversity, i.e., only one type of environment at that locality (Brower *et al.* 1990).

To convert these measurements into ecological distances, a matrix of pairwise distances was constructed with elements equal to $(1 - I_M)$, with values ranging from zero, indicating that the two localities possess identical environments, to 1, indicating that the two localities have no environments in common (Table A.IV.2).

Statistical Methods

The following methods were used to assess statistical associations between geographical and

environmental variables, and observed patterns of genetic and morphometric variation.

Association between shape, geographical, ecological, and genetic distances

Partial Mantel tests (Manly 1986) were used to assess associations among shape, geographical, ecological, and genetic distances among sampled populations. This test extends the basic Mantel test for correlation between two matrices by using the rationale of multiple regression (Thorpe *et al.* 1995). In a partial Mantel test, an independent, response variable matrix is simultaneously compared to multiple dependent predictor matrices, after each has been controlled statistically for the effects of the others. This approach was used to evaluate (1) the regression of morphometric distances (both mandible shape and size) on geographical, ecological, and genetic distances.

For these tests, shape distances were measured as pairwise Euclidean distances between population means computed from average Procrustes residuals projected onto a space of adequate dimensionality (see Chapter II for details), and size distances were measured as pairwise Euclidean distances between population mean centroid sizes. Geographical distances between the geographical midpoints of populations were calculated as geodesics, i.e., shortest distances over the Earth's surface (Table A.IV.3). Ecological distances were computed as $1 - I_M$, where I_M is the Morisita's index of community similarity described above (Table A.IV.2). Two different genetic distance metrics were considered in these analyses, pairwise Nei's distances *D* (Nei 1987) and *F*st estimates (Reynolds *et al.* 1983), which were computed using Arlequin 3.11 (Excoffier *et al.* 2005). Significance levels were adjusted by Bonferroni correction for multiple comparisons. Additionally, dendrograms were constructed using an average distance-based linkage function (UPGMA method) to graphically visualize distance patterns in these matrices.

Effect of climate, geography, and land cover on mandible shape

The pattern of covariance between mandible shape and ecogeographical variables (i.e., climate, geography, and land cover) was investigated using two-block Partial Least Squares (2B-PLS). Two-block PLS is a regression technique in which two multivariate sets of variables are decomposed into pairs of linear combinations that maximally account for the covariance between the original variables (Rohlf & Corti 2000). PLS resembles ordinary regression in that both methods produce coefficients that can be used to describe associations between two sets of variables. However, unlike regression, PLS does not specify a predictor-response structure between the sets of variables, but instead treats them symmetrically (Zelditch *et al.* 2004). Computationally, PLS is also similar to Principal Component Analysis (PCA) in that both methods perform an eigendecomposition of the variance-covariance matrix, and thus produce a series of orthonormal axes that each account for a progressively smaller proportion of the original information. The two methods differ, however, in that in PCA, the variance-covariance matrix among all variables is decomposed, thus producing a single set of eigenvectors, whereas PLS produces more than one set of eigenvectors, as it is based on the matrix of cross-covariances between variables in multiple data blocks (Zelditch et al. 2004). In two-block PLS, pairs of axes are produced, each with an associated singular value (λ_i) that measures the proportion of the squared covariance between the two data blocks that is captured by those axes. To assess consistency between blocks, correlations between corresponding PLS axes can be measured using a standard correlation coefficient. In each analysis, interpretation was restricted to the set of PLS axes capturing a cumulative total of 90% of the square covariance between the blocks.

To investigate the covariation between mandible shape and climate, two PLS analyses

were conducted: first, shape variables were regressed on a composite matrix comprising annual, winter, spring, summer, and fall average temperatures; and second, shape variables were regressed on a composite precipitation matrix containing total annual snow- and rainfall records. These two sets of climatic variables were analyzed separately because temperature and precipitation are expressed in different units, and standardization would not preserve their variation structure.

To investigate the pattern of covariance between mandible shape and geography and environment, two additional PLS analyses were conducted. Here shape variables were regressed on the geographical coordinates (i.e., latitude and longitude) of the midpoint of each population, and on the population environmental variable (defined by the relative abundance of the 20 land cover/use types identified in Fig. IV.1).

Analyses were performed in Matlab R2006a (The MathWorks, Inc.), using a combination of tools for multivariate and graphical analysis included in Matlab's Statistics Toolbox.

Results

Association between morphometric, geographical, ecological, and genetic distances Partial Mantel tests reveal different patterns of correlations between mandible shape and geographical, ecological, and genetic factors for the two lineages analyzed in this study (Table IV.1). Because of a very high positive correlation between the two metrics of genetic distance computed herein, i.e., Nei's *D* and *F*st (r = 0.98, P < 0.001), only the latter is reported. In the LP-EUP lineage, shape distances between populations are not significantly correlated with geographical, ecological, or genetic distances when the others factors are held constant (P >0.016; Table IV.1). In contrast, shape distances between populations in the WUP-WI lineage are

significantly correlated with all three of these factors when the others are held constant (P < 0.003, Table IV.1). Pooling the two lineages and repeating these tests results in high positive correlations between shape distances and both geographical and ecological distances (P < 0.001), and a non-significant positive correlation between shape and genetic distances (P = 0.03). The differences between lineages found here might be explained in part by the much larger WUP-WI sample (18 populations versus 6 for LP-EUP), but might also reflect historical and ecological differences between lineages.

The relationships between mandible size distances and geographical, ecological, and genetic distances are broadly consistent across lineages (Table IV.2). Size distances are significantly correlated with ecological distances (P < 0.001), but not with geological or genetic distances. This result suggests that mandible size, and possibly overall body size, is responsive to ecological differences, after geographical and genetic differences have been taken into account.

Despite significant positive correlations, there are no obvious patterns of correspondence between dendograms representing the three sets of distances for either lineage (Fig. IV.2), i.e., dendograms based on morphometric (shape), genetic, and ecological distances do not resemble one another.

Association between genetic and geographical and ecological distances

Geographical distances are positively and significantly correlated with genetic distances (based on pairwise F_{st}). This result is concordant with the pattern of isolation by distance suggested by genetic analyses (Chapter II). In contrast, pairwise genetic distances are not significantly correlated with ecological distances, after controlling for geographical distances, suggesting that environmental similarity does not affect gene flow in a predictable manner (Table IV.3).

Effect of climate on shape

Table IV.4 shows results from PLS analyses of multivariate covariation between shape and temperature variables within each lineage. For the LP-EUP lineage, PLS produces an essentially one-dimensional solution, where a single axis (labeled PLS1) explains 96% of the squared covariance between the two sets of variables, although only a small proportion of the square covariation is captured by those axes ($\lambda_1 = 0.0320$). This dimension receives similar contributions from most temperature variables, and therefore can be broadly interpreted as an axis of variation in overall temperature. The largest contribution is from winter temperature, which shows a slightly higher coefficient along this vector than the other temperature variables. A plot of the temperature dimension (y-axis) against the shape dimension (x-axis, Fig. IV.3a), contrasts mandible shape in localities with high temperature values (e.g., Charlevoix, CX) to that in localities with relatively low temperature values (e.g., Chippewa, CW). Increasing temperatures are associated with expansion of the angular and condyloid processes, contraction of the caudal end of the masseteric ridge, and slight contraction of the incisor alveolus.

For the WUP-WI data, PLS supports a two-dimensional solution representing covariation between shape and temperature variables, with both axes being required to account for 90% of the squared covariance (Table IV.4). The square covariation captured by those axes is also small $(\lambda_{1, LP-EUP} = 0.0105 \text{ and } \lambda_{1, WUP-WI} = 0.007)$. The first of these axes is very similar to PLS1 for the LP-EUP lineage, whereas the second contrasts temperature in winter to that in the warmer seasons. A plot of the temperature dimension against the shape dimension for PLS1 (Fig. IV.3b) contrasts mandible shape in localities with high temperature values (e.g., Buffalo, BF) to that in localities with relatively low temperature values (e.g., Gogebic, GO). Increasing temperatures are associated with a slight expansion of the condyloid; and contraction of the coronoid, the caudal margin connecting condyloid and angular processes, the masseteric ridge, and the molar alveolus. The temperature axis for PLS2 (Fig. IV.3c) contrasts localities where winters are milder and springs and summers are generally cooler (e.g., Marquette, MA), with localities where winters are severe and spring and summer temperatures are warmer (e.g., Buffalo, BF). Shape changes in transitioning from the former to the latter include the molar and incisor alveoli, and the caudal margin connecting condyloid and angular processes, the latter resembling variation in the coronoid process captured by PLS1 (Fig. IV.3c).

Analyses of covariation between shape and precipitation variables suggest that snowfall has consistently had a much stronger effect on mandible shape than rainfall in both the LP-EUP and WUP-WI lineages (Table IV.5). Increasing snowfall in LP-EUP populations is associated with contraction of the anterior angular and condyloid processes, expansion of the coronoid process, and expansion of the caudal end of masseteric ridge relative to the base of the mandible (Fig. IV.4). The opposite pattern is found in the WUP-WI, where increasing snowfall is associated with expansion of the condyloid process and molar alveolus, and contraction of the caudal end of masseteric ridge. A larger proportion of the square covariation between shape and precipitation is captured by these axes, in comparison to the covariation between shape and temperature captured by PLS ($\lambda_{1, LP-EUP} = 0.2413$ and $\lambda_2 = 0.1247$).

Effect of geographical location on shape

PLS analyses of covariation between shape and geographical variables produced contrasting results for the two lineages; in the LP-EUP, shape covaries almost exclusively with latitude, whereas in the WUP-WI, shape covaries strongly with longitude, and only weakly with latitude

(Table IV.6). The square covariation captured by those axes is also small ($\lambda_{1, LP-EUP} = 0.0029$; $\lambda_{1, WUP-WI} = 0.0048$ and $\lambda_{2, WUP-WI} = 0.0030$). As expected, the effect of latitude on mandible variation in LP-EUP populations is very similar to the effect of temperature (Figs. IV.3a and IV.5a). In WUP-WI, movement from east to west (i.e., PLS1, Fig. IV.5b) is associated with narrowing and straightening of the coronoid process and sharpening of the curvature of the caudal margin connecting the angular and condyloid processes. Unlike what is observed in LP-EUP populations, the effect of latitude in the WUP-WI lineage (i.e., PLS2, Fig. IV.5c) does not resemble the effect of temperature, but is instead characterized by a reduction in curvature of the caudal margin connecting the angular and condyloid processes, dorsal expansion of the molar alveolus in transitioning from southern (e.g., Adams, AD) to northern (e.g., Marquette, MA) localities (Fig. IV.5c).

Effect of land cover on shape

Results from a PLS analysis of covariation between shape and land cover variables are shown in Table IV.7. The 90% threshold of the squared covariance between the two factors for the LP-EUP lineage was obtained in two dimensions. The environments mostly responsible for the association between shape and land cover on PLS1 are northern hardwoods and pine, which appear to have opposite effects on mandible shape. On PLS2, northern hardwoods and pine both have high positive loadings that contrast with high negative loadings for spruce-fir and lakes.

The 90% threshold of the squared covariance between the two factors for the WUP-WI lineage was obtained in four dimensions. The dominant types in the first two dimensions (i.e., PLS1 and 2) are northern hardwoods and conifers, which show a similar pattern of covariation to

that found in the LP-EUP data. The third and fourth dimensions contrast central hardwoods to all other environment types (PLS3), and pine to lowland conifers and scrubs/ shrubs/wetlands (PLS4).

Plots of the land use dimension (y-axis) against the shape dimension (x-axis) suggest that vegetation type has a substantial effect on mandible shape (Fig. IV.6). In the LP-EUP lineage, there is a nearly linear gradient of environment-shape covariation for PLS1 (Fig. IV.6a), from localities characterized primarily by northern hardwoods (e.g., Charlevoix, CX) to those where pine is prevalent (e.g., Crawford, CR). The former habitats are associated with mandibles that are more robust, with more prominent processes, and a proximal contraction of the mandibular alveoli. PLS2 (Fig. IV.6b) contrast regions where hardwoods and pine are abundant (e.g. Chippewa, CW) to regions characterized by lakes and spruce-fir forests (e.g., Cheboygan, CH). Shape changes in transitioning from the former to the latter include expansion of the condyloid and angular processes and contraction of the caudal end of the masseteric ridge and coronoid process.

In the WUP-WI lineage, PLS1 (Fig. IV.6c) contrasts localities in which conifers are the prevalent vegetation and hardwoods are scarce (e.g., Marquette, MA) to those with the opposite configuration (e.g. Ontonagon, ON). Shape change along this axis is characterized by contraction of the angular and condyloid processes, posterior displacement of the caudal end of the masseteric ridge, contraction of the molar and ventral displacement of the frontal end of the masseteric ridge. PLS 2 (Fig. IV.6d) contrasts environments where pine is abundant (e.g., Ontonagon, ON, and Marquette, MA) to localities where northern hardwoods and spruce-fir prevail (e.g., Schoolcraft, SC). The change in mandible morphology along this axis includes expansion of the dorsal margin connecting the coronoid and the condyloid processes and the

caudal end of the masseteric ridge, and contraction of the condyloid, and molar and incisor alveoli.

The next dimension (PLS3, Fig. IV.6e) separates Marinette (MR) from all other populations, as the only one where central hardwoods are abundant. The major effects on mandible shape associated to this type of forest are increased curvature of the caudal margin connecting condyloid and angular processes, and contraction of the caudal end of the masseteric ridge, and of the molar and incisor alveoli, yielding a relatively shorter mandible. The last dimension (PLS4, Fig. IV.6f) separates populations where pine forests are more abundant (e.g., Schoolcraft, SC) from those where lowland conifers, scrubs/shrubs/wetland predominate (e.g., Outagamie, OU, and Buffalo, BF). Morphological changes along this axis include expansion of the coronoid process and of the curvature of the caudal margin connecting the angular and condyloid process, and contraction in the angular process.

In all cases, the square covariation captured by those axes is also small ($\lambda_{1, \text{ LP-EUP}} = 0.0025$ and ($\lambda_{2, \text{ LP-EUP}} = 0.0011$; $\lambda_{1, \text{ WUP-WI}} = 0.0012$ and $\lambda_{2, \text{ WUP-WI}} = 0.0010$, $\lambda_{3, \text{ WUP-WI}} = 0.0006$, $\lambda_{2, \text{ WUP-WI}} = 0.0005$).

Discussion

As shown in previous analyses, as much as 85% of the variation observed in the shape of mandible within each lineage was explained by differences among populations, including genealogy, expansion history (i.e., old established vs. newly founded), and characteristics of the populations, such as local ecology (Chapter III). In this Chapter, some aspects of the local ecology were analyzed to assess their role in the variation in shape observed across populations. As expected, results from this study show a consistent association between shape differentiation

and latitudinal distribution and temperature and precipitation variables, whereas land cover is unrelated to variation accumulated during the expansion process. The following discussion focuses on the relationship between those geographical and environmental variables and observed changes in the shape of the mandible, with particular emphasis on the potential role of these factors in explaining the differentiation between old and new populations.

Distance-based comparisons between shape and geographical, ecological, and genetic variables Partial correlations between morphometric distances and geographical, ecological, and genetic distances among populations in the LP-EUP lineage were not significant in any case, which suggests that similarity in mandible shape among populations is not strongly influenced by geographical proximity, environmental similarity, or gene flow. Alternatively, lack of statistical power due to small sample sizes could hinder the discovery of patterns of association among the examined variables, which remains plausible given the statistical significance of the same associations in the better-sampled WUP-WI lineage.

Results based on WUP-WI data suggest greater similarity in mandible shape among specimens from localities separated by shorter distances, after controlling for the effects of genetic and ecological distances. This pattern of positive correlation between morphometric and geographical distances suggests that gene flow has played a role in the process of phenotypic divergence, which is consistent with expectations of a model of isolation by distance, and further supported by observed positive correlations between morphometric and genetic distances (see below). Mice from ecologically similar localities also have mandibles that are more similar than mice from ecologically dissimilar localities, both in shape and size, after controlling for the effects of genetic and geographical distances. A number of factors might explain this association.

For example, mice inhabiting similar habitats are likely to exploit similar resources, which could lead to morphological similarity due to either shared epigenetic input and interactions during the development of the mandible (Atchley 1993; Atchley & Hall 1991). Similarity could be due to developmental plasticity (Schlichting and Pigliucci 1998), shared selective pressures (Rychlik *et al.* 2006), or both.

Morphometric and genetic distances in the WUP-WI lineage appear to be significantly correlated after partialing out the effects of ecological and geographical distances. This contrasts with previous results from pairwise correlation analyses, which showed these distances to be uncorrelated (Fig. IV.2; see also Chapter III). Similarly, pairwise correlations between morphometric and ecological distances are non-significant before partialing out the effects of genetic and geographic variation (r = 0.27, P = 0.33; data in Appendices IV and VI). In both cases, these results suggest a complex pattern of association between causal factors and phenotypic variation, which is likely a consequence of the high dimensionality of the latter. As demonstrated by PLS analyses (see below), different dimensions of mandible shape variation may be associated with disparate factors, and patterns of distances among populations may reflect the added effect of multiple causal agents. Thus, whereas shape differences over all possible dimensions may be uncorrelated with genetic or ecological factors, a multiple-predictor analysis such as the partial Mantel tests carried out herein suggests that there are dimensions of shape variation that, in fact, can be explained by either of these factors. Therefore, these results complement the findings reported in Chapter III by suggesting that at least some aspects of variation in mandible shape are consistent with expectations from phenotypic drift, whereas others are consistent with ecological differentiation.

Genetic and geographical distances show a significant positive partial correlation, after controlling for the effect of ecological distance, in agreement with previous results (Chapter II). Because genetic distances are based on mtDNA polymorphism, which is maternally inherited, this pattern of isolation by distance might be the consequence of the limited dispersal capabilities and philopatry of females in *Peromyscus leucopus*, which supports results from field studies that have found that dispersal in this species is male biased (Jacquot & Vessey 1995), as it is usually seen in mammals (Johnson 1986). The pattern of isolation by distance associated with morphometric distances is caused by a different process. For all morphometric analyses, males and females were pooled together, since there were no differences between sexes (Chapter III). Thus, that pattern is not caused by female philopatry, but might be explained by the fact that, in general, Pln does not typically disperse over great distances (Krohne *et al.* 1984; but see Maier 2002 for a report of an exceptional dispersion distance in this mouse).

The absence of association between genetic and ecological distances, after controlling for the effect of geographical distance, suggests that gene flow among these populations might not be limited by the characteristics of environments of this regions. This result is supported by evidence that *Peromyscus leucopus* is able to disperse over great distances (Maier 2002), and presumably exchange genes, regardless of whether the habitat is continuous or fragmented (Mossman& Waser 2001). However, this is not true for urbanized areas, like New York City, where populations have very limited dispersal ability and are genetically differentiated (Munshi-South & Kharchenko 2010).

Effect of climate, geography, and land cover on shape

In this study, Partial Least Squares regression was used to find the dimensions of shape variation

within each of the sampled lineages that most strongly covary with a number of geographical and environmental variables. These dimensions can be interpreted as components of shape variation that have been chosen to maximize the explanatory power of specific extrinsic predictors, in contrast to the distance matrix approaches discussed above in which associations are studied without prior decomposition.

Thus, even though comparisons based on distance matrices in the LP-EUP lineage returned no significant associations, PLS decomposition found directions in shape space that are highly correlated with climatic and environmental factors. Three of the considered predictors, temperature, precipitation, and geographical location, capture similar signals, with CW population bearing the most extreme scores for snowfall, average annual temperature, and latitude. Along these dimensions, the temperature and snowfall axes describe similar patterns of contraction of the angular and condyloid processes, accompanied by an anterior expansion of the alveoli and a dorsal expansion of the masseteric ridge at colder and high-precipitation localities. The latitude axis reflects some of the same patterns, i.e., contraction of the angular process coupled with dorsal displacement of the masseteric ridge at northernmost localities, whereas others, e.g., contraction of the condyloid process, were not seen.

The contraction/expansion of one or more mandibular processes is a recurrent pattern captured by these predictors. The mandibular processes were the regions of the mandible that captured most of the differences between old (LP) and new (CW) populations (Chapter III). Together, these results suggest that climate rather than vegetation may be the most important driver behind such divergence. In contrast, land cover (Table 7) captures the contrast between a few major types of environment and is not strongly associated with geographical location. The extreme value obtained for the CW population along the second axis, however, deserves further attention upon acquisition of additional samples from the UP region, which might reveal a more discrete separation between old versus new populations than is presently possible.

As previously discussed, distance analyses based on WUP-WI mandible data suggest that only some dimensions of shape variation are correlated with geographical distribution (i.e., geographical distances), whereas other dimensions are correlated with environmental features (i.e., ecological distances). PLS complements these results by providing estimates for some of these dimensions. The novel application of fine-grained land cover/use maps to explore the association between patterns of morphometric and habitat similarity has proven a valuable tool for gaining insight regarding details of habitat structure that could be responsible for gross patterns of similarity suggested by correlation analyses in this study.

Unlike PLS results for the LP-EUP lineage, PLS axes based on the WUP-WI shape data do not show a predominant contrast among populations or a recurrent motif of shape variation. Instead, predictors are associated with different, albeit partly overlapping, aspect of shape variation. For instance, annual snowfall is associated with variation in the posterior masseteric ridge, where average temperature has little effect, instead showing a marked influence on the coronoid and angular processes; on the other hand, both of these factors exert an effect on the molar alveolus.

The comparison between the mean shapes of old and new populations in the WUP-WI lineage (Chapter III, Fig. III.6) indicates that the most prominent changes in the new populations correspond to an expansion of the condyloid process, and a contraction of both the angular process and the molar alveolus. These changes closely correspond to dimensions of variation captured by oscillations in annual temperatures (Fig. IV.3a) and snow precipitation (Fig. IV.4a), and are only partly related to patterns of shape variation associated with latitude (Fig. IV.5c). A

simple ANOVA testing the similarity between old and new populations for the projections of morphometric data on the PLS axis of temperature, precipitation, and geographic predictors reveals that PLS1 significantly accounts for differences between these groups (F = 6.93, P =0.018), whereas PLS2 does not (F = 1.88, P = 0.190). Snowfall (captured by axis 2 of precipitation-shape PLS) fails to reject the null hypothesis of equality of means (F = 4.02, P =0.062), possibly due to the presence of localities with both extremely high and extremely low snowfall rates among the new populations, whereas most of the populations that define this axis (e.g., MA, ON, GO, AS, IR), are in fact new. Finally, latitude (F = 10.78, P = 0.005), but not longitude (F = 0.05, P = 0.827), shows an association with the expansion process. Shape variation associated with land cover/use, although displaying a contrast among environment types (i.e., hardwood vs. conifer forests), is largely unrelated to the expansion process of the LP-EUP or WUP-WI lineages, a result confirmed by ANOVAs on PLS scores ($P \ge 0.05$ in all axes).

Cold temperature and snow fall have also been causally linked to changes in the shape of the skull and mandible in the common shrew *Sorex araneus* over a period of only 27 years, presumably these reflect changes in foraging strategies (Poroshin *et al.* 2010). The effect of cold temperature on morphology has been investigated in several previous studies, especially the association between temperature gradients (or latitude) and postcranial morphology (Ashton *et al.* 2000; Heath 1984; Rae *et al.* 2006; Steegman & Platner 1968; Weaver & Ingram 1969). Craniofacial features change in response to cold temperature (Nicholson & Harvati 2006; Perez & Monteiro 2009; Poroshin *et al.* 2010; Rae *et al.* 2006; Steegman & Platner 1968). Those morphological changes can be induced very rapidly, as has been shown experimentally by Steegmann and Platner (1968). In their study, animals reared under cold stress (5° C) showed a reduction in the surface of insertion of masticatory muscle in comparison to animals grown under ambient temperature (22° C). Although those results might have limited explanatory power due to the simplicity of the analysis, they suggest possible changes in the mandible associated with colder temperatures. The results obtained in this study also show a reduction in mandibular processes, which are the places of insertion of masticatory muscles, in the new populations of Pln (from both lineages) that are found in colder localities.

Peromyscus leucopus is an omnivore that feeds mostly on seeds and arthropods (Wolff *et al.* 1985), and its dietary breadth is determined not only by the abundance (Ivan & Swihart 2000), but also the nutritional value of seeds (Lewis *et al.* 2001). Animals in colder environments have higher metabolic requirements that demand eating (and chewing) more often, and because of the scarcity of resources during the colder seasons, these animals might increase their diet breadth and/or feed on less-preferred resources during periods when their preferred foods are unavailable (i.e., fallback foods ; Lambert 2009; Marshall & Wrangham 2007). Hence, a possible explanation for the reduction in mandibular processes observed in specimens from new populations in colder climates could be that these animals are feeding on softer items (e.g., insects, softer-shell seeds) that might offer a higher net nutritious value. There is experimental evidence showing that animals fed on a soft diet show a significant reduction in mandibular processes reflecting the lower masticatory strength required to chew softer foods (Corruccini & Beecher 1982; Maki *et al.* 2002; Renaud *et al.* 2010), which is consistent with patterns observed in this study.

Even though land cover is a poor predictor of differences between old and new populations, more detailed community characterizations could help to expose causal local factors behind observed morphological divergence. An obvious candidate is diet, which is considered a determinant factor in the diversification of primates (Anapol and Lee 1994), bats (Nogueira *et al.*

2009; Monteiro and Nogueira 2010), and rodents ((Michaux *et al.* 2007; Samuels 2009). Incorporating detailed dietary information with geographical expansion and seasonal and nonseasonal climatic fluctuations, might significantly improve our understanding of the causal factors behind phenotypic divergence via local adaptation/acclimation to novel environmental regimes.

Conclusions

Analyses carried out in this study attempted to dissect patterns of morphometric and genetic variation in the light of geographical and environmental variables. Distance-based methods were used to test for broad associations among these variables, and then PLS regression was used to find actual directions of variation responsible for these associations. Together, these analyses suggest that among the considered factors, climatic variables, specifically temperature and snow precipitation, are the best predictors for the divergence between old and new populations. However, it remains unclear whether these factors have a direct effect on morphology, or if alternatively, their association with shape reflects the latitudinal component of the geographic expansion. Shape deformations suggest that the latter could be partly the case, but the majority of mandible changes associated with temperature and precipitation are unrelated to latitude. Besides these climatic factors, vegetation and land use variables failed to show an association with the expansion, suggesting that they instead contribute to non-geographical variation. Previous results showed that differences among populations explain over 80% of the overall variation in shape; the results reported here indicate that this component of variation might be partly accounted for by the differences in land features mapped in this study. These effects, however, account for disparate directions of variation. Given that the expansion process is a relatively recent
phenomenon, it is plausible that variation in shape is in fact highly noisy and difficult to predict based on regional-scale factors. Careful modeling and increasing the level of detail, for instance by adding species-level community information, could be a promising approach to explain morphological differences among populations.

I found substantial differences between the two lineages in their responses to geographical and environmental factors, further supporting previous findings that the expansion process has differed between them (Chapters 1 and 2). In the LP-EUP lineage, the observed association with climate variables is largely due to the extreme differences observed between the new (CW) and old populations (LP), not only in terms of genetic and morphological differentiation (see Chapters II and III), but also in relation to geographical and climatic factors. In contrast, in the WUP-WI lineage, old and new populations are not sharply differentiated, perhaps because they are more continuously distributed that in the LP-EUP lineage. In this case, the association between morphological and genetic variation and geographical and climatic factors is more gradual.

Have populations of Pln in the northern Great Lakes been tracking their habitat during their recent geographical expansion? These results suggest that morphological variation in the mandible has responded to climatic shifts encountered in the newly occupied localities, which counters the notion of habitat tracking. Similarly, other environmental factors, such as land cover/use, contribute to population differences, although such differences are unrelated to geographical expansion. Combined, these findings are consistent with the fact that both intrinsic (phenotypic, genetic) and extrinsic (geographical, environmental) factors comprise multifactorial phenomena, whereas the notion of habitat tracking is better suited for one-dimensional responses to one-dimensional environmental shifts. For instance, whereas climate encompasses such

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meteorological elements as temperature, precipitation, atmospheric pressure, wind, and humidity, phenotypes are also multidimensional and therefore can simultaneously respond to disparate selective pressures along multiple directions of variation, such as those found by PLS analyses. Therefore, the suggestion of some authors that range expansion in response to current climate change has been possible due to populations tracking the expansion of their habitat (Hughes 2000; Thomas *et al.* 2001; Walther *et al.* 2002; Salamin *et al.* 2010) is an oversimplification of a complex and multidimensional process for which passive tracking may be a reasonable assumption only for a limited set of traits.

Tables

Table IV.1. Relationship between mandible shape distances and geographical (Geo), ecological (Eco), and genetic (F_{st}) distances based on partial Mantel tests. See text for details about how distances were calculated. The correlation (r) is between shape distance and the corresponding distance variable after controlling for the other two factors. Tests of significance were based on 1,000 permutations. The Bonferroni-adjusted alpha level is 0.006. Significant correlations are indicated in boldface.

		Factor									
		Geo		Eco		Fst					
	r	Р	r	Р	r	Р					
LP-EUP	0.128	0.319	0.615	0.016	0.324	0.120					
WUP-WI	0.506	< 0.001	0.483	< 0.001	0.317	0.003					
Pooled	0.349	< 0.001	0.582	< 0.001	0.185	0.030					

Table IV.2. Relationship between mandible size distances and geographical (Geo), ecological (Eco), and genetic (F_{st}) distances base on partial Mantel tests. See text for details about how distances were calculated. The correlation (r) is between size distance and the corresponding distance variable after controlling for the other two factors. Significance tests were based on 1,000 permutations. The Bonferroni-adjusted alpha level is 0.006. Significant correlations are indicated in boldface.

		Factor									
		Geo		Eco		Fst					
	r	Р	r	Р	r	Р					
LP-EUP	0.154	0.284	0.870	0.002	0.015	0.450					
WUP-WI	0.140	0.075	0.386	< 0.001	0.178	0.073					
Pooled	0.128	0.097	0.409	< 0.001	0.092	0.191					

Table IV.3. Relationship between genetic distances based on pairwise F_{st} and geographical (Geo) and ecological (Eco) distances base on partial Mantel tests. . See text for details about how distances were calculated. The correlation (r) is between genetic distance and the corresponding distance variable after controlling for the other factor. Significance tests were based on 1,000 permutations. The Bonferroni-adjusted alpha level is 0.0083. Significant correlations are indicated in boldface.

	G	eo	Eco			
	r	Р	r	Р		
LP-EUP	0.912	0.004	-0.559	0.029		
WUP-WI	0.524	0.001	0.104	0.231		
Pooled	0.767	0.001	-0.121	0.151		

Townsonsteine	LP-EUP	WU	P- WI
Temperature variables –	PLS1	PLS1	PLS2
Annual Temp	0.442	0.442	0.063
Winter Temp	0.573	0.563	-0.676
Spring Temp	0.398	0.378	0.556
Summer Temp	0.350	0.392	0.470
Fall Temp	0.443	0.437	-0.096
Singular value	0.0320	0.0104	0.007
%Explained covariance	95.95	68.90	29.18
Correlation	0.94	0.66	0.75

Table IV.4. Results of a partial least-square analysis of the covariation between mean mandible shape and temperature for populations of *Peromyscus leucopus* in the LP-EUP and WUP-WI.

Presinitation variables	LP- EUP	WUP-WI
Frecipitation variables	PLS1	PLS1
Snowfall	0.998	1.000
Rainfall	0.069	-0.002
Singular value	0.2413	0.1247
%Explained covariance	99.91	99.87
Correlation	0.89	0.64

Table IV.5. Results of a partial least-square analysis of the covariation between mean mandible shape and precipitation for populations of *Peromyscus leucopus* in the LP-EUP and WUP-WI.

Casarranhiaslaurrishlas	LP-EUP	WUP	- WI
Geographical variables	PLS1	PLS1	PLS2
Latitude	1.000	0.081	-0.997
Longitude	-0.023	0.997	0.081
Singular value	0.0029	0.0048	0.0030
%Explained covariance	90.75	72.05	27.95
Correlation	0.82	0.80	0.79

Table IV.6. Results of a partial least-square analysis of the covariation between mean mandible shape and geographical coordinates for populations of *Peromyscus leucopus* in the LP-EUP and WUP-WI.

	LP- E	UP		WUP	-WI	
Land use variables	PLS1	PLS2	PLS1	PLS2	PLS3	PLS4
Residential	0.499	3.730	0.675	-1.984	-14.543	-1.656
Commercial, Services and Institutional	0.000	0.000	-3.303	-1.682	0.163	2.032
Transportation, Communication and Utilities	-1.074	-0.323	0.000	0.000	0.000	0.000
Extractive	0.000	0.000	0.657	0.372	-1.174	0.429
Open Land and Other	0.000	0.000	-8.492	-4.922	-0.322	4.701
Cropland	0.000	0.000	-1.694	-10.246	-0.563	-0.429
Permanent Pasture	1.651	3.178	-1.266	-7.513	1.635	1.900
Other Agricultural Land	0.000	0.000	-0.265	-1.229	0.374	0.260
Grasses and Forbs	-11.255	0.957	0.161	-1.381	-5.031	0.751
Shrubs	-1.977	-0.595	1.227	-2.590	-4.418	-7.295
Lakes	1.623	-42.156	0.243	0.746	-2.912	-0.105
Northern Hardwoods	77.360	44.481	70.939	53.334	22.530	-14.725
Central Hardwoods	0.000	0.000	2.797	10.953	-86.732	2.004
Aspen-Birch	-12.178	-27.590	-4.875	-13.975	22.076	-15.762
Lowland Hardwoods	0.000	0.000	3.734	-1.984	-3.762	9.870
Pine	-60.907	58.357	8.499	-53.552	10.158	-61.212
Other Upland Conifers (Spruce-Fir)	3.667	-45.025	-66.245	57.744	17.249	-28.484
Lowland Conifers	2.590	4.987	-16.611	-20.725	20.233	48.194

Table IV.7. Results of a partial least-square analysis of the covariation between mean mandible shape and land cover/use for populations of *Peromyscus leucopus* in the LP-EUP and WUP-WI.

Table IV.7. Continued.

Table I V.7. Continued.								
I and use merichles	LP- E	UP	WUP-WI					
Land use variables	PLS1	PLS2	PLS1	PLS2	PLS3	PLS4		
Scrub Shrub Wetland	0.000	0.000	7.060	-1.100	18.661	48.389		
Emergent Wetland	0.000	0.000	6.763	-0.268	6.379	11.140		
Singular value	0.0025	0.0011	0.0012	0.0010	0.0006	0.0005		
%Explained covariance	75.87	16.86	42.39	31.85	11.82	8.58		
Correlation	0.96	0.90	0.63	0.73	0.68	0.70		

Note: PLS vector coefficients multiplied x100 to facilitate interpretation. The largest loadings within vectors are indicated by boldface.

Figures



Land Use / Land Cover Classification



Figure IV.1. Land Cover/Use of populations sampled in this study. Each circle covers a radius of 454 m from the geographical midpoint of each locality. See text for additional information.

Morphometric Distances LP-EUP



Figure IV.2. UPGMA dendrograms based on morphometric, genetic, and ecological distances in LP-EUP and WUP-WI lineages.

Morphometric Distances WUP-WI



Distance (Euclidean)

Genetic Distances LP-EUP



Genetic Distances WUP-WI



Ecological Distances LP-EUP



Ecological Distances WUP-WI





Figure IV.3a. Correlation between temperature and shape on PLS1 for LP-EUP, and the corresponding deformation grid. Two-letter acronyms are as in Table A.IV.1.



Figure IV.3b. Correlation between temperature and shape on PLS1 for WUP-WI, and the corresponding deformation grid. Two-letter acronyms are as in Table A.IV.1.



Figure IV.3c. Correlation between temperature and shape on PLS2 for WUP-WI, and the corresponding deformation grid. Two-letter acronyms are as in Table A.IV.1.



Figure IV.4a. Correlation between precipitation and shape on PLS1 for LP-EUP, and the corresponding deformation grid. Two-letter acronyms are as in Table A.IV.1.



Figure IV.4b. Correlation between precipitation and shape on PLS1 for WUP-WI, and the corresponding deformation grid. Two-letter acronyms are as in Table A.IV.1.



Figure IV.5a. Correlation between latitude and shape on PLS1 for LP-EUP, and the corresponding deformation grid. Twoletter acronyms are as in Table A.IV.1.



Figure IV.5b. Correlation between longitude and shape on PLS1 for WUP-WI, and the corresponding deformation grid. Two-letter acronyms are as in Table A.IV.1.



Figure IV.5c. Correlation between latitude and shape on PLS2 for WUP-WI, and the corresponding deformation grid. Two-letter acronyms are as in Table A.IV.1.



Figure IV.6a. Correlation between land cover/use and shape on PLS1 for LP-EUP, and the corresponding deformation grid. Twoletter acronyms are as in Table A.IV.1.



Figure IV.6b. Correlation between land cover/use and shape on PLS2 for LP-EUP, and the corresponding deformation grid. Twoletter acronyms are as in Table A.IV.1.



Figure IV.6c. Correlation between land cover/use and shape on PLS1 for WUP-WI, and the corresponding deformation grid. Twoletter acronyms are as in Table A.IV.1.



Figure IV.6d. Correlation between land cover/use and shape on PLS2 for WUP-WI, and the corresponding deformation grid. Twoletter acronyms are as in Table A.IV.1.



Figure IV.6e. Correlation between land cover/use and shape on PLS3 for WUP-WI, and the corresponding deformation grid. Twoletter acronyms are as in Table A.IV.1.



Figure IV.6f. Correlation between land cover/use and shape on PLS4 for WUP-WI, and the corresponding deformation grid. Twoletter acronyms are as in Table A.IV.1.

APPENDIX

Table A.IV.1. Temperature and precipitation variables and coordinates of the geographical midpoint for all populations in this study. Temperatures are in Fahrenheit degrees; snow and rain precipitations in mm^2 . In Timing, O and N stand for old and new, respectively. *l* is Simpson's Index of Dominance.

Population	ID	Timing	Lat	Long	Annual T	Winter T	Spring T	Summer T	Fall T
Adams	AD	0	43.9012	-89.8676	44.50	18.30	44.63	68.10	46.87
Ashland	AS	Ν	46.1568	-90.4755	40.90	13.77	41.20	65.10	43.33
Bayfield	BY	Ν	46.3705	-91.3376	41.60	14.53	41.30	66.00	44.57
Buffalo	BF	0	44.5892	-91.8022	45.70	18.23	46.43	69.93	48.10
Charlevoix	CX	0	45.2615	-84.8037	44.80	22.93	42.67	65.70	48.03
Cheboygan	CH	0	45.5634	-84.6637	42.50	19.87	39.03	64.67	46.50
Chippewa	CW	Ν	46.4320	-84.7266	39.00	15.83	36.40	61.00	42.90
Crawford	CR	0	44.7888	-84.7340	41.90	18.50	39.87	64.30	44.80
Delta	DE	Ν	46.0788	-86.8345	42.00	18.83	38.67	64.30	46.17
Emmet	EM	0	45.5431	-85.0478	43.70	22.10	40.13	64.73	47.67
Gogebic	GO	Ν	46.3437	-89.3678	39.00	12.83	38.03	63.00	42.30
Iron	IR	Ν	46.3579	-90.3774	39.60	13.43	38.80	63.13	43.03
Marathon	MH	0	44.8175	-89.7509	43.60	16.90	43.67	67.83	46.00
Marinette	MR	0	45.5900	-87.9464	41.30	15.63	40.73	64.90	43.77
Marquette	MA	Ν	46.8736	-87.8958	43.00	20.87	40.50	64.13	46.40
Menominee	MN	0	45.1580	-87.7000	44.60	19.83	43.00	68.00	47.67
Oneida	OD	Ν	45.8709	-89.6527	39.20	12.97	38.43	63.13	42.23
Ontonagon	ON	Ν	46.7364	-89.7394	42.40	18.40	41.17	64.67	45.53
Otsego	OT	0	45.0549	-84.5688	43.30	20.00	41.60	65.40	46.07
Outagamie	OU	0	44.3961	-88.6663	44.20	18.10	43.90	68.10	46.80
Portage	PO	0	44.5412	-89.5654	43.10	16.43	43.07	67.43	45.40
Schoolcraft	SC	Ν	46.0886	-86.2278	43.20	19.23	41.40	65.67	46.47
Taylor	TA	0	45.3127	-90.6268	41.10	14.13	41.83	64.73	43.80
Wood	WO	0	44.3785	-90.1090	44.30	18.20	44.77	67.53	46.87

Table A.IV.1. Continued.

Population	ID	Timing	Snow	Rain	l
Adams	AD	Ο	50.10	33.28	0.3068
Ashland	AS	Ν	41.20	32.09	0.7482
Bayfield	BY	Ν	68.10	34.25	0.5013
Buffalo	BF	Ο	47.80	33.56	0.3938
Charlevoix	CX	Ο	106.50	32.14	0.9761
Cheboygan	CH	Ο	90.00	29.56	0.3664
Chippewa	CW	Ν	187.80	35.80	0.4908
Crawford	CR	Ο	104.70	33.42	0.3550
Delta	DE	Ν	46.70	28.53	0.7972
Emmet	EM	Ο	120.80	31.15	0.5026
Gogebic	GO	Ν	98.30	30.34	0.2533
Iron	IR	Ν	139.40	34.40	0.3088
Marathon	MH	Ο	59.10	33.36	0.2216
Marinette	MR	Ο	53.10	29.83	0.6876
Marquette	MA	Ν	120.90	30.03	0.8937
Menominee	MN	Ο	53.70	32.40	0.3139
Oneida	OD	Ν	58.00	32.09	0.7490
Ontonagon	ON	Ν	187.80	33.56	0.9672
Otsego	OT	Ο	149.60	36.59	0.3178
Outagamie	OU	Ο	42.50	30.82	0.6242
Portage	PO	Ο	44.50	32.13	0.2480
Schoolcraft	SC	Ν	97.90	30.76	0.5195
Taylor	TA	Ο	56.90	32.81	0.3712
Wood	WO	0	41.30	31.92	0.1808

Adams	0											
Ashland	0.45	0										
Bayfield	0.46	0.07	0									
Buffalo	0.82	0.97	1.00	0								
Charlevoix	0.48	0.01	0.13	1.00	0							
Cheboygan	0.52	0.22	0.12	1.00	0.29	0						
Chippewa	0.13	0.17	0.21	1.00	0.20	0.32	0					
Crawford	0.28	0.87	0.87	1.00	0.88	0.89	0.41	0				
Delta	0.46	0.00	0.09	0.99	0.01	0.24	0.18	0.87	0			
Emmet	0.45	0.07	0.07	1.00	0.11	0.20	0.20	0.78	0.08	0		
Gogebic	0.64	0.49	0.28	0.88	0.58	0.30	0.57	0.85	0.51	0.36	0	
Iron	0.53	0.29	0.22	0.86	0.36	0.29	0.37	0.72	0.31	0.10	0.23	0
Marathon	0.81	0.76	0.76	0.94	0.78	0.78	0.76	0.92	0.77	0.75	0.86	0.79
Marinette	0.98	1.00	1.00	1.00	1.00	1.00	0.98	0.97	1.00	1.00	1.00	1.00
Marquette	1.00	0.91	0.63	0.99	0.99	0.63	0.97	1.00	0.94	0.84	0.37	0.73
Menominee	0.34	0.15	0.17	0.90	0.21	0.24	0.17	0.74	0.15	0.16	0.44	0.30
Oneida	0.98	0.87	0.59	0.97	0.95	0.57	0.93	0.99	0.90	0.80	0.33	0.69
Ontonagon	0.48	0.01	0.13	0.99	0.00	0.29	0.20	0.88	0.01	0.11	0.59	0.36
Otsego	0.46	0.19	0.21	0.93	0.24	0.30	0.27	0.79	0.19	0.20	0.46	0.33
Outagamie	0.94	0.95	1.00	0.49	1.00	1.00	1.00	1.00	0.94	1.00	0.73	0.93
Portage	0.49	0.28	0.26	0.86	0.34	0.33	0.34	0.86	0.29	0.26	0.46	0.35
Schoolcraft	0.36	0.99	0.99	1.00	0.99	0.99	0.50	0.06	0.99	0.87	0.90	0.77
Taylor	0.45	0.21	0.25	0.70	0.27	0.33	0.32	0.89	0.21	0.24	0.38	0.34
Wood	0.29	0.71	0.70	0.99	0.74	0.67	0.38	0.30	0.72	0.70	0.82	0.74

Table A.IV.2. Matrix of pairwise ecological distances, $(1-I_M)$, were I_M is Morista's Index of community similarity.

Table A.IV.2. Continued

Adams												
Ashland												
Bayfield												
Buffalo												
Charlevoix												
Cheboygan												
Chippewa												
Crawford												
Delta												
Emmet												
Gogebic												
Iron												
Marathon	0											
Marinette	0.99	0										
Marquette	1.00	1.00	0									
Menominee	0.66	0.99	0.99	0								
Oneida	0.99	1.00	0.01	0.96	0							
Ontonagon	0.78	1.00	1.00	0.20	0.96	0						
Otsego	0.62	0.98	0.99	0.07	0.96	0.24	0					
Outagamie	1.00	1.00	0.96	0.75	0.96	1.00	0.73	0				
Portage	0.73	1.00	0.94	0.16	0.91	0.34	0.19	0.80	0			
Schoolcraft	0.97	0.97	1.00	0.88	1.00	0.99	0.99	1.00	0.99	0		
Taylor	0.78	1.00	0.94	0.09	0.91	0.27	0.12	0.44	0.21	0.99	0	
Wood	0.61	0.94	1.00	0.55	0.98	0.74	0.61	1.00	0.61	0.41	0.72	0
Adams	0.0											
-------------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
Ashland	255.3	0.0										
Bayfield	297.8	70.4	0.0									
Buffalo	172.0	202.8	201.4	0.0								
Charlevoix	428.5	451.4	521.0	555.9	0.0							
Cheboygan	450.6	454.8	523.4	570.7	35.3	0.0						
Chippewa	491.4	442.7	506.8	587.9	130.3	96.7	0.0					
Crawford	419.9	472.7	543.0	559.0	52.8	86.3	182.7	0.0				
Delta	339.8	280.8	347.9	422.1	182.1	177.7	166.8	217.8	0.0			
Emmet	422.2	425.8	494.7	540.8	36.7	30.0	101.9	87.4	150.7	0.0		
Gogebic	274.4	87.7	151.2	272.2	373.6	373.8	356.1	399.9	197.1	345.6	0.0	
Iron	276.1	23.6	73.7	225.9	448.8	450.3	433.3	472.5	274.3	421.8	77.5	0.0
Marathon	102.3	159.3	212.3	164.1	391.8	407.1	429.9	395.8	267.2	377.3	172.3	178.1
Marinette	241.4	205.7	276.0	322.5	247.9	255.5	265.7	267.0	101.9	225.7	138.2	206.3
Marquette	364.6	212.9	268.7	395.5	298.4	288.2	246.8	337.2	120.1	264.4	126.9	198.0
Menominee	221.5	242.6	312.7	329.3	227.2	241.5	270.5	236.9	122.5	211.6	184.7	246.9
Oneida	219.7	71.0	141.2	220.5	383.4	388.8	384.5	402.8	219.0	359.4	57.0	77.8
Ontonagon	315.4	85.6	128.8	287.6	414.9	412.1	384.5	444.5	234.4	385.0	52.1	64.4
Otsego	439.4	475.4	545.3	572.7	29.4	57.0	153.6	32.3	209.9	66.0	399.2	473.6
Outagamie	110.5	241.6	302.8	249.7	319.4	340.5	381.8	314.4	235.7	311.9	223.4	255.8
Portage	75.1	193.2	245.9	177.3	383.4	401.4	431.7	383.0	273.5	372.0	201.0	211.7
Schoolcraft	375.5	327.4	394.2	466.4	143.9	134.5	121.6	185.7	46.8	109.7	243.2	320.6
Taylor	168.1	94.6	129.9	122.6	455.5	466.0	473.3	466.5	306.6	436.0	150.5	117.8
Wood	56.5	199.8	241.4	136.3	429.7	448.1	478.1	428.0	318.5	418.7	226.1	221.1

 Table A.IV.3. Matrix of pairwise geographic distances, in km.

Table A.IV.3. Continued.

Adams												
Ashland												
Bayfield												
Buffalo												
Charlevoix												
Cheboygan												
Chippewa												
Crawford												
Delta												
Emmet												
Gogebic												
Iron												
Marathon	0.0											
Marinette	165.4	0.0										
Marquette	270.0	142.8	0.0									
Menominee	165.7	51.7	191.4	0.0								
Oneida	117.4	136.1	174.9	171.6	0.0							
Ontonagon	213.4	187.9	141.1	235.9	96.5	0.0						
Otsego	408.7	270.7	327.1	246.0	406.7	441.6	0.0					
Outagamie	97.8	144.3	281.9	114.0	181.3	273.3	331.9	0.0				
Portage	34.0	172.5	289.9	162.3	148.0	244.5	398.3	73.2	0.0			
Schoolcraft	309.0	144.2	154.7	154.3	265.7	278.7	172.9	268.1	312.6	0.0		
Taylor	88.1	211.3	272.9	229.8	98.0	172.5	475.5	185.1	119.7	352.3	0.0	
Wood	56.5	217.0	326.4	209.0	169.8	263.8	444.1	114.7	46.8	358.4	111.6	0.0

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