ABSTRACT

EFFECTS OF RANGE CONTRACTION AND HABITAT FRAGMENTATION ON GENETIC VARIATION OF THE WOODLAND DEER MOUSE (*PEROMYSCUS MANICULATUS GRACILIS*)

by Sarah E. Curry

In recent years, the range of *Peromyscus maniculatus gracilis* in Michigan’s Lower Peninsula (LP) contracted simultaneously with the northward expansion of the closely related *Peromyscus leucopus*. Recent trapping shows that the range of *P. m. gracilis* has shrunk to two remaining areas: one in Cheboygan and Otsego Counties and another to the east in Alpena Co. (AP). I used a Geographic Information System habitat analysis to predict where *P. m. gracilis* would be found across the region, trapped at selected locations, and analyzed 11 microsatellite loci for all individuals. Genetic diversity and bottleneck analyses indicate that LP populations once had good gene flow and were continuous across the area; however, trapping data and assignment tests show that the AP population is in the process of becoming geographically and genetically isolated from all other populations. The AP population is currently genetically viable, but is showing signs of becoming genetically distinct from the western populations.
EFFECTS OF RANGE CONTRACTION AND HABITAT FRAGMENTATION ON GENETIC VARIATION OF THE WOODLAND DEER MOUSE
(Peromyscus maniculatus gracilis)

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DEDICATION

To my parents, Jim and Nancy, for your unwavering encouragement, love, and support.
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INTRODUCTION

Overview

Ever since the Laurentide ice sheet receded from the Great Lakes region approximately 11,000 years ago, the terrestrial landscape has remained fragmented into large peninsulas and numerous islands (Dyke et al. 2002). The glacial recession was quickly followed by the development of forests throughout the region; these forests are primarily of a mixed conifer-hardwood type, forming a transition zone between the conifer-based northern boreal forests and the southern deciduous forests. This transitional zone across the middle of the Great Lakes has been a range limit for many species of forest-dwelling mammals in the region (Myers et al. 2005, 2009).

In historical times, deforestation and climate change have affected the vegetation and small mammal distribution within the region. Deforestation and patterns of human settlement through the late 19th/early 20th centuries (Botti & Moore 2006) fragmented the habitat available to forest-dwelling animals into relatively small stands. This large-scale destruction of forests ended about eighty years ago, and since then human land use has resulted in a fairly stable mixture of well-grown secondary forest, wetlands, and agricultural fields throughout the area. More recently, however, climate warming has become evident in the region—for example, daily maximum and minimum temperatures across Michigan’s Upper Peninsula (UP) increased significantly from 1970 to 2005 (mean increase of 2.1°C; Myers et al. 2009). Another indication of regional warming is provided by the dates of ice break-up taken from Grand Traverse Bay weather station, which is located on the northwest coast of the Lower Peninsula (LP) of Michigan. The date of ice break-up has become progressively earlier over the past 110 years, and the number of ice-free years increased from 24% during the 20th century to 78% from 2000 to 2009 (modified from Myers et al. 2005).
Possibly in response to recent climate warming, the small mammal species that are characteristic of the transitional and boreal forests are being displaced by the northward population expansion of southern deciduous forest species (Myers et al. 2009). Examples of this include the replacement of the woodland deer mouse (*Peromyscus maniculatus gracilis*), northern flying squirrel (*Glaucomys sabrinus*), and least chipmunk (*Tamias minimus*) by the white-footed mouse (*P. leucopus*), southern flying squirrel (*G. volans*) and eastern chipmunk (*T. striatus*), respectively, throughout the northern part of Michigan (Myers et al. 2009).

Of particular interest to my research is *P. m. gracilis*, whose range appears to be contracting simultaneously with the northward expansion of the closely related *P. leucopus*. *P. m. gracilis* and *P. leucopus* are both forest-dwelling mice that are very similar morphologically and ecologically, and that co-inhabit many forest patches. Despite their many similarities, the two species show marked differences in their winter adaptations; *P. m. gracilis* builds larger and better-insulated nests, stores more food, and utilizes torpor more efficiently than *P. leucopus* (Pierce & Vogt 1993). *P. m. gracilis* thus has significantly better overwinter survival in cold climates with harsh winters, whereas *P. leucopus* tends to flourish where winters are more mild (Wolff 1996). It is thus possible that shorter/warmer winters in Michigan have facilitated the decline of *P. m. gracilis*, while allowing *P. leucopus* to reproduce successfully and replace it ecologically at higher latitudes (Wolff 1985a; 1985b; Myers et al. 2005; 2009), though direct competitive displacement of one species by the other has not been demonstrated.

*P. m. gracilis* was historically the most common woodland mouse in the Great Lakes transitional forest, whereas *P. leucopus* has dominated the deciduous forests to the south (Hall 1981; Baker 1983). In Michigan, *P. m. gracilis* ranged across the entire UP and the northern half of the LP, south to around the 45th parallel (Figure 1; Hall 1981; Myers et al. 2009). *P. leucopus* was historically common throughout the LP but was found only in the southernmost corner of
the UP (Hall 1981). In the last few decades, *P. leucopus* has substantially increased in abundance across the northern LP (Myers 2009), and has expanded its range northward by about a hundred kilometers to become common throughout most of the UP (Myers et al. 2005, 2009). In addition to the expansion observed in Michigan, *P. leucopus* has also moved northward in other parts of the Great Lakes region (Long 1996; Deitloff et al. 2010). In Wisconsin, the range of *P. leucopus* began to expand northward in the 1970’s, concurrent with the decline of *P. m. gracilis* in that state (Long 1996). In northern Minnesota, *P. leucopus* has displayed an overall increase in population sizes over the past couple of decades; however, the effects of this on *P. m. gracilis* in Minnesota have not been analyzed (Deitloff et al. 2010).

In this study, I will examine the relative status of *P. m. gracilis* in the northern LP of Michigan and observe the genetic consequences of what has happened to this declining species. The findings will serve as a model of what can happen to small mammals as conditions change and the range of a species is constricted.

**Background**

**Current distribution of *Peromyscus* across the LP of Michigan**

The combination of warmer temperatures and the increasing numbers of *P. leucopus* may explain the decline in *P. m. gracilis* populations in the LP of Michigan. Currently, there are only two known areas in the LP that harbor *P. m. gracilis* (Figure 2). Several populations are located in and around the Pigeon River State Forest, within Cheboygan and Otsego Cos., in the north-central part of the LP. Within this well-forested area of approximately 230 km², five specific sites have been trapped yearly since 2001, while three additional sites have been trapped less frequently, and both *P. m. gracilis* and *P. leucopus* have been consistently present at all the sites. This general area will subsequently be referred to as the CH/OT region. A few *P. m. gracilis*
were also trapped in 2005 and 2009 in a small forested area in western Alpena Co. (AP). Covering a total area of approximately 0.2 km$^2$, this Alpena Co. South Swamp site is located approximately 50 km east of the Pigeon River State Forest. Eleven other sites from across the northern LP that used to support *P. m. gracilis* have been retrapped within the last ten years, with only *P. leucopus* being caught at those locations.

Historical trapping records have shown that *P. m. gracilis* was widely distributed across this region of the LP less than a century ago (Figure 1; Myers *et al.* 2009). Though it appears that *P. m. gracilis* is currently restricted to the CH/OT and AP regions of the LP, very little trapping has been done recently between the two locations, even though a large amount of fairly continuous forest exists across the area. *P. m. gracilis* was trapped in 1921 near Valentine Lake, in 1940 at Clear Lake State Park, and in 1940 at the Hunt Creek Research Station, all locations that are between the CH/OT and AP regions. Also, a site near Camp 30 Road was trapped in 2003 and was found to contain a few *P. m. gracilis*. The Hunt Creek Research Station has since been retrapped, with no *P. m. gracilis* found (P. Myers, pers. comm.). Forests near the other historical sites were trapped as part of this study.

**Current distribution of forest habitats**

Most of the land between the CH/OT and AP areas is a patchwork of agricultural fields and second-growth forest. Patches of forested land are well distributed across the area except for an almost treeless band about 8 km wide that runs north to south, about 5 km west of the AP site, subsequently referred to as the farmland belt. This habitat break is composed of agricultural fields, open land with herbaceous plant cover, and other habitat types that are unlikely to harbor any *P. m. gracilis* or *P. leucopus*. The forest patches on either side of this break are substantially different: forests west of the habitat break are primarily well-drained upland deciduous and
mixed forest, and are expected, based on past trapping records, to be optimal *P. m. gracilis* habitat. The forests to the east of the habitat break are primarily lowland deciduous and coniferous types that mostly occur within a matrix of non-forested wetlands. Although these eastern forest patches are not typical *P. m. gracilis* habitat, the existence of the Alpena population demonstrates that this forest type can indeed support deer mice.

In Michigan, both species of mice are often caught in the same patches of forest. *P. m. gracilis* tends to thrive best in dense upland forests that include maple, yellow birch, beech, and other hardwoods (Hooper 1942), but a survey conducted in Charlevoix Co. and surrounding counties in the 1920’s reported that *P. m. gracilis* was also found in bogs, conifer swamps, jackpine, and various virgin and second-growth hardwood forests (Dice 1925). In the UP, *P. m. gracilis* has been found in mature upland forest in Marquette and Baraga Cos. (Manville 1949; Robinson & Werner 1975), conifer swamps and upland deciduous forest in Alger and Schoolcraft Cos. (Ozoga & Phillips 1968), isolated woodlots and surrounding open habitat of lichen-grass associations in the Kingston Plains of Alger Co. (Fitch 1979), and various forest and shrub habitats in Ontonagon and Gogebic Cos. (Dice & Sherman 1922). *P. leucopus* has also been found in these different types of forest, except for the wettest types (Osgood 1909; Wrigley 1969).

In addition to the forest types that they share, *P. leucopus* has been found to thrive in other habitats where *P. m. gracilis* is seldom or never found. These are habitats that are near forest stands and that are primarily used for foraging, and they include prairies, beaches, fields, hedgerows, and sandy brushland (Getz 1961; Long & Long 1993; Long 1974, 1978; Jackson 1961). Historically in the Great Lakes region, *P. leucopus* is mostly strongly associated with oak-hickory forests with a dense understory (Allen 1938; Burt 1940; Getz 1961). It is also associated with other well-grown forest types in the LP (Myers et al. 2009), including pine
barrens and coniferous swamps in Montmorency Co. (Green 1925). It must be noted, however, that older trapping records cannot always be relied upon to correctly assign mice to a particular habitat, due to the difficulties in accurately discriminating between the two similar species.

*Peromyscus* dispersal

Forest habitats range from small to large patches across the LP of Michigan; therefore, it is important to understand the vagility and dispersal ability of *P. m. gracilis* in order to determine how the species is affected genetically by habitat fragmentation. Mice in the genus *Peromyscus* have repeatedly been shown to migrate well across poor habitat; for example, the genetic structure of *P. leucopus* is not significantly affected by the extreme fragmentation of forest habitats in northwest Indiana (Mossman & Waser 2001) and Ohio (Cummings & Vessey 1994; Anderson & Meikle 2010). In general, *Peromyscus* are able to readily disperse across unsuitable habitats such as open fields and swamps (Cooke & Terman 1977; Cummings & Vessey 1994; Krohne & Hock 1999). Members of the genus have also been known to swim short distances and to cross major rivers (Sheppe 1965; Mossman & Waser 2001). With regard to the effect of roads on *Peromyscus* movement across a landscape, Rytwinski & Fahrig (2007) found a positive relationship between road densities and the abundance of *P. leucopus*, while McDonald & St. Clair (2007) found that roads do not appear to be barriers for *P. maniculatus*. In addition, individual *P. leucopus* have demonstrated the ability to travel nearly 700 m within a two-night period (Krohne *et al.* 1984) and to disperse several kilometers within a period of months (Maier 2002).
Genetic consequences of population fragmentation

It is presumed that when *P. m. gracilis* was widely distributed across the LP (Figure 1), populations had extensive gene flow between them (Myers *et al.* 2009; Taylor & Hoffman 2010). If there is currently good gene flow between continuous populations in the LP, it is expected that the populations would have uniform genetic diversity, few or no private alleles, and low levels of genetic differentiation, so that individual mice could not be confidently assigned to specific populations. If, however, there is not substantial gene flow between populations distributed across the LP, they should show non-uniform genetic diversity, characteristic private alleles, elevated levels of differentiation, and high levels of genetic structure.

Comparisons of genetic diversity are used to obtain information about current or recent population sizes and connectivity. Mouse populations that become small and isolated can lose genetic diversity by having reduced gene pools (Amos & Harwood 1998; Frankham, Ballou, & Briscoe 2002). Taylor and Hoffman (2012) found uniformly high levels of genetic diversity in earlier samples of *P. m. gracilis* from the CH and AP populations. These levels are very similar to those seen in *P. m. gracilis* populations throughout the UP, where populations are separated by distances comparable to the distance between the CH and AP mice, but are located within more continuous forests. However, these analyses were conducted using a very limited sample (n=11 individuals) of the AP population and must be considered preliminary.

Genetic differentiation measures are used to measure the extent to which populations are separated. If there is currently little to no gene flow across the LP, then the smaller AP population should be in the process of becoming genetically distinct from other populations due to drift or inbreeding. If significant gene flow is still occurring, however, differentiation should remain relatively low between all populations in the region. Compared to most mammals, *Peromyscus* populations typically display relatively low levels of genetic differentiation.
(Mossman & Waser 2001; Vucetich et al. 2001; Anderson & Meikle 2010; Yang & Kenagy 2009), presumably due to the extraordinary vagility of these mice. Taylor and Hoffman (2012) recently examined levels of differentiation between populations within and across the UP, LP and various islands of Michigan. Differentiation was found to be low for *P. m. gracilis* between the CH/OT and AP sites, which is what would be expected if gene flow between these populations is ongoing. This comparison between LP mice must also be considered preliminary, however, since the sample size from the Alpena site was relatively small and obtained from a single very limited area (Taylor & Hoffman 2012).

Measures of private allelic richness show the number of alleles that are unique to a population. A high frequency of private alleles within a population can indicate that gene flow with other populations is highly restricted (Slatkin 1985). A recent study (Munshi-South & Kharchenko 2010) analyzed private allelic richness of isolated *P. leucopus* populations and found that the geographically farthest and most isolated population contained the highest number of private alleles. The actual distance between that population and the other populations in that study is similar to the distance seen between the AP and CH/OT populations in Michigan’s LP. If the AP population is genetically isolated and possibly inbreeding, it would be expected to have a higher number of unique alleles than do other populations.

Assignment tests are used to examine population structure and current gene flow through the assignment of individuals to populations or clusters (Paetkau et al.1995). Immigration can be identified when an individual’s genotype is associated with a different population from that within which it was found. If extensive gene flow is currently occurring between the LP populations, individuals from each population should show a mixture of assignments or ambiguous assignments to two or more populations. The more geographically isolated the populations are, however, the more distinct the genetic structure will be between populations.
Taylor and Hoffman (2012) used several mice from the CH and AP populations when performing assignment tests that compared *P. m. gracilis* across northern Michigan. However, because these analyses were performed on a much larger geographical scale, results are not particularly relevant to what is happening among the LP populations.

**GIS habitat analysis**

A Geographic Information System (GIS) is a computer-based system for storing, managing, and displaying geospatial data (Chang 2010). Since the late 1970’s, GIS has become an important tool in many fields, including ecology, conservation biology, and natural resource management (Chang 2010). Although habitat-related GIS analyses are primarily used for correlational or managerial purposes (e.g. Arbuckle & Downing 2002; Carrière *et al.* 2006; Kashaigili *et al.* 2006; Liu *et al.* 1995), some studies have used GIS as a predictive tool for determining current and future species distributions (e.g. Bloomberg *et al.* 2009; Joy & Death 2004; Kumar *et al.* 2009; Peterson 2001; Sperduto & Congalton 1996). In addition, GIS has been used in several recent studies to make range predictions for endangered mammal species (Doko 2011; Falcucci *et al.* 2008; Smith *et al.* 2012). This study gives us a chance to potentially test the predictive power of GIS to locate sites that are likely to support additional populations of *P. m. gracilis* in the LP. By analyzing the forest types that are currently inhabited by *P. m. gracilis*, GIS can be used to compare habitat patches between the CH/OT and AP sites and to rank their potential for supporting *P. m. gracilis* populations.

**Research Objectives**

The first research objective for this study was to use GIS habitat analysis to predict where *P. m. gracilis* is most likely to be found in between the CH/OT and AP locations. Although all
selected forest locations were likely to be inhabited by *P. leucopus*, I aimed to optimize for the presence of *P. m. gracilis* over *P. leucopus*. Most of the selected locations were trapped during the summer to 2011 to determine if any *P. m. gracilis* remain.

Based on the wide range of possibilities for if and where *P. m. gracilis* might be found, three general scenarios were considered regarding the distribution of the species across the study region. The first scenario is where *P. m. gracilis* populations are distributed across most or a significant fraction of the area between the known CH/OT and AP sites. The second scenario is where one or more *P. m. gracilis* populations would be found in between the known sites; however, they would not be well distributed across the corridor between the CH/OT and AP sites. The third scenario is where no *P. m. gracilis* would be found at any of the targeted locations between the CH/OT and AP sites. Each of these scenarios would lead to different expectations with regard to the distribution of genetic variation among the remaining populations of *P. m. gracilis* in the northern LP.

After initially using GIS as a predictive tool, I then performed more advanced GIS analyses with the goal of making finer-scale discriminations between the habitat types than could be done using the preliminary habitat classifications. These analyses were used to focus our efforts during a second trapping trip on sites that were most likely to harbor *P. m. gracilis*.

The second research objective was to collect data from microsatellite DNA and perform genetic analyses on all *P. m. gracilis* caught outside of the two established locations, then to compare these mice to the CH/OT and AP populations. If trapping results were in accordance with scenario one, with well-distributed *P. m. gracilis* populations, I hypothesized that extensive gene flow currently exists within the LP, with some gradient of genetic differences found across the region. If scenario two (few new populations) was found to be correct, I hypothesized that the newly discovered populations would be genetically similar to the previously known
populations to which they are geographically nearest, but that the eastern and western regions would be genetically distinct. If scenario three (no new populations) was found to be correct, I hypothesized that the established populations would have become isolated from each other genetically as well as geographically in recent years.

In summary, I planned to do the following: 1. Establish *P. m. gracilis* distribution in the LP of Michigan by identifying likely optimal habitat and then trapping at optimal sites near the known populations. Identifying optimal habitat was performed using GIS analyses combined with ground-truthing to determine fine-scale variation between habitat types. 2. Genetically analyze all new populations of *P. m. gracilis* found across the region and compare them to previously known CH, OT, and AP populations.
MATERIALS AND METHODS

Preliminary Landscape Analyses

Landscape analyses were performed in ArcGIS version 10.0. The 2001 Integrated Forest Monitoring, Assessment, and Prescription (IFMAP) land cover dataset was obtained for the Lower Peninsula of Michigan (Michigan Department of Natural Resources, 2003). IFMAP uses thirty-three cover types to define specific habitats within Michigan’s Lower Peninsula. In addition to the IFMAP, road shapefiles and 2001-2010 *P. m. gracilis* trapping data were imported into ArcGIS 10 via separate layer files. All layers were converted into the NAD83 State Plane Michigan North FIPS2111 projected coordinate system and added together to create one map (Figure 2).

Three habitat classifications were created for the land west of the farmland belt, based on their suitability for identifying forests that are capable of harboring *P. m. gracilis*. These included optimal, suitable, and unsuitable habitat types. Optimal habitats were determined from sites where *P. m. gracilis* has consistently been found in recent years in Michigan. These forest types included lowland deciduous, lowland mixed, lowland coniferous, mixed upland deciduous, northern hardwood association, oak association, and upland mixed forests. Although lowland forests were included in this classification, the vast majority of forests in this region are of the upland type. Suitable habitats included forest types in which *P. m. gracilis* could potentially be found and have been found in the past by other researchers (Dice 1925; Ozoga & Philips 1968), but which are not typical of the habitat of known populations. These included mixed upland coniferous, other upland coniferous, and pine forests. Large isolated areas of these conifer-based habitats are probably insufficient to support *P. m. gracilis* populations; however, when located near more optimal habitats, the chances of these coniferous forest patches harboring *P. m.*
gracilis greatly improve. Finally, unsuitable habitats included all habitat types that were not categorized as optimal or suitable.

Once the three habitat categories were created, each was converted into a separate plot type using the ‘plot xy’ function, then each was exported as a separate permanent layer. Potential trapping points were determined by locating each area in between the CH/OT and farmland belt that appeared to have the highest density of optimal habitat in a relatively large patch. Twenty-eight locations were chosen that covered both an east-west and north-south distribution. Fewer points were placed near the farmland belt, as it was composed mostly of unsuitable habitat. All new points were created using the point edit command and were placed into a separate created layer from a new geodatabase. The buffer command was then performed on each proposed sample point. Buffer circles around each point included radius levels of 175, 350, 500, 750, and 925 meters. A radius of 925 meters was used as the largest buffer region because anything larger could overlap the regions of two geographically close points and alter the analysis output substantially. For every buffer radius increment at each sample point, the ‘tabulate area’ function was used to quantify the amount of optimal, suitable and unsuitable habitat found within the circular area. Various buffer radii were compared between sample points to determine which proposed areas contain the largest amount of optimal habitat. Each point was then evaluated based on its distance to the nearest road and on whether it is on publically owned land. Also, sites at or near where P. m. gracilis had been trapped <60 years ago were given preference over other alternative sites. All these variables were taken into consideration before selecting the exact locations to visit in early summer 2011.

It is important to note that three of the chosen sites were located at or near places where P. m. gracilis had been trapped 10-60 years ago. Two of my sites were near the historic
Valentine Lake and Clear Lake sites, while one site (MSW22) was located slightly east of the OT site and was trapped in 2003, when only two *P. m. gracilis* were found.

Similar analyses were conducted for the habitat east of the farmland belt and around the South Swamp site. Optimal habitat in this area consisted mostly of lowland conifer, mixed and deciduous forests, since the forests are more low-lying than those west of the farmland belt. To conduct this analysis, several potential trapping sites were selected in optimal habitat at each of five-, ten-, and fifteen-kilometer radii around the S. Swamp site. Site accessibility and patch size were taken into consideration before selecting exact trapping locations.

**Trapping Session 1**

The first summer trapping session took place from June 13th to June 20th 2011. Of the twenty-eight sites selected between the CH/OT region and the farmland belt as potential trapping sites, fifteen were visited (Figure 3). Nine additional sites were visited east of the farmland belt around the S. Swamp site, although only those that were trapped are shown in Figure 3 (n=4). Not all of these sites were located along a transect between the CH/OT and S. Swamp sites; instead, they were trapped to see if the AP population range extended in other directions. In total, nine sites were chosen for trapping along a regional transect between the CH/OT and AP areas based on both accessibility and the suitability of habitat. The remaining sites were not trapped due to either access issues (n = 7), because I deemed the woodlands to be inferior in terms of either plant species composition or the distribution of mature trees (n = 5) based on previous trapping experience, because the forest patch was not large enough (n=1), or because the site was near another site that had superior forest cover (n= 3; Table 1). In addition, one site (MSW23) was visited because it was mistakenly thought to have been trapped previously; it was later determined that the coordinates for this location were incorrect (Table 1).
Secondary GIS Analyses

Based on trapping success during the early summer, additional GIS analyses were performed with the goal of refining the identification of cover types for all the western sites. Although all of these forest sites correlated to optimal habitat as defined using the land cover dataset, there was a difference in tree composition between those that had *P. m. gracilis* versus those that did not. Specifically, sites that harbored both *Peromyscus* species were dominated by sugar maples, whereas those that were more oak-dominant harbored only *P. leucopus*.

To further refine the land cover analysis, the Landsat 7 Geocover Data Set produced by the USGS Geological Survey (USGS Geological Survey) EROS Data Center was obtained and downloaded from the NASA Global Land Cover Facility at the University of Maryland, from a web site accessible at http://glcf.umiacs.umd.edu. A scene acquired from World Reference System 2 (WRS-2) Path 021, Row 029 on 2005-08-07 was used. The scene’s center is located at approximately 44 degrees, 36 minutes, 48 seconds north latitude, and 84 degrees, 17 minutes, 4 seconds west longitude. The image was orthorectified by the USGS as part of the Global Land Survey 2005 data set. The scene’s GLCF Online Identification Number is 216-151. Bands 1, 2, 3, 4, 5 and 7 (not 6 or 8) were combined into an ENVI standard data file for unsupervised classification using the ISODATA technique. An unsupervised ISODATA classification was performed for 10 through 80 classes, with images of 50, 60, and 70 classes appearing to differentiate the habitat and forests most adequately. These images clearly differentiated between the forest types to the east versus west of the farmland belt, but also appeared to differentiate between the maple and oak dominated forests in Montmorency Co.

These new Landsat images were then compared to ground observations, to satellite images provided by GoogleEarth (2012) and Google Maps (2012), and to several other land
cover classifications. These land cover classifications included the IFMAP Landcover dataset used in the preliminary landscape analyses, and the National Land Cover Gap Analysis Project (Gap Analysis Program). After all comparisons were made, two additional sites were selected slightly east of the Montmorency site (MT) at which *P. m. gracilis* had been previously trapped. These sites were used to better identify the eastern edge of the western part of the *P. m. gracilis* range (Figure 3) and represented ideal *P. m. gracilis* habitat based on the GIS and satellite image comparisons.

**Second Trapping Trip and Final GIS Analyses**

The second summer trapping session occurred from July 28th to July 30th 2011. Mice were collected from the two newly selected locations (Figure 3), and the original MT site was re-trapped to increase the total sample size from this site.

After the second trapping trip, additional ground-truthing of all sites was compared back to the GIS land cover dataset and the custom processed Landsat data, and to the Mi-HUNT Interactive Mapping Application (State of Michigan DNR), which is based on the Michigan DNR’s ground-truthing of cover types on state land.

To determine if oak versus maple-dominated forests could be distinguished from one another, a dataset search was conducted in USGS Earth Explorer (2012). Data were selected only from the Thematic Mapper (TM) satellites 4 and 5 in the Landsat Archive. Search criteria included path 21 and row 29, or the area surrounding Montmorency Co., MI. Six datasets from the following dates were selected: August 26th, 2009; September 30th, 2004; October 2nd, 2005; November 14th, 2009; December 24th, 2006; January 22nd, 2006.

ERDAS Imagine software (Erdas, Inc., Norcross, Georgia) version 9.3 was used to combine the raw satellite data into a usable image for GIS analysis. For each dataset, six out of
seven bands were stacked using the “layer stack” option within the “interpreter” window. Bands 1-4, which represent wavelengths of 0.45-0.90µm in the visible spectrum, were added first. Bands 5 and 7, which represent wavelengths between 0.76 and 2.35µm in the infrared spectrum, were also added. Band 6 was not stacked because it senses thermal infrared radiation that was not useful to analysis. Once all layers were stacked, a composite image of all the wavelengths was produced.

All composite images were imported into ArcGIS version 10.0 and projected onto the NAD83 State Plane Michigan North FIPS2111 projected coordinate system mapping project. For each image, the RGB Composite was stretched to the option of “standard deviations” with n=2. Layer 6 was selected to be the red channel, layer 4 the green channel, and layer 2 the blue channel. Images were visually compared using the Image Analysis tool and the “layer swipe” method.

Finally, the surrounding habitats around the S. Swamp, MSW22, OSM1 and Tin Shanty Road sites (see Table 2) were measured and compared to determine the proportion of optimal, sufficient, and unsuitable habitat around and between sites. These four sites were chosen because each harbored P. m. gracilis and contained samples used in the genetic analyses. The analysis was performed by using the buffer command and creating buffer circles around each point at radius levels of 250, 500, 1000, 1500, and 2000 meters. For every buffer radius increment at each sample point, the ‘tabulate area’ function was used to quantify the amount of optimal, suitable and unsuitable habitat found within the circular area. Habitat composition within each site and between the four sites could then be compared.
Sampling

Trapping sessions occurred between June and August of 2011. Between 100 and 240 non-lethal traps (H.B. Sherman Traps, Tallahassee, Florida) baited with whole oats were placed in the evening at each trapping site and checked the following morning. Each location was trapped for three nights or until at least 25 mice were captured, since previous studies have shown that this is sufficient for catching the majority of mice in a forest patch (Stickel 1949). Data were taken from all captured mice, including ear length, weight, age, reproductive condition, sex, and tentative species identification, and saliva and tissue samples were obtained. Age was determined from pelage characteristics (e.g. Millar et al. 1979), and preliminary species identifications were based on a combination of morphological and pelage characters. Due to the many morphological similarities between *P. leucopus* and *P. m. gracilis* in this region of Michigan (Feldhamer et al. 1983; Long & Long 1993; Myers et al. 2005; Rich et al. 1996), species identifications were later confirmed using cellulose acetate electrophoresis of salivary amylase protein from saliva samples (Aquadro & Patton 1980; Bruseo et al. 1999). In addition to the saliva sample collection, a small piece of ear tissue was removed from each individual using sterile dissecting scissors. This tissue removal marked the animals in case of re-capture, and the ear clips were saved for subsequent DNA extraction and analysis. All animals were handled under a protocol approved by Miami University’s Institutional Animal Care and Use Committee, and all methods were in accordance with guidelines established by the American Society of Mammalogists (Gannon et al. 2007). In addition to the mice captured during the 2011 trapping sessions, tissue and DNA samples collected from CH, OT and AP mice in recent years were also used (see Table 2).
Sample Preparation and Genotyping

DNA was isolated from ear tissue fragments using the e.Z.N.A. Tissue DNA Kit (Omega Bio-Tek, Norcross, Georgia). The polymerase chain reaction (PCR) using Promega Go-Taq DNA polymerase and the Flexi buffer system was used to amplify microsatellites. Primer sets were selected from the literature and optimized for this species in our laboratory (Taylor & Hoffman 2012; Table 3). Typical PCR reactions contained 20 ng template DNA, 1.5-2 mM MgCl$_2$, and 0.2 mM of each dNTP. The PCR cycle consisted of 94°C for 2 min.; 40 cycles of 30 sec. at 94°C, 30 sec. at 50-65°C, and 1 min. at 72°C; 5 min. at 72°C. Specific annealing temperatures and MgCl$_2$ concentrations are shown in Table 3. Forward primers labeled with the G5 dye set were obtained from Applied Biosystems (Foster City, California). Products were run on an Applied Biosystems 3130 DNA Analyzer with the 600LIZ internal size standard (Applied Biosystems). Product peaks were identified manually using Peak Scanner v1.0 software (Applied Biosystems, Foster City, California), (Matschiner & Salzburger 2009). In addition to data obtained from samples genotyped in this study, data generated from Taylor & Hoffman (2012) was also incorporated into the CH and AP population samples to increase the number of individuals analyzed from each population. Genotypes for several (n=10) of the CH samples were incomplete. Therefore, some of the genetic analyses were run both with and without four of the most problematic individuals.

Statistical Analyses

Check for Null Alleles

Data were first checked for null alleles using the program Micro-Checker (Van Oosterhout et al. 2004). Genotypes and allele frequencies were corrected using the method of Brookfield (1996) when null alleles were detected at frequencies greater than 5% at each locus.
within a population. The corrected genotypes were used for all analyses except assignment tests and clustering analyses, which require the use of unmodified multilocus genotypes. A side effect of the null allele correction is that it corrects by locus rather than individual, thereby inhibiting the ability to distinguish between homozygous genotypes and heterozygotes incorporating a null allele (Van Oosterhout et al. 2004).

Analyses of Genetic Diversity

Allelic richness (\(A_R\)) and heterozygosity (\(H_O\) and \(H_E\)) under conditions of Hardy-Weinberg equilibrium were first calculated to provide measures of genetic diversity. The number of alleles observed was determined for each locus using the software program HP-Rare. The average number of alleles per locus (\(\bar{A}\)) and the allelic richness standardized for sample size using the repeated sampling procedure (\(A_R\)) with a sample size of eight were implemented in HP-Rare (Kalinowski 2006). Private allelic richness (\(A_P\)), which represents the number of unique alleles in a population, was also calculated in HP-Rare, using a sample size of eight individuals. This sample size of eight was selected because this was the number of individuals sampled from the OT population, which was the smallest of the four main populations. \(H_O\) and \(H_E\) were calculated in Arlequin v3.5 (Excoffier et al. 2010) for each locus within each population. An ANOVA was conducted in Minitab version 15 (Minitab Inc., State College, Pennsylvania) to calculate differences in genetic diversity among populations. A Tukey’s Honestly Significant Difference (HSD) post hoc test was then conducted to determine significant differences for \(A_R\) and \(H_O\) values. It is important to note that because of their close proximity to one another (see Figure 3) and the small sample sizes of the BTT and Rouleau Farm populations (\(n=8\) and \(6\), respectively), I combine all three Alpena Co. populations (those east of the large farmland belt)
into one population (AP) in subsequent analyses. Genetic diversity indices were rerun after four CH individuals were removed from the sample.

**Genetic Differentiation, Deviation from HWE, and Linkage Disequilibrium**

Population genetic analyses were conducted in the program GenePop version 4.0.10 (Rousset 2008). These include measurements of significance of population differentiation, deviation from Hardy-Weinberg expectations (HWE), and linkage disequilibrium. These tests were conducted using log likelihood ratio tests (Goudet et al. 1996) after using the sequential Bonferroni correction to adjust for multiple comparisons (Rice 1989).

Wright’s F-statistics are used to characterize population genetic structure, with $F_{ST}$ representing the fixation index. This statistic provides a measure of genetic differentiation between subpopulations by calculating the proportion of genetic diversity due to differences in allele frequencies (Holsinger & Weir 2009). Values range from zero to one, with values close to zero indicating no differentiation and values close to one indicating high differentiation. Assumptions of $F_{ST}$ included the following: non-overlapping generations, random mating within populations, subpopulations evolving independently, equal sizes of subpopulations, and symmetrical migration rate (Holsinger & Weir 2009). It therefore assumes the infinite island model of population structure.

Rho-statistics, represented by values of $\rho_{ST}$, are a similar measure of population differentiation but are calculated from the variances of allele sizes. Values also range from zero (no differentiation) to one (complete differentiation). $\rho_{ST}$ pairwise estimates have the same assumptions as $F_{ST}$, but also assume the generalized stepwise mutation model (SMM), since this is how microsatellites are thought to most frequently mutate. Pairwise estimates of $F_{ST}$ and $\rho_{ST}$
were conducted according to the method of Weir & Cockerham (1984) using log likelihood ratio tests (Goudet et al. 1996) to measure significance of differentiation in GenePop.

Mantel tests (Mantel 1967) were used to calculate isolation-by-distance of the four population groups. Tests were conducted in GenAlEx (Peakall & Smouse 2006), version 6, using Rousset’s (1997) isolation-by-distance procedure. Pairwise geographical distances using geographic coordinates (see Table 2) represented the $X$ matrix, while either $F_{ST}$ or $\rho_{ST}$ pairwise values represented the $Y$ matrix.

Deviations from HWE were calculated for each population in GenePop using the Markov chain (MC) algorithm (Guo & Thompson 1992). Regarding the parameters, the dememorization number was 1,000, the number of batches was 100, and the number of iterations per batch was 1,000. Both heterozygote deficit and excess were tested for all loci in each population. Global tests across all populations were then conducted using Fisher’s method (Fisher 1925) and the sequential Bonferroni technique was used to adjust for multiple comparisons (Rice 1989).

Linkage disequilibrium was calculated in GenePop to determine if genotypes at one locus are independent from genotypes at the other loci (Ho). The model tests for the association between diploid genotypes at the two loci being compared. It assumes independence between the two loci and random mating; however, no assumption is made about one-locus HWE. The model tested for linkage disequilibrium between each pair of loci in each population using the log likelihood ratio statistic. Regarding the MC parameters, the dememorization value was set at 1000, the number of batches at 100, and the number of iterations per batch at 1000. The sequential Bonferroni correction was then implemented to adjust for multiple comparisons (Rice 1989).
Check for Genetic Bottlenecks

Two methods were used to test for bottleneck events in the population using the program BOTTLENECK v1.2.02 (Cornuet & Luikart 1996). The first method was developed by Cornuet & Luikart (1996) and consists of three heterozygosity excess tests. These included the Sign test, the Standardized differences test, and the Wilcoxon sign-rank test. The sign test measures the excess of heterozygosity by calculating the difference between observed and expected heterozygosity across all loci in the population (Cornuet & Luikart 1996). It assumes mutation drift equilibrium, no immigration, no population substructure, selectively neutral loci, and independent results among pairs of loci, and that the same mutation model fits all loci in a given sample (Cornuet & Luikart 1996). The Standardized differences test is similar to the Sign test and has the same assumptions; however, it also takes into account the magnitude of heterozygosity excess or deficiency by dividing the difference (observed - expected) in heterozygosity by the standard deviation of the corresponding distributions of heterozygosities (Cornuet & Luikart 1996). The Wilcoxon sign-rank test is a nonparametric test that controls for differences in heterozygosity among polymorphic loci (Luikart et al. 1998b). It has the same assumptions as the other tests and is powerful and robust when used with a small number (<20) of loci (Piry et al. 1999).

Three models were run for each of the three tests in order to establish probability distributions. These models included the Infinite Allele Model (IAM; Kimura & Crow 1964), the Stepwise Mutation Model (SMM; Ohta & Kimura 1973) and the Two-Phase Model of mutation (TPM; Di Rienzo et al. 1994). The IAM is based on a formula created by Watterson (1984), which assumes that populations each evolve under the Wright-Fisher model of random mating and that individuals are at mutation drift equilibrium before any bottleneck occurs. This model allocates a single mutation at a time and then computes the resulting number of alleles.
The SMM uses a Bayesian approach that is based on the size of alleles, in which each mutation is assumed to cause either a gain or loss of one repeat unit (Kimura & Ohta 1973). The model is based on the formula provided by Chakraborty & Nei (1977) and also assumes that all alleles at each locus are selectively equivalent and that there is random mating within the population. The TPM combines both the IAM and the SMM, with the user being able to define the ratio of IAM to SMM in the model. The probability distribution was established using 1,000 simulations for each model. The proportion of SMM in the TPM was set at 70%, meaning that alleles can arise through large mutation steps (IAM) only 30% of the time. These TPM settings are therefore analogous to the SMM but also allow for occasional multistep mutations (Selkoe & Toonen 2006). They are the default settings commonly used in studies analyzing genetic bottlenecks of mammal populations using the program BOTTLENECK (e.g. Amirinia et al. 2007; Hoelzer et al. 2002; Muwanika et al. 2003) and are the most appropriate settings for analyzing microsatellite DNA data (Di Rienzo et al. 1994).

The second method used was the allele frequency distribution test. This is a graphical representation of the mode shift indicator proposed by Luikart et al. (1998b) to determine whether a genetic bottleneck has occurred in the past few dozen generations. A “nonbottlenecked” population is defined as one that has not recently been bottlenecked and is therefore likely to be near mutation-drift equilibrium (Luikart et al. 1998b). Assumptions for this model include the following: each population is randomly mating, has no substructure or recent immigration, has selectively neutral loci, and has sampling that is representative of the population (Luikart et al. 1998b). No parameters for this model can be manipulated, and the test was scaled so that allele frequency classes are based on equal 0.1 increments for each population. All bottleneck analyses were rerun after four CH individuals were removed from the sample.
**Assignment Tests**

I examined overall genetic structure using two Bayesian clustering algorithms. I first used the program STRUCTURE (Pritchard *et al*. 2000) to examine the number of genetically supported populations. I then used the program GeneClass2 (Piry *et al*. 2004) to examine population structure by performing assignment tests.

The program STRUCTURE, version 2.3.3 (Pritchard *et al*. 2000), was used to assign all individuals to a user–defined number of clusters (K) based on similarities among genotypes (microsatellite data). The model assumes that within populations/subpopulations, loci are at Hardy-Weinberg equilibrium and linkage equilibrium. The model does not assume a particular mutation process, so it can be applied easily to microsatellite markers.

Three replicate runs with a burn-in of 100,000 and 1,000,000 subsequent iterations were performed for each value of K from 1 to 10 using the admixture model. To determine the number of clusters (K) that best fit the data, the results from all runs were imported into the program STRUCTURE Harvester (Earl & VonHoldt 2012) using the methods derived by Evanno *et al*. (2005). Ten long runs were then performed in STRUCTURE using a burn-in of 100,000 and 2,000,000 iterations at fixed values of K. Results from all ten runs were combined in the program CLUMPP, version 1.1.2 (Jakobsson & Rosenberg 2007), using the Greedy algorithm. This program fixes label switching and genuine multimodality problems that arise from the stochastic simulations in the clustering algorithms of STRUCTURE. Finally, the combined results were imported into the program Dstruct, version 1.1 (Rosenberg 2004), to visually represent the data.

GeneClass2 (Piry *et al*. 2004) was used to perform assignment tests on individuals across the four major population groups. The chosen model assumes Hardy-Weinberg equilibrium and linkage equilibrium within contributing populations. It also assumes the Stepwise Mutation
Model (SMM), a mutation rate ($\mu$) greater than zero, the sampling of all baseline populations, and no admixture of individuals in the observed or baseline data.

The option to assign/exclude the population as the origin of individuals was chosen and the Bayesian methods of Rannala & Mountain (1997) were used. The probability computation of Monte-Carlo resampling using the simulation algorithm from Paetkau et al. (2004) was enabled, the number of simulated individuals was set at 10,000, and the Type 1 error threshold was set at 0.01.
RESULTS

Trapping Success:

Of the nine total sites that were trapped during the summer of 2011, *P. m. gracilis* was found at only three of them (Figure 3; Table 2) while *P. leucopus* was found in high numbers (n=25-73) at all eight. The first of the sites harboring *P. m. gracilis* is located on the western edge of Montmorency Co. (MT site); it was last trapped in 2003, with fourteen *P. leucopus* but only two *P. m. gracilis* captured at that time. We trapped sixteen *P. m. gracilis* and seventy-three *P. leucopus* at this site during the two 2011 trapping sessions. Because of the recent loss of *P. m. gracilis* from many locations in this area of the LP, and the fact that only two individuals were found at the MSW22 site in 2003, we did not have high confidence that we would find *P. m. gracilis* at that location. In this respect, MSW22 was unlike the OT, CH and AP populations, which have been trapped multiple times in the last 10 years, and at which *P. m. gracilis* is consistently found. We therefore classify the MSW22 site as representing a newly verified population of *P. m. gracilis*.

The other two sites containing *P. m. gracilis* (BTT and Rouleau Farm; Table 2) were located in Alpena Co. just east of the farmland belt and slightly west of the S. Swamp site (Figure 3). In addition to the three *P. m. gracilis* trapped at the S. Swamp site, thirteen more individuals were trapped at the BTT and Rouleau Farm sites. Although several other trapping sites were located in well-grown mature maple-dominated hardwood forest that appeared to be prime *P. m. gracilis* habitat, they yielded no *P. m. gracilis*, even though many *P. leucopus* were captured at those sites.
GIS Analysis Results

Initial habitat analyses correctly identified well-grown forest stands and detected a distinct difference between the forest types to the east versus west of the farmland belt (Figure 2). Secondary GIS analyses using ISODATA classifications detected a distinct difference in habitat composition between forests east versus west of the farmland belt (Figure 4). Differences in pixel coloration could also be detected between oak and maple dominated forests with the ISODATA images of 60 and 70 classes; however, these differences could not be quantified since only unsupervised classifications were used. Final GIS analyses comparing satellite imagery from various months distinguished broad-leaf deciduous forests from other types of forest habitat, but were unable to distinguish oak- versus maple-dominated forest types. An example of two sites being compared is shown in Figure 5, with site MS7 being the oak-dominated site and MSW22 the more maple-dominated site. Final analyses comparing the proportions of optimal, suitable, and unsuitable habitat around and between representative sites (OSM 1, Tin Shanty Rd., MSW22, S. Swamp) for each of the populations (see Table 2) showed that the area around the AP site contained a higher proportion of unsuitable habitat compared to the other sites at all radius increments (250 m-2 km) (Table 4).

Statistical Analyses

Ninety-four individuals were genotyped from four populations at eleven loci. All loci were polymorphic in all populations. All loci were successfully genotyped for the MT and OT populations, whereas 98.9 and 95.8 percent of loci were successfully genotyped for the AP and CH populations, respectively.
**Check for Null Alleles**

The Brookfield (1996) method in the program Micro-Checker (Van Oosterhout et al. 2004) detected the presence of null alleles in three of the four populations (OT, AP, and CH). Out of the eleven loci analyzed in each population, one was corrected for null alleles in the OT population, nine in the AP population, and seven in the CH population.

**Analyses of Genetic Diversity**

Results for genetic diversity for the loci and populations analyzed are represented in Tables 3 and 5. Expected heterozygosities were high for all loci ($H_E$ between 0.79 and 0.89). My point estimates of average genetic diversity were slightly higher in the western populations (average $H_O=0.74$; $H_E=0.90$; $\bar{A}_R=9.57$, $\bar{A}_P=4.86$) than in the AP population (average $H_O=0.66$; $H_E=0.87$; $\bar{A}_R=8.09$, $\bar{A}_P=3.01$). However, the ANOVA and Tukey’s HSD tests found that there were no significant differences between populations when comparing $\bar{A}$ to $\bar{A}_R$ or $H_O$ to $H_E$.

Private allelic richness analysis showed that $\bar{A}_P$ was largest for the CH ($\bar{A}_P=3.39$) and AP ($\bar{A}_P=3.01$) populations. Private alleles were found in high numbers across all loci in the CH population, but each variant was represented by only a few individuals ($n<4$). In the AP population, private alleles were not as common across loci, but most of these alleles were present in many individuals ($n>13$). Results of the genetic diversity analyses were not significantly different after they were performed a second time with four CH individuals removed.

**Genetic Differentiation, Deviation from HWE, and Linkage Disequilibrium**

Levels of genetic differentiation between populations represented by $F_{ST}$ and $\rho_{ST}$ values are displayed in Table 6. Isolation-by-distance calculated using Mantel tests was not significant
between populations for $F_{ST}$ (Figure 6A; $r=-0.274$; $P=0.490$; $R^2=0.075$) or $\rho_{ST}$ (Figure 6B; Mantel: $r=0.334$; $P=0.100$; $R^2=0.112$).

Regarding deviations from HWE, significant heterozygosity excess ($P<0.05$) was not found in any of the populations. Heterozygosity deficit was significant for all loci in the AP population, nine loci in the CH population, and one locus in the OT population. For the H-W probability tests, AP had seven loci, CH had eight loci, and OT had one locus that deviated from HWE.

Linkage disequilibrium was significant ($P<0.05$) for only three out of 194 pairwise comparisons across all populations. Twenty-seven pairwise comparisons between loci from the OT population were not included in the results because of the lack of complete genotype data.

**Check for Genetic Bottlenecks**

All four populations showed a normal L-shaped allele frequency distribution (Table 7). This distribution is expected under mutation drift equilibrium and indicates there was no recent bottleneck to provoke a mode-shift in the distribution. No population exhibited signs of undergoing a recent bottleneck in the TPM model (Table 7). However, all populations exhibited signs under the IAM and SMM models, although the tests that determined significance were different for each population (Table 7). Results of all bottleneck analyses were not significantly different after they were performed a second time with four CH individuals removed.

**Assignment Tests**

The STRUCTURE analyses (Pritchard *et al.* 2000) using the method of Evanno *et al.* (2005) determined the ideal number of clusters to be 4 (Figure 7). The AP and MT individuals
belong almost entirely to their own distinct clusters, whereas the CH and OT individuals are more mixed.

Regarding the results of the assignment tests performed in GeneClass2 (Piry et al. 2004), 88.3% (83/94) of individuals were assigned to the correct source population. Of the mis-assignments, one individual from OT, two individuals from AP, and one individual from MT were assigned to the CH population. In addition, two individuals found in MT were assigned to the OT population. The remaining three individuals were assigned to no population since the value of their probability in each reference population was lower than the previously defined threshold.
DISCUSSION

Habitat Analyses and Trapping Data

My first research objective was to use GIS habitat analysis to predict where *P. m. gracilis* was most likely to be found between the established CH/OT and AP locations. Initial habitat analyses correctly identified well-grown forest stands across the study region and also detected a distinct difference between the forest types to the east versus west of the farmland belt. I visited or trapped at a large number of these sites across the study region (Figure 3) and captured high numbers of *P. leucopus* at each site. Past studies have found that live trapping over a two-night period will capture 80% of resident mice in a forest patch (Stickel 1949). Although it is possible that there could be small numbers of *P. m. gracilis* at some of these sites that were not detected by my trapping, they would be unlikely to constitute a functional population. Therefore, it is very likely that I have correctly approximated the current range boundaries of *P. m. gracilis* in the LP, and it is improbable that there are any viable populations between the two range fragments of this species.

Trapping results most closely matched the second of the three possible scenarios I originally envisioned. Three new populations of *P. m. gracilis* were found in between the known sites, but these newly established populations were not well distributed across the corridor between the CH/OT and AP regions. Instead of being near the middle of the study area, the three newly confirmed populations were located near the original OT and AP sites, slightly expanding the known range of *P. m. gracilis* in the LP and bringing the two regions closer together, but not connecting the two disjunct parts of the range.

The newly established populations expanded the known range fragments of *P. m. gracilis* in the LP towards each other. The MSW22 (MT) site was the most eastern site at which *P. m.*
*gracilis* was found on the west side of the farmland habitat break. When this site was trapped for a single night in 2003, only two *P. m. gracilis* were found, and these individuals might have been transients. This site was therefore not considered to be an established part of the species’ range until we caught 16 more *P. m. gracilis* there in 2011, including overwintered and pregnant adults. Two other sites (MSW7 and MSW8) were trapped in optimal forests slightly to the east of the MSW22 site. These additional sites were in forests near the Clear Lake State Park and Valentine Lake locations where *P. m. gracilis* was trapped in the 1940’s, approximately 14 km and 12 km, respectively, northeast of the MSW22 site. However, only *P. leucopus* was found at the MSW7 and MSW8 sites, indicating that the MSW22 site likely represents the extreme eastern edge of this part of *P. m. gracilis*’ range (Figure 3).

In the eastern part of the range, the most western site that contained *P. m. gracilis* was Rouleau Farm (see Table 2, Figure 3), which is located less than 1 km east of the farmland belt. Another site (GL1) was trapped directly across the 8 km wide farmland belt from Rouleau Farm, and only *P. leucopus* was found there. Thus the gap between the two parts of the LP range of *P. m. gracilis* has been reduced from about 50 km to the slightly more than 40 km that separates the MSW22 (MT) site from the Rouleau Farm site. Together, the absolute distance between the two parts of the range and the presence of the strong habitat break created by the farmland belt are likely to prevent the natural migration of mice between the AP population and any of the western populations.

In addition to using established GIS data and maps as a predictive tool for this study, I created my own datasets and fine-tuned habitat classifications by ground-truthing various forms of satellite data. Primary GIS analyses correctly distinguished between the lowland mixed and coniferous forests around the AP area versus the upland deciduous and mixed forest stands west of the farmland belt. This indicates that land cover classifications, such as the IFMAP, are a
good predictor of the general types of forest habitat in which *P. m. gracilis* can be found. These GIS analyses, plus the summer 2011 trapping data, have determined that there are many forest patches that could support *P. m. gracilis* across the study region, and correctly predicted that when trapping was conducted in those forest stands, large populations of at least one species of *Peromyscus* were found.

Although preliminary GIS analyses identified two general types of *P. m. gracilis*-eligible forest across the study region, our ground-truthing indicated that my original habitat classification system could be further refined to better classify differences in forests west of the farmland belt. In particular, I had the goal of seeing if there was a way to differentiate between oak- and maple-dominated forests. Preliminary GIS analyses could not differentiate between these two types of forests, and classified them both as upland deciduous/mixed forests stands. Both the trapping done for this study and older trapping records show that maple-dominated transitional forests, such as the forest at the MSW22 site, are ideal habitat for *P. m. gracilis* (Grimm 1983; Hooper 1942). While *P. m. gracilis* might be able to inhabit more oak-dominated forests, we do not have any evidence that they regularly do so. *P. leucopus*, however, are known to thrive in oak-dominated forests (Allen 1938; Burt 1940; Getz 1961), as shown in this study by their high capture rates at several oak forest sites such as MSW7.

The secondary GIS analyses were therefore aimed at refining the identification of cover types for all the western sites to differentiate between habitats that are ideal for *P. m. gracilis* (maple-dominated forests) and for *P. leucopus* (oak-dominated forests). These analyses revealed differences in pixel color combinations between the two types of forests (Figure 4) that corresponded to both our ground-truthing and to the highly specific habitat classifications of state land provided by the Michigan DNR data (also derived from ground-truthing), and these differences were used to select the best sites for the second round of trapping. However, these
differences in pixel color combinations could not be quantified, since habitat classifications were not initially assigned to the satellite imagery. Therefore, in order to classify all pixel colors within potential trapping sites, more extensive and specific ground-truthing in all types of habitat across the region would have to be performed.

Because results could not be easily quantified from the secondary GIS analyses, I explored a different method for distinguishing oak- versus maple-based forests in my final GIS analyses. This approach used satellite imagery to analyze differences in the two forest types over a period of four months, since some species of oaks retain their leaves longer than maple trees in the fall or early winter (Grimm 1983; Williams 2005). These qualitative analyses did not detect differences between the forest types for any of the fall or early winter months, although differences between months could be detected across each site (a visual example comparing two sites is in Figure 5). Because only one satellite image was available for each month studied, and because the maps were obtained from different years between 2004 and 2009, differences in images between months could be caused by a number of factors other than tree type. Soil composition, amount of leaf litter, rainfall, wind, viewing angle of the sensor and other factors all contribute to vegetation reflectance and therefore to differences in satellite imagery (Frank 1984; Gratto-Trevor 1996; Kaufman 1989). Subsequent and more intense work could be done that might allow researchers to differentiate between the maple- and oak-dominated forests using this satellite data. For example, it is possible that each pixel color could be categorized and quantified around each trapping site for each month. However, this analysis would require extensive work to produce very advanced classification assignments and was not within the scope of this study.

Though analyses did not discriminate between two relevant tree types, the GIS analyses used throughout this study have shown that we can use satellite imagery to make accurate
predictions of where *P. m. gracilis* might be found within a region. Initial predictions based on the IFMAP classifications detected virtually all well-grown forest stands, as confirmed by ground-truthing, that could potentially harbor large *Peromyscus* populations. Comparing satellite imagery to ground-truthing can create much more accurate predictions (Fretwell et al. 2012) and better define habitat suitability across the range of a species (Singh, Joshi, & Kumar 2009).

Although many studies use GIS and satellite imagery to make predictions about a species’ potential range (e.g. Bloomberg *et al.* 2009; Joy & Death 2004; Kumar *et al.* 2009; Peterson 2001; Sperduto & Congalton 1996), this study was more detailed in that GIS analyses were used before, between, and after the two summer trapping sessions to better define trapping sites based on a very fine-scale classification of forest habitats. The more detailed secondary GIS analysis identified the best forest stands for *P. m. gracilis*, including all the patches in which this species was found between 1920 and 2011. However, none of the more detailed analyses were able to take the final step of separating out the oak- versus maple-dominated patches. Even though these analyses can greatly reduce the time spent searching for suitable trapping sites in the field, they cannot completely replace on-the-ground inspection of field sites by experienced investigators.

**Genetic Analyses**

*P. m. gracilis* populations were not well distributed across the corridor between the CH/OT and AP regions, demonstrating that there is a strong geographic separation between the eastern and western population regions. Because of this separation and the consequent lack of contemporary gene flow, the two population regions should show non-uniform genetic diversity, characteristic private alleles, elevated levels of differentiation, and high levels of genetic
structure. However, within each region, populations are expected to have uniform genetic diversity, few or no private alleles, low levels of genetic differentiation, and low levels of genetic structure. While my results do show the latter, with evidence of substantial gene flow within regions, the comparison between the regions was more ambiguous, with some tests indicating good gene flow and others consistent with genetic isolation of the AP mice.

I first tested the internal integrity of the populations by testing for linkage disequilibrium, deviations from HWE, and indications that the populations had recently undergone genetic bottlenecks. The results of the HW tests indicate no linkage disequilibrium for the loci selected. This is expected, since the selected loci are independently segregating and are well distributed across the *P. m. gracilis* genome. Regarding deviations from HWE, significant heterozygote excess was not found in any of the populations; however, a significant heterozygote deficit was found for all loci in the AP population and for nine out of eleven loci in the CH population, which can indicate that those populations are suffering from inbreeding. This apparent deficit may also be an artifact due to the smaller sample sizes from some populations, to the existence of an underlying structure (Wahlund effect), or to the presence of null alleles. I found evidence for null alleles in the form of ungenotyped individuals and heterozygote deficiencies in the CH and AP populations. Although null alleles were restricted to one specific locus in the OT population, null alleles were distributed across multiple loci in the AP and CH populations, and may be the primary reason why those two populations deviate from HWE.

Identification of recently bottlenecked populations (i.e., populations bottlenecked within the past few dozen generations) is important because such populations may have not yet had time to adapt to the problems associated with small population size (Luikart et al. 1998a). Since the AP population shows signs of being genetically distinct and potentially genetically isolated from
the other populations, there is a possibility that it has recently undergone a bottleneck, and so I used multiple tests to look for bottlenecking.

The allele frequency distribution test determined that all populations were near mutation-drift equilibrium and had large proportions of alleles with low frequencies, indicating that the populations had not bottlenecked recently (Table 7). Results of the heterozygosity excess tests were not significant for any population under the TPM model and were only significant for the AP population under the SMM model using the standardized differences test (Table 7). Because microsatellites are thought to evolve by a combination of mechanisms (Amirinia et al. 2007; Selkoe & Toonen 2006), results from the TPM model are the most relevant for this study. Also, because of the low statistical power often associated with the sign test and the fact that a modest number of loci were used (<20), results from the Wilcoxon-signed-ranks test are probably the most relevant for this study (Luikart et al. 1998b). The results from the bottleneck analyses therefore indicate that the AP population has not been reduced enough in the recent past to show signs of having gone through a genetic bottleneck.

Since the populations did not seem as though they have undergone any extreme events recently, I wanted to see how they compared to one another genetically. If the AP population has been small and geographically isolated from the western populations for a significant length of time, I would expect to see an indication of this in the genetic diversity indices due to the effects of drift. However, genetic diversity indices showed no significant difference in $A_R$ or heterozygosity between populations. There were more total alleles found in the western populations than in the eastern population, likely because the large sample of CH mice inflated the western sample size (Table 5). However, after implementation of the Tukey’s HSD test, allelic richness was not found to be significantly different between populations. I found high levels of both $H_O$ and $H_E$ in all the populations, with slightly higher levels in the western
populations, but those differences were also not statistically significant (Table 5). Genetic diversity indices were the only analyses to look at the three AP populations individually and as a group. These populations could be distinguished by the diversity indices, with the values being an artifact of small sample size (Table 5). Even when the sample sizes were corrected for across all LP populations, the small sample sizes of the AP populations seemed to still have influence over the values. Also, the AP populations were very geographically close together, indicating that migration between them was very possible. The results of the diversity indices indicate that all populations are exhibiting high levels of diversity and that the AP population is not currently showing signs of being genetically depauperate due to its geographic isolation.

Because there is presumably no current gene flow between the eastern and western populations, the AP population is expected to be genetically distinct from the western populations as shown by indices of genetic differentiation. However, genetic differentiation among all four populations using F statistics was found to be low (Table 6), with results of the Mantel test indicating no isolation by distance (Figure 6). Relative to the levels of genetic variation typically detected between geographically disjunct populations of other mammals, including other Peromyscus (Mossman & Waser 2001; Vucetich et al. 2001; Anderson & Meikle 2010; Yang & Kenagy 2009), the F_{ST} and ρ_{ST} values found in this study are very low. However, these low values are very similar to those seen for UP populations of P. m. gracilis that are also separated by 10-40 km distances (Taylor & Hoffman 2012). The UP mice are almost continuously distributed throughout a more well-forested landscape, similar to the situation that existed in the LP for P. m. gracilis as recently as the 1940’s (Myers et al. 2009). Therefore, the current separation between the P. m. gracilis from the two LP regions must be quite recent, and the F_{ST} and ρ_{ST} data probably reflect the recent past rather than the present. Even though there is unlikely to be any gene flow currently between the AP and western populations, not enough time
has passed for genetic drift, mutation, or other processes to significantly affect these measures of
differentiation.

Private allelic richness ($A_P$), another indicator of differentiation, should be relatively high
in the AP population, since the population is currently geographically isolated from the western
populations. Values were found to be high for both the CH and AP populations and low for both
the OT and MT populations (Table 5). The high $A_P$ found in the CH population is probably in
part an artifact of sample size–the private alleles were found for a number of loci, likely because
larger samples can capture more of the underlying allelic variation in a population, but most of
the alleles were in only a small number of individuals. Private alleles in the AP population,
however, were widespread throughout the population, which might be a sign that genetic drift
and/or inbreeding have begun to affect this population in the short time since it has been isolated.

The assignment tests for genetic structure showed that the vast majority of both the AP
and MT mice could be correctly assigned to the populations in which they were found (Figure 7).
Assignments of the CH and OT individuals, however, were much more mixed, which
presumably reflects higher levels of gene flow between these two populations (Figure 7). The
genetic hypothesis based on the second scenario posited that the newly established populations
would be genetically indistinguishable from the previously known populations to which they are
geographically nearest. This hypothesis was not supported based on the results of these
assignment tests, since the MT population, although geographically closest to the OT population,
is clearly genetically distinct from the OT population. However, STRUCTURE analyses show
some gene flow occurring between the OT and MT populations (Figure 7), indicating that there
is some migration between them. The assignment tests also show that, while all populations had
high numbers of alleles, the AP population is different from the western populations in its
assortment of alleles. This makes sense in terms of habitat continuity across the region, with the
CH and OT mice located in a more continuous forest corridor and the AP mice geographically separated from the western populations both by distance and by the large farmland habitat break (Figures 2 and 3). The assignment tests therefore seem to be relatively sensitive to what has been happening in the recent past, indicating that little or no gene flow is presently occurring between the AP and western mice, and that the AP mice are becoming genetically distinct.

Conclusions

As recently as the 1940’s, *P. m. gracilis* were well distributed across the northern LP of Michigan, with a number of known populations in the study region that presumably had good gene flow between them (Myers *et al.* 2009). Since that time, forest composition and habitat fragmentation have not altered significantly across the study region (Myers *et al.* 2009). Aside from the 8 km wide farmland belt, a fairly continuous distribution of optimal and suitable habitat for *P. m. gracilis* remains across the study region, with high numbers of *P. leucopus* found at all sites trapped. However, populations of *P. m. gracilis* have been lost from most suitable forest patches between the CH/OT and AP areas, which has begun to alter the genetics of the remaining populations. Genetic diversity measures and bottleneck analyses indicate that *P. m. gracilis* populations once had extensive gene flow and were continuous across the area. Trapping data, deviations from HWE, private allele analysis and assignment tests, however, show that the AP populations are currently geographically isolated and are beginning to show signs of genetic isolation. Therefore, *P. m. gracilis* populations in the AP region now appear to be at a transitional stage—they are not yet so reduced that they are becoming unhealthy, but genetic drift and/or selection is making them more distinct from the western populations. Also, the widespread private alleles and the heterozygote deficiencies in the AP population may indicate that inbreeding is just beginning to have an effect.
There are several possibilities regarding what could happen to these mice in the near future (the next 20-30 years). It is possible that if the forests within the eastern region expand as a percentage of land cover, the Alpena Co. \textit{P. m. gracilis} would have a chance to become a more viable group and to potentially expand their range enough to reestablish gene flow with the western populations. There is a low probability of this happening, however, since land use in and near the farmland belt is unlikely to change radically in the next few decades. It is more likely that due to global warming, temperatures across the region will continue to increase and forest stands will become drier. Because the AP \textit{P. m. gracilis} populations seem to be limited to very wet lowland forests, any drying of the eastern forest stands could imperil their local survival, though whether or not they cross a critical threshold in population size would depend on the extent of drying. Alternatively, warmer temperatures could continue to favor the growth of populations of \textit{P. leucopus} at the expense of \textit{P. m. gracilis}. These forces could lead to a greater loss of genetic diversity in \textit{P. m. gracilis}, possibly resulting in their complete extirpation from the eastern area.

I have been able to track the recent history of these mouse populations with unusual completeness because of the very detailed records available on habitat fragmentation and deforestation in the LP, and the well-documented trapping of both \textit{P. m. gracilis} and \textit{P. leucopus} in Michigan over the last 100 years. I have also been able, by employing many different types of genetic analyses on \textit{P. m. gracilis} populations in the LP, to show that we have a sensitive way of detecting a population that is at risk but still genetically viable. Although many studies have genetically analyzed populations and observed their responses to changes in habitat or climate, most have focused on species that are already highly inbred or otherwise genetically abnormal. This research shows what can happen to the genetics of a limited population when it is in the very early stages of being geographically isolated, and might therefore serve as an example of
how to detect range contractions and genetic consequences for potentially endangered populations of other mammal species before they are critically endangered.


Google Earth. 2012. 44.893660°N; 83.112605°W. [2010 Oct].


Table 1. List of sites that were visited but not trapped. Sites are listed in the order which they are located along a west-east transect across the study region. Western sites above dotted line, eastern sites below.

<table>
<thead>
<tr>
<th>Site Code</th>
<th>GPS Coordinates</th>
<th>Reason for not trapping</th>
</tr>
</thead>
<tbody>
<tr>
<td>U3</td>
<td>45.1122, -84.3231</td>
<td>Accessibility: road</td>
</tr>
<tr>
<td>U4</td>
<td>45.0626, -84.3103</td>
<td>Very little with tall trees; patchy</td>
</tr>
<tr>
<td>U5</td>
<td>45.1502, -84.2732</td>
<td>Accessibility: road</td>
</tr>
<tr>
<td>MSW23</td>
<td>45.0861, -84.2500</td>
<td>Incorrect past trapping record</td>
</tr>
<tr>
<td>MWO7</td>
<td>45.1501, -84.2261</td>
<td>Accessibility: road; on private land</td>
</tr>
<tr>
<td>U9</td>
<td>45.1959, -84.1885</td>
<td>Not well-grown; predominantly conifers; dry</td>
</tr>
<tr>
<td>MC11</td>
<td>45.1443, -84.1623</td>
<td>Redundant with other nearby site</td>
</tr>
<tr>
<td>U10</td>
<td>45.1256, -84.1461</td>
<td>Not well-grown</td>
</tr>
<tr>
<td>MNC20</td>
<td>45.1923, -84.1094</td>
<td>Not well-grown; predominantly conifers; dry</td>
</tr>
<tr>
<td>LW21</td>
<td>45.1404, -84.0724</td>
<td>Redundant with other nearby site</td>
</tr>
<tr>
<td>U13</td>
<td>45.1993, -84.0342</td>
<td>Accessibility: road; on private land</td>
</tr>
<tr>
<td>ACSW17</td>
<td>45.1076, -83.8491</td>
<td>Redundant with other nearby site</td>
</tr>
<tr>
<td>AEC</td>
<td>45.1291, -83.6814</td>
<td>Patch of woods too small</td>
</tr>
<tr>
<td>ACN</td>
<td>45.1462, -83.8119</td>
<td>Not well-grown</td>
</tr>
<tr>
<td>ACSE</td>
<td>45.0952, -83.9496</td>
<td>Accessibility: road</td>
</tr>
<tr>
<td>AEN</td>
<td>45.1844, -83.6924</td>
<td>Accessibility: road; on private land</td>
</tr>
<tr>
<td>ASE</td>
<td>45.0871, -83.6358</td>
<td>Accessibility: road</td>
</tr>
</tbody>
</table>
Table 2. Samples of *P. m. gracilis* from Michigan’s LP

<table>
<thead>
<tr>
<th>Location</th>
<th>Site</th>
<th>Year</th>
<th>Number</th>
<th>GPS Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cheboygan (CH)</strong></td>
<td>OSM 1</td>
<td>2006</td>
<td>17</td>
<td>45.2953, -84.4258</td>
</tr>
<tr>
<td></td>
<td>Webb Rd.</td>
<td>2005</td>
<td>12</td>
<td>45.2720, -84.4363</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2006</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td><strong>Otsego (OT)</strong></td>
<td>Tin Shanty Rd.</td>
<td>2005</td>
<td>1</td>
<td>45.1099, -84.4132</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2008</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>**Montmorency (MT)</td>
<td>MSW22</td>
<td>2011</td>
<td>16</td>
<td>45.0861, -84.3170</td>
</tr>
<tr>
<td><strong>Alpena (AP)</strong></td>
<td>BTT</td>
<td>2011</td>
<td>8</td>
<td>45.1061, -83.8562</td>
</tr>
<tr>
<td></td>
<td>Rouleau Farm</td>
<td>2011</td>
<td>6</td>
<td>45.1513, -83.8610</td>
</tr>
<tr>
<td></td>
<td>N. Swamp</td>
<td>2005</td>
<td>7</td>
<td>45.1297, -83.8077</td>
</tr>
<tr>
<td></td>
<td>S. Swamp</td>
<td>2009</td>
<td>11</td>
<td>45.1238, -83.8078</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2011</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Microsatellite loci selected for analysis. Total number of alleles, expected heterozygosity ($H_E$) and observed heterozygosity ($H_O$) are shown for each locus. References are indicated by superscripts: 1, Prince et al. 2002; 2, Schmidt 1999; 3, Chirhart et al. 2000.

<table>
<thead>
<tr>
<th>Locus</th>
<th>[MgCl$_2$]</th>
<th>Temp.</th>
<th>Size Range</th>
<th>Alleles</th>
<th>$H_E$</th>
<th>$H_O$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO-09$^1$</td>
<td>1.5mM</td>
<td>58° C</td>
<td>151-247</td>
<td>23</td>
<td>0.81</td>
<td>0.77</td>
</tr>
<tr>
<td>PO-21$^1$</td>
<td>2mM</td>
<td>55° C</td>
<td>86-260</td>
<td>31</td>
<td>0.82</td>
<td>0.66</td>
</tr>
<tr>
<td>PO-26$^1$</td>
<td>2mM</td>
<td>56.5° C</td>
<td>137-235</td>
<td>33</td>
<td>0.79</td>
<td>0.68</td>
</tr>
<tr>
<td>PO-35$^1$</td>
<td>1.5mM</td>
<td>59° C</td>
<td>245-331</td>
<td>30</td>
<td>0.83</td>
<td>0.67</td>
</tr>
<tr>
<td>PO-40$^1$</td>
<td>1.5mM</td>
<td>59° C</td>
<td>154-372</td>
<td>31</td>
<td>0.87</td>
<td>0.62</td>
</tr>
<tr>
<td>PO3-68$^1$</td>
<td>1.5mM</td>
<td>58° C</td>
<td>230-290</td>
<td>28</td>
<td>0.79</td>
<td>0.52</td>
</tr>
<tr>
<td>PO3-85$^1$</td>
<td>2mM</td>
<td>58° C</td>
<td>182-254</td>
<td>22</td>
<td>0.85</td>
<td>0.71</td>
</tr>
<tr>
<td>PLGT-15$^2$</td>
<td>1.5mM</td>
<td>58° C</td>
<td>234-270</td>
<td>18</td>
<td>0.84</td>
<td>0.76</td>
</tr>
<tr>
<td>PLGT-62$^2$</td>
<td>2mM</td>
<td>53° C</td>
<td>141-247</td>
<td>22</td>
<td>0.84</td>
<td>0.76</td>
</tr>
<tr>
<td>Pml-01$^3$</td>
<td>2mM</td>
<td>53° C</td>
<td>146-194</td>
<td>21</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>Pml-04$^3$</td>
<td>2mM</td>
<td>50° C</td>
<td>174-302</td>
<td>28</td>
<td>0.89</td>
<td>0.84</td>
</tr>
</tbody>
</table>
Table 4: Percentage of unsuitable habitat around each of the four population centers, for circular areas using 5 different radii.

<table>
<thead>
<tr>
<th>Population</th>
<th>250m</th>
<th>500m</th>
<th>1km</th>
<th>1.5m</th>
<th>2km</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheboygan (CH)</td>
<td>26</td>
<td>22</td>
<td>25</td>
<td>27</td>
<td>31</td>
</tr>
<tr>
<td>Otsego (OT)</td>
<td>6</td>
<td>16</td>
<td>33</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>Montmorency (MT)</td>
<td>15</td>
<td>16</td>
<td>29</td>
<td>35</td>
<td>37</td>
</tr>
<tr>
<td>Alpena (AP)</td>
<td>51</td>
<td>45</td>
<td>45</td>
<td>43</td>
<td>40</td>
</tr>
</tbody>
</table>
Table 5. Genetic diversity indices for LP populations of *P. m. gracilis*. N: sample size (total individuals). $\bar{A}$: average number of alleles per locus. $\bar{A}_R$: allelic richness corrected by rarefaction with a sample size of 8 individuals. $\bar{A}_P$: private allelic richness corrected by rarefaction with a sample size of 8 individuals. $H_E$: expected heterozygosity. $H_O$: observed heterozygosity.

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>$\bar{A}$</th>
<th>$\bar{A}_R$</th>
<th>$\bar{A}_P$</th>
<th>$H_E$</th>
<th>$H_O$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Western</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheboygan (CH)</td>
<td>35</td>
<td>14.81</td>
<td>9.47</td>
<td>3.93</td>
<td>0.90</td>
<td>0.71</td>
</tr>
<tr>
<td>Otsego (OT)</td>
<td>8</td>
<td>7.54</td>
<td>7.55</td>
<td>2.46</td>
<td>0.80</td>
<td>0.67</td>
</tr>
<tr>
<td>Montmorency (MT)</td>
<td>16</td>
<td>7.91</td>
<td>6.23</td>
<td>1.63</td>
<td>0.77</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>Eastern (Alpena; AP)</strong></td>
<td>35</td>
<td>13.72</td>
<td>8.09</td>
<td>3.01</td>
<td>0.87</td>
<td>0.66</td>
</tr>
<tr>
<td>BTT</td>
<td>8</td>
<td>6.36</td>
<td>6.64</td>
<td>1.77</td>
<td>0.80</td>
<td>0.72</td>
</tr>
<tr>
<td>Rouleau Farm</td>
<td>6</td>
<td>4.36</td>
<td>4.36</td>
<td>0.48</td>
<td>0.62</td>
<td>0.56</td>
</tr>
<tr>
<td>Swamp (N &amp; S)</td>
<td>21</td>
<td>11.18</td>
<td>7.96</td>
<td>2.43</td>
<td>0.86</td>
<td>0.66</td>
</tr>
</tbody>
</table>
Table 6. Interpopulation differentiation for LP populations of *P. m. gracilis* represented by $F_{ST}$ and $\rho_{ST}$ values.

<table>
<thead>
<tr>
<th>Populations</th>
<th>$F_{ST}$</th>
<th>$\rho_{ST}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH &amp; OT</td>
<td>0.036</td>
<td>0.152</td>
</tr>
<tr>
<td>CH &amp; MT</td>
<td>0.068</td>
<td>0.169</td>
</tr>
<tr>
<td>OT &amp; MT</td>
<td>0.081</td>
<td>0.096</td>
</tr>
<tr>
<td>AP &amp; CH</td>
<td>0.037</td>
<td>0.086</td>
</tr>
<tr>
<td>AP &amp; MT</td>
<td>0.102</td>
<td>0.188</td>
</tr>
<tr>
<td>AP &amp; OT</td>
<td>0.046</td>
<td>0.255</td>
</tr>
</tbody>
</table>
Table 7. Results from BOTTLENECK for four different tests: sign test, standardized differences test, Wilcoxon sign-rank test, and allele-frequency distribution test.

<table>
<thead>
<tr>
<th>Pop.</th>
<th>Sign test</th>
<th>Standardized differences test</th>
<th>Wilcoxon sign-rank test</th>
<th>Allele frequency distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAM</td>
<td>0.117</td>
<td>0.017</td>
<td>0.012</td>
<td>0.966</td>
</tr>
<tr>
<td>SMM</td>
<td>0.033</td>
<td>0.458</td>
<td>0.083</td>
<td>0.966</td>
</tr>
<tr>
<td>TPM</td>
<td>0.053</td>
<td>0.440</td>
<td>0.123</td>
<td>L-shaped</td>
</tr>
<tr>
<td>IAM</td>
<td>0.056</td>
<td>0.000</td>
<td>0.147</td>
<td>0.765</td>
</tr>
<tr>
<td>SMM</td>
<td>0.123</td>
<td>0.147</td>
<td>0.083</td>
<td>L-shaped</td>
</tr>
<tr>
<td>TPM</td>
<td>0.271</td>
<td>0.083</td>
<td>0.765</td>
<td>L-shaped</td>
</tr>
<tr>
<td>IAM</td>
<td>0.012</td>
<td>0.966</td>
<td>0.320</td>
<td>L-shaped</td>
</tr>
<tr>
<td>SMM</td>
<td>0.012</td>
<td>0.966</td>
<td>0.240</td>
<td>L-shaped</td>
</tr>
<tr>
<td>TPM</td>
<td>0.012</td>
<td>0.966</td>
<td>0.240</td>
<td>L-shaped</td>
</tr>
</tbody>
</table>

*Numbers in bold show rejection of the null hypothesis (bottleneck)*
Fig. 1. Locality records for *P. m. gracilis* populations between 1883-1980 (A) and 1981-2006 (B). Some of the populations in the LP prior to 1981 have since disappeared. (Adapted from Myers et al. 2009)
Figure 2. Map of habitat distribution across the study region. Yellow circles represent locations where *P. m. gracilis* were trapped between 1980 and 2010. *P. m. gracilis* were trapped in Oak Association, Northern Hardwood Association, all Mixed Forest types, and all Deciduous Forest types.
Figure 3. 2011 trapping results. Black circles represent the previously known distribution of *P. m. gracilis*. Black triangles represent sites that were visited but not trapped. White triangles represent locations that were trapped but only contained *P. leucopus*. White circles represent locations where *P. m. gracilis* were trapped. Green represents optimal habitat, beige represents suitable habitat, and white represents unsuitable habitat.
Figure 4. Habitat composition around the AP and MT trapping areas using the 60Tiff image map produced from the unsupervised ISODATA classification (A) AP locations of *P. m. gracilis*. Black circle: S. Swamp site; White circle: BTT site; Grey circle: Rouleau Farm site. (B) MT trapping area. White circle: MSW22 site
Figure 5. Comparison of an oak-dominated (MS7) and maple-dominated (MSW22) site across a four-month period. Maps were downloaded from USGS Earth Explorer Landsat Archive. White circles represent approximate center of each trapping area.
Figure 6. Genetic differentiation among LP populations of P. m. gracilis compared to distances between populations. (A) Pairwise $F_{ST}$ values for the CH, OT, MT and AP populations (Mantel: $r = -0.274$; $P = 0.490$; $R^2 = 0.075$). (B) Pairwise $\rho_{ST}$ values for the CH, OT, MT and AP populations (Mantel: $r = 0.334$; $P = 0.100$; $R^2 = 0.112$).
Figure 7. Clustering of LP populations of *P. m. gracilis* by the program STRUCTURE with k=4. Cluster 1: Purple; Cluster 2: Yellow; Cluster 3: Blue; Cluster 4: Green. Bars represent average assignment of each individual sampled in a population to the indicated clusters. Population abbreviations are given in Table 2.