

# Microsatellite Genetic Structure and Cytonuclear Discordance in Naturally Fragmented Populations of Deer Mice (*Peromyscus maniculatus*)

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## Abstract

The Great Lakes impose high levels of natural fragmentation on local populations of terrestrial animals in a way rarely found within continental ecosystems. Although separated by major water barriers, woodland deer mouse (*Peromyscus maniculatus gracilis*) populations on the islands and on the Upper Peninsula (UP) and Lower Peninsula (LP) of Michigan have previously been shown to have a mitochondrial DNA contact zone that is incongruent with the regional landscape. We analyzed 11 microsatellite loci for 16 populations of *P. m. gracilis* distributed across 2 peninsulas and 6 islands in northern Michigan to address the relative importance of geographical structure and inferred postglacial colonization patterns in determining the nuclear genetic structure of this species. Results showed relatively high levels of genetic structure for this species and a significant correlation between interpopulation differentiation and separation by water but little genetic structure and no isolation-by-distance within each of the 2 peninsulas. Genetic diversity was generally high on both peninsulas but lower and correlated to island size in the Beaver Island Archipelago. These results are consistent with the genetic and demographic isolation of Lower Peninsula populations, which is a matter of concern given the dramatic decline in *P. m. gracilis* abundance on the Lower Peninsula in recent years.

**Key words:** Great Lakes, microsatellites, population genetics, Rodentia, small mammals

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Habitat fragmentation due to either natural or anthropogenic barriers can result in genetic drift by limiting both population size and gene flow between nearby populations (Wright 1931; Templeton et al. 1990; Gilpin 1991). Over time, this should result in interpopulation differentiation that can be measured by variations in marker allele frequencies and diversity (Wright 1931). However, the robust dispersal ability of many small mammals tends to overcome the effects that landscape barriers might be expected to have on their genetic structure (McCullough and Chesser 1987; Mossman and Waser 2001). Therefore, in order to measure the effects of landscape barriers on the genetics of small mammal populations, one must look to areas where putative geographical barriers are highly resistant to animal migration.

As melting glaciers retreated and formed the Great Lakes, they fragmented the surrounding region into peninsulas and islands, a process that ended about 11 000 years ago (see Dyke et al. 2002). The flora and fauna now inhabiting the region appear to have colonized it shortly thereafter, from one or

more southern refugia (Brant and Ortí 2003; Rowe et al. 2006; Taylor and Hoffman 2010). Among the colonizers was the woodland deer mouse (*Peromyscus maniculatus gracilis*), a strict woodland specialist inhabiting boreal and mixed hardwood forests in the most northern parts of the contiguous US and in southern Canada, from the Midwest to the East Coast (Hall 1981).

Populations of *P. m. gracilis* in Michigan face 2 potential barriers to migration. First, Lake Michigan separates populations in the Upper Peninsula (UP) and Lower Peninsula (LP) from each other, whereas Lake Superior and Lake Huron separate the 2 peninsulas from Ontario, Canada. Second, the southern range limit of this subspecies bisects the LP of Michigan latitudinally, roughly along the 45th parallel (Hall 1981; Myers et al. 2009); the unsuitable habitat south of that line presumably inhibits migration around the shores of the Great Lakes.

Studies of mitochondrial DNA (mtDNA) sequences from the woodland deer mouse indicate that 2 distinct

lineages are present in the Great Lakes region (Lansman et al. 1983; Dragoo et al. 2006), suggesting origins in 2 different refugia (Taylor and Hoffman 2010). Most of the populations in Michigan belong to an eastern lineage that probably colonized the region shortly after the glaciers retreated, about 9000–11 000 years ago, crossing the lakes multiple times in the process (Taylor and Hoffman 2010). A second mitochondrial lineage dominates the westernmost end of the UP and is found as far west as Minnesota. The zone marking the transition from one lineage to another does not correspond to any obvious geographical barrier (Figure 1) and instead most likely represents contact between previously separated groups (Taylor and Hoffman 2010). Thus, the lakes do not appear to have played a significant role in structuring mouse populations during colonization.

Although the Great Lakes appear to have been porous barriers on the timescale of postglacial colonization, their imposing presence in the landscape of Michigan suggests that they have the potential to act as significant genetic and demographic barriers for terrestrial animal populations in the short term. The potential for the Great Lakes to deter migration is especially relevant for small mammals at the present time: Many species in the region have experienced range expansions or contractions correlated with recent climate change (Long 1996; Jannett et al. 2007; Myers et al. 2009), so strong barriers could interfere with those species' adaptive adjustments to range limits. The woodland deer mouse is among the species experiencing the most dramatic range shifts; its abundance and distribution on the LP of Michigan appear to have declined sharply in the last 100 years (Myers et al. 2009). Because *P. m. gracilis* is not found in

the deciduous forests on the southern half of the LP (Hall 1981; Figure 1), northern LP mouse populations are effectively cut off from populations in the surrounding states by unsuitable habitat on one side and lakes on the other 3 sides. Therefore, if the Great Lakes are indeed effective barriers, they could threaten the maintenance of genetic diversity and the long-term demographic persistence of these populations.

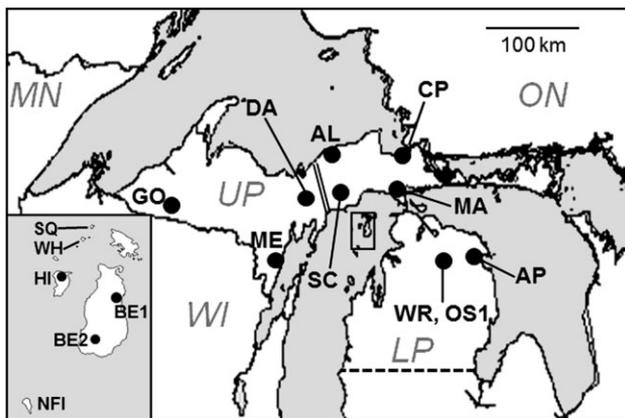
In the absence of major barriers, North American field mouse (*Peromyscus* spp.) populations typically have little nuclear differentiation (Mossman and Waser 2001; Yang and Kenagy 2009; Anderson and Meikle 2010). The limited genetic differentiation observed in *Peromyscus* species has been attributed in part to their ready dispersal across apparently hostile terrain, such as swampy areas, open fields (Cooke and Terman 1977; Cummings and Vessey 1994; Krohne and Hoch 1999), or even short stretches of open water (Sheppe 1965). Mossman and Waser (2001) found very low levels of microsatellite marker differentiation over distances up to 30 km, suggesting that mice disperse readily through all matrix types. Because many small mammals have more stringent habitat requirements (Rosenblatt et al. 1999; Nupp and Swihart 2000) and can therefore exhibit more divergence between populations (Smith and Fujio 1982), *Peromyscus* species may serve as conservative models for mammalian population genetics and provide a useful baseline for estimating the effects of geographical barriers.

We have analyzed microsatellite markers from Great Lakes populations of *P. m. gracilis* to address the roles of the lakes in restricting postcolonization gene flow in this subspecies. First, we hypothesized that genetic diversity would be lower on the islands and LP of Michigan than on the UP, corresponding to the lower diversity seen in mitochondrial sequences (Taylor and Hoffman 2010). Second, we hypothesized that we would find an east–west population structure in Michigan's UP, corresponding to the 2 lineages defined by mtDNA sequences (Taylor and Hoffman 2010). Finally, we hypothesized that we would find significant population structure among the mice on the islands and peninsulas of northern Michigan, due to inhibition of dispersal by the Great Lakes.

## Materials and Methods

### Sampling

Tissue samples were obtained from 16 *P. m. gracilis* populations trapped in northern Michigan from 2002 to 2009 (Figure 1; exact sampling locations are shown in Supplementary Table 1), using the same nonlethal methods described previously (Taylor and Hoffman 2010). Animals were handled in accordance with guidelines established by the American Society of Mammalogists (Gannon et al. 2007), using a protocol approved by the Institutional Animal Care and Use Committee of Miami University. For the Gogebic population only, samples from 2 sites 8.3 km apart were pooled for analyses due to small sample sizes; all other



**Figure 1.** Trapping locations for *Peromyscus maniculatus gracilis* in the Great Lakes region. Double line indicates the approximate location of contact zone between mitochondrial lineages from Taylor and Hoffman (2010). Dashed line indicates historical southern range limit of *P. m. gracilis* after Hall (1981). Inset box shows the Lake Michigan islands included in this study. The UP and LP of Michigan are indicated, as are the surrounding states/provinces of Wisconsin (WI), Minnesota (MN), and Ontario (ON), Canada.

populations had  $\geq 6$  individuals and represent individual trapping sites. Samples for North Fox Island (Leelanau County), Alger County, and the Beaver Island group (Charlevoix County) were obtained from the collections of the Michigan State University Museum and the University of Michigan Museum of Zoology (Supplementary Table 2).

### Sample Preparation and Genotyping

DNA was isolated from ear tissue using the E.Z.N.A. Tissue DNA Kit (Omega Bio-Tek, Norcross, GA). We genotyped 11 microsatellite loci from 183 individuals representing 16 populations. Microsatellites were amplified by PCR using Promega Go-Taq DNA polymerase and the Flexi buffer system. Primer sets were selected from the literature and amplified under published or experimentally determined conditions (Supplementary Table 3). Typical PCR reactions contained 20 ng template DNA, 1.5–2 mM  $MgCl_2$ , and 0.2 mM each dNTP. The PCR cycle used consisted of 94 °C for 2 min; 40 cycles of 30 s at 94 °C, 30 s at 50–65 °C, and 1 min at 72 °C; and 5 min at 72 °C. Specific annealing temperatures and  $MgCl_2$  concentrations are shown in Supplementary Table 3. Forward primers labeled with the G5 dye set were obtained from Applied Biosystems (Foster City, CA), and products were run on an Applied Biosystems 3130 or 3730 DNA Analyzer with the 600LIZ internal size standard (Applied Biosystems). Product peaks were identified manually using Peak Scanner v1.0 software (Applied Biosystems). Samples that produced ambiguous or negative results on a first attempt were repeated; samples producing consistently ambiguous or negative genotypes after 3 repetitions were treated as null at that locus.

### Analyses of Genetic Diversity

Genetic diversity was measured using both allelic richness ( $A_R$ ) and common heterozygosity measures. The expected heterozygosity ( $H_E$ ) from Hardy–Weinberg calculations is presented in the text and in Tables 1 and 2, whereas observed heterozygosity ( $H_O$ ) is presented only in the tables for brevity.  $A_R$  was standardized for sample size using the repeated sampling procedure implemented in HP-Rare (Kalinowski 2005).  $H_O$  and  $H_E$  were calculated in Arlequin v3.11 (Excoffier et al. 2005). Differences in genetic diversity among populations were determined using an ANOVA in Minitab version 15 (Minitab Inc., State College, PA), with Tukey's Honestly Significant Difference (HSD) post hoc test to determine which  $A_R$  values were distinct. Tukey's HSD is a conservative test that adjusts the individual confidence intervals to maintain the specified 95% simultaneous confidence interval.

### Analyses of Genetic Structure

We examined overall genetic structure using 2 Bayesian clustering algorithms. First, we examined the number of genetically supported populations using the program STRUCTURE, version 2.3.3 (Pritchard et al. 2000), which assigns individuals to a user-defined number of genetic clusters  $K$  based on similarities among multilocus genotypes.

We performed 3 replicate runs with a burn-in of 100 000 and 1 000 000 subsequent iterations for each value of  $K$  from 1 to 10. Because the posterior probability is not always an accurate indicator of the number of clusters in gradually structured populations (Evanno et al. 2005; Pritchard et al. 2010), we estimated the number of clusters that best fit the data using the  $\Delta K$  metric derived by Evanno et al. (2005) from the second-order rate of change of  $\ln P(D)$ . We then performed 10 long runs of 2 000 000 iterations at fixed values of  $K$  and combined the results from these runs using the program CLUMPP, version 1.1.2 (Jakobsson and Rosenberg 2007) for assignment of individuals to clusters. To examine the possibility of subtle genetic structure within the 2 peninsulas, we also performed structure runs for the UP and LP samples separately; these analyses are available as Supplementary Figure 1.

We also used assignment tests implemented in the program GeneClass2 (Piry et al. 2004) to examine population structure and the possibility of contemporary gene flow. Higher levels of interpopulation structure increase the correct assignment of individuals to their source populations (Paetkau et al. 1995), so correct assignment rates can be seen as a measure of population structure (Rannala and Mountain 1997). We performed assignment using the criterion of Rannala and Mountain (1997) to examine the accuracy of assignment to the correct population and to the correct landmass (for instance, the assignment of an individual from a UP population to any of the populations on the UP). For individuals that were assigned to nonsource populations, we examined the probability of membership in the source population using the algorithm developed by Paetkau et al. (2004) to simulate 10 000 individual genotypes. Individuals were considered to be correctly assigned to nonsource populations only if the estimated  $P$  value for membership in the source population was below the desired  $\alpha$  of 0.05 or 0.01.

For comparison with traditional methodologies, we also conducted population structure analyses using the program GenePop version 4.0 (Rousset 2008). Significance of population differentiation was determined using log likelihood ratio tests (Goudet et al. 1996) after adjusting for multiple comparisons using the sequential Bonferroni correction (Rice 1989). We used pairwise estimates of  $F_{ST}$  ( $\theta$ ) according to the method of Weir and Cockerham (1984) as well as the allele size–dependent estimator  $\rho_{ST}$ , which incorporates a high-rate stepwise mutation model.

### Geographical Distribution of Genetic Structure

We examined isolation-by-distance using Mantel tests (Mantel 1967) as implemented in Arlequin version 3.11 (Excoffier et al. 2005). We tested for isolation-by-distance within a given landmass using a Mantel test with pairwise  $F_{ST}$  values between populations as the  $Y$  matrix and pairwise geographical distances as the  $X$  matrix.

We also used a categorical Mantel test to determine whether separation by the Great Lakes was associated with increased genetic differentiation. We constructed a matrix in which the geographical distance between each population

**Table 1** Molecular diversity indexes for populations of *Peromyscus maniculatus gracilis*

Population	N	$\bar{A}$	$\bar{A}_R$	$\bar{A}_{RT}$	$\bar{H}_E$	$\bar{H}_{ET}$	$\bar{H}_O$
UP	80	8.11	6.38		0.80		0.74
Alger (AL)	19	10.63	6.92	A	0.79	AB	0.66
Chippewa (CP)	13	7.91	6.55	A	0.78	AB	0.75
Delta (DA)	14	8.91	6.75	A	0.81	AB	0.67
Gogebic (GO)	8	8.91	6.57	A	0.80	AB	0.72
Mackinac (MA)	9	7.27	6.53	A	0.82	AB	0.77
Menominee (ME)	4	4.73	N/D	N/D	0.78	AB	0.82
Schoolcraft (SC)	13	8.45	6.62	A	0.81	AB	0.76
LP	29	8.24	6.55		0.82		0.67
Alpena (AP)	11	10.64	6.55	A	0.82	AB	0.67
Osmun 1 (OS1)	6	6.09	N/D	N/D	0.83	A	0.74
Webb Road (WR)	12	8.00	6.38	A	0.79	AB	0.62
Islands	74	4.20	3.64		0.58		0.52
Beaver Island 1 (BE1)	7	3.82	3.82	B	0.59	ABC	0.68
Beaver Island 2 (BE2)	9	5.36	4.94	AB	0.74	ABC	0.66
High Island (HI)	16	4.55	3.63	B	0.58	ABC	0.52
North Fox	20	4.73	3.49	B	0.61	BC	0.47
Island (NFI)							
Squaw Island (SQ)	11	3.64	3.17	B	0.51	C	0.39
Whiskey Island (WH)	10	3.09	2.81	B	0.47	C	0.42

N: sample size (individuals);  $\bar{A}$ : average number of alleles per locus;  $\bar{A}_R$ : allelic richness corrected by rarefaction with a sample size of 7;  $\bar{A}_{RT}$ : Significance groupings for  $\bar{A}_R$ , according to Tukey's HSD; populations not sharing a letter designation are significantly different.  $\bar{H}_E$ : expected heterozygosity;  $\bar{H}_{ET}$ : Significance groupings for  $\bar{H}_E$ , according to Tukey's HSD;  $\bar{H}_O$ : observed heterozygosity. N/D, not determined.

pair was replaced by the number 1, 2, or 3, determined as follows: 1) populations located on the same landmass, 2) populations separated by <10 km of Lake Michigan (e.g., all comparisons between the UP and the LP or between islands), and 3) populations separated by  $\geq 10$  km of Lake Michigan (all island-mainland comparisons). The categorical matrix of separation classes was used as the X matrix for comparison with a Y matrix of  $F_{ST}$  values. For comparison, we also examined the importance of over-water distance by combining categories 2 and 3 to produce a matrix in which the only categories were 1) not separated by Lake Michigan and 2) separated by Lake Michigan.

For comparison with mitochondrial genetic structure, we used population-level comparisons to circumvent the lack of an exact one-to-one correspondence of individual mitochondrial and nuclear genotypes between studies. Specifi-

cally, we used a Mantel test to compare a modified  $F_{ST}$  matrix to a pairwise  $\Phi_{ST}$  distance matrix derived from previously described mitochondrial D-loop sequences (Taylor and Hoffman 2010). To allow direct comparisons between the 2 studies, the Alpena, Beaver Island, and High Island populations were removed from this analysis.

**Other Population Genetic Analyses**

Deviation from Hardy-Weinberg expectations (HWE) and linkage disequilibrium were determined in GenePop version 4.0 (Rousset 2008) using log likelihood ratio tests (Goudet et al. 1996) after adjusting for multiple comparisons using the sequential Bonferroni correction (Rice 1989).

We examined the data for null alleles using the program Micro-Checker (Van Oosterhout et al. 2004). Where null alleles were detected at frequencies over 5% within a population, we corrected genotypes and allele frequencies using the method of Brookfield (1996). However, a side effect of correction for null alleles is the disruption of multilocus genotypes because one cannot determine exactly which genotypes are from homozygotes as opposed to heterozygotes incorporating a null allele (Van Oosterhout et al. 2004). Therefore, we used the original genotypes for clustering analyses, which require the information present in the unmodified multilocus genotypes.

**Results**

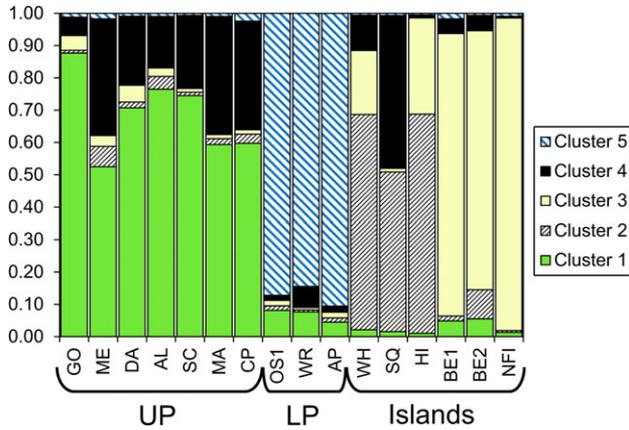
**Genetic Diversity**

All loci were polymorphic in all populations, with the lone exception of locus PO-09 on Squaw Island, which was fixed for a single allele. Genetic diversity was relatively high in all

**Table 2** Clustering parameters of *Peromyscus maniculatus gracilis* samples determined by STRUCTURE analyses

K	Ln P(D)	Var[Ln P(D)]	$\Delta K$
1	-9572.4	121.1	N/A
2	-8931.2	264.3	17.06
3	-8567.3	391.0	7.24
4	-8346.6	444.7	9.66
5	-7922.2	580.3	19.86
6	-7976.2	673.6	4.80
7	-7905.5	802.1	8.91
8	-7582.5	876.4	14.68
9	-7694.2	1307.0	0.92
10	-7772.8	2503.0	1.57

The average values from 3 structure runs at each value of K are given for the log probability of data (Ln P(D)), variance (Var [Ln P(D)]), and the  $\Delta K$  statistic recommended by Evanno et al. (2005). N/A, not applicable.



**Figure 2.** Clustering of Michigan populations of *Peromyscus maniculatus gracilis* by STRUCTURE. Bars represent the average assignment of individuals in a population to the indicated clusters, over 10 runs at  $K = 5$  ( $\ln P(D) = -7922.2$ ; variance  $\text{Var}[\ln P(D)] = 580.3$ ;  $\Delta K = 19.9$ ). Population abbreviations are given in Table 1.

mainland populations, with  $\bar{H}_E = 0.80$  and  $\bar{A}_R = 6.4$  on the UP and  $\bar{H}_E = 0.82$  and  $\bar{A}_R = 6.6$  on the LP (Table 1). Diversity was lower on the islands ( $\bar{H}_E = 0.58$ ;  $\bar{A}_R = 3.6$ ) and was correlated with island size for both  $\bar{H}_E$  ( $r = 0.98$ ;  $P = 0.0014$ ) and  $\bar{A}_R$  ( $r = 0.95$ ;  $P = 0.013$ ). Allelic richness for all island populations except Beaver Island 2 was significantly different from that of mainland populations (Table 1; ANOVA: degrees of freedom = 15;  $F = 10.19$ ;  $P < 0.001$ ).  $\bar{H}$  followed a similar trend, except that the Squaw and Whiskey Island heterozygosities were significantly lower than those of the other islands (Table 1;  $P < 0.001$ ).

### Genetic Structure

Analyses of our microsatellite data using the STRUCTURE clustering algorithm (Pritchard et al. 2000) indicated the presence of 5 distinct genetic clusters ( $\ln P(D) = -7922.2$ ; variance  $\text{Var}[\ln P(D)] = 580.3$ ;  $\Delta K = 19.9$ ), according to the  $\Delta K$  metric developed by Evanno et al. (2005). At  $K = 5$ , the LP individuals clustered together, whereas UP individuals formed a second distinct group of clusters, and island mice formed a third cluster (Figure 2). The clustering of island populations was more complex, as 2 internal clusters were identified; the small northern islands of Squaw,

**Table 3** Gene flow among Great Lakes populations of *Peromyscus maniculatus gracilis*, as measured by GeneClass2

	Total	$\alpha_{0.05}$	$\alpha_{0.01}$
Expected type I errors	N/A	9.15	1.83
Nonsource individuals	43	24	11
Assigned to same landmass	38	19	8
Assigned to separate landmass	5	5	3

The expected number of Type I errors is given for  $N = 183$ . Significance values for assignment of individuals were determined by comparing genotypes to those of 10 000 simulated individuals. N/A, not applicable.

Whiskey, and High formed by one cluster, and the southern Beaver and North Fox islands were characterized by the other.

In the assignment tests performed in GeneClass2, 77% (140/183) of individuals were assigned to the correct source population. Of the 43 incorrectly assigned individuals, 38 were assigned to a population on the same landmass as the source population, so 98% (178/183) of individuals were assigned to the correct landmass and only 2% (5/183) were misassigned. At the 95% confidence level ( $\alpha_{0.05}$ ), 9 erroneous assignments are expected, so all 5 misassignments could be due to error (Table 3). At the 99% confidence level, the number of misassignments (3) just exceeds the number expected (1.83).

Levels of differentiation as measured by  $F$ -statistics and Fisher's exact  $G$ -test were moderate and significant (Supplementary Table 4; overall  $\rho_{ST} = 0.15$ ;  $F_{ST} = 0.17$ ;  $P < 0.0001$ ). The population pairs with the lowest  $P$  values were significant under the adjusted threshold value of  $P = 0.00042$ .

### Geographical Distribution of Genetic Structure

Overall differentiation in the Great Lakes region showed an inverse pattern of isolation-by-distance (Figure 3A;  $r = -0.30$ ;  $P = 0.045$ ), due to very high pairwise  $F_{ST}$  values among nearby islands ( $\rho_{ST} = 0.58$ ;  $F_{ST} = 0.27$ ;  $P < 0.0001$ ) and low values among the dispersed populations on the UP ( $\rho_{ST} = 0.044$ ;  $F_{ST} = 0.036$ ;  $P < 0.0001$ ). Within the UP, populations showed no isolation-by-distance (Figure 3B;  $r = 0.12$ ;  $P = 0.31$ ). Differentiation between populations separated by water was always statistically significant and on average much higher (average pairwise  $\rho_{ST} = 0.28$ ;  $F_{ST} = 0.19$ ) than was seen for populations within the same landmass (Figure 3C). Correlation between separation by water and genetic differentiation was highly significant, both when over-water distance was divided into 2 different categories ( $r = 0.61$ ;  $P < 0.0001$ ) and when all populations separated by water were included in the same category ( $r = 0.63$ ;  $P < 0.0001$ ).

No correlation was observed between pairwise microsatellite  $F_{ST}$  values and mitochondrial genetic distances ( $r = 0.092$ ;  $P = 0.036$ ) measured in a previous study using the same animals (Taylor and Hoffman 2010). We also found that populations on either side of the mitochondrial contact zone reported previously (Taylor and Hoffman 2010) were no more different in terms of microsatellites than were populations on the same side of the zone ( $r = -0.13$ ;  $P = 0.74$ ).

### Other Population Genetic Analyses

Linkage disequilibrium was significant for only 1 of 880 pairwise comparisons between populations. When populations were tested for HWE, 13 of 176 tests distributed across 7 populations resulted in significant deviations from equilibrium. Four loci deviated in the Delta (UP) population and 2 in the Webb Road (LP) population; no other population deviated at more than one locus. As heterozygote deficiencies were not consistent within populations, they

are more likely to be due to null alleles than to an overall absence of HWE within any specific population.

## Discussion

### Natural Barriers and Genetic Structure in *Peromyscus* Populations

Populations of *P. m. gracilis* on Michigan's LP are geographically separated from surrounding populations by the Great Lakes to the north and by the southern range limit to the south. Therefore, we expected limited gene flow to or from the LP and differentiation from populations elsewhere. All analyses of our microsatellite data indicated little dispersal and describe a genetic structure consistent with the isolation of *P. m. gracilis* populations on the LP from all other populations tested, as we initially hypothesized.

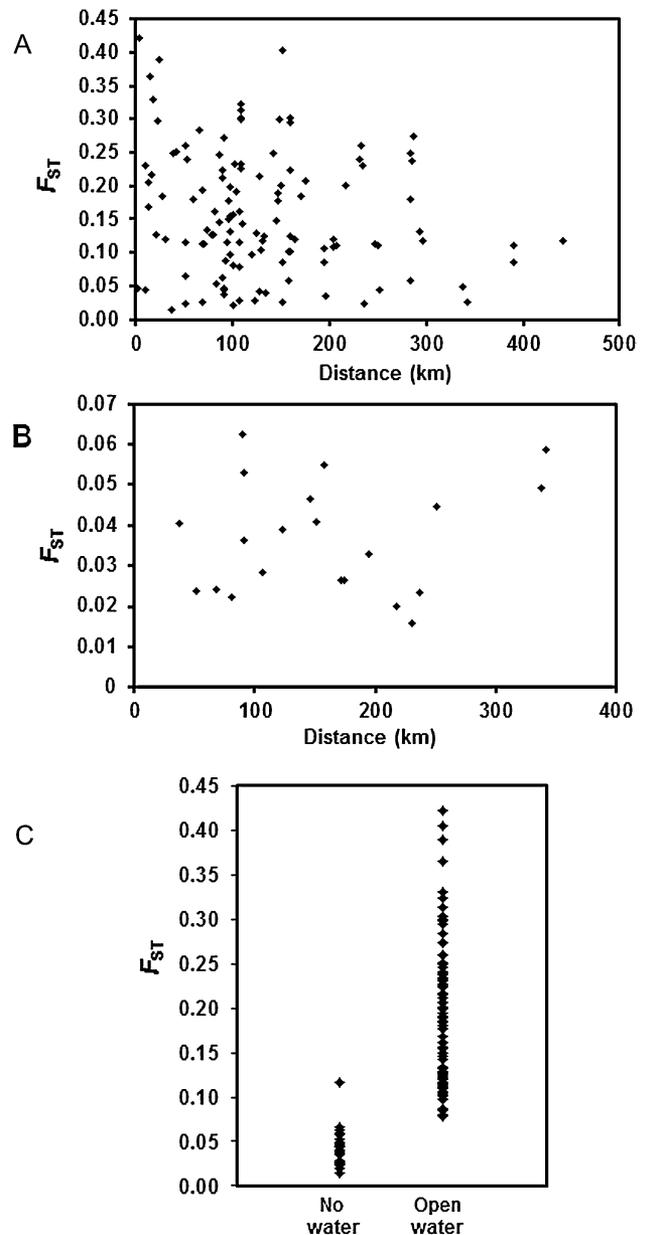
Similarly, we found very high levels of divergence among the Lake Michigan islands, even though the over-water distances separating many island pairs are less than the distance separating the 2 peninsulas (Figure 3C). This finding is highlighted by the fact that including over-water distance in our categorical Mantel tests did not increase the correlation between  $F_{ST}$  and separation by water.

### Cytoneuclear Discordance in Great Lakes *Peromyscus*

Our second hypothesis, that we would find significant east-west genetic structure in the UP corresponding to the distribution of the 2 distinct mitochondrial lineages (Taylor and Hoffman 2010), was refuted by the microsatellite data. We found no genetic structure among populations of *P. m. gracilis* on the LP and minimal structure on the UP (Figure 2), in contrast to the significant divergence between the 2 peninsulas. Our results are consistent with the general lack of geographically defined genetic structure shown by *Peromyscus* populations elsewhere (Mossman and Waser 2001; Yang and Kenagy 2009; Anderson and Meikle 2010) and with the established high vagility of these mice. Even though the typical dispersal distance for a female *Peromyscus* between birth and breeding is likely only around 250 m (Blair 1940; Krohne et al. 1984; Neigel et al. 1991), males tend to disperse about 50% farther (Dice and Howard 1951; Keane 1990), and rare long-distance dispersal events are known to occur in this genus. Krohne et al. (1984) documented an individual traveling nearly 700 m over 2 nights; Maier (2002) found different tagged females 7 and 15 km from their original capture locations, after 8 months or a single month, respectively. In this context, the genetic separation between the peninsulas provides a rare example of a geographical barrier that significantly inhibits gene flow in these highly vagile mice.

### Genetic Isolation and Drift in Island Mouse Populations

The high levels of genetic divergence we observed among islands (Figure 3C; Supplementary Table 4) suggest a strong role for genetic drift in structuring island populations. Island populations are expected to be more susceptible to the effects of genetic drift because of small and/or fluctuating



**Figure 3.** Spatial distribution of genetic structure for *Peromyscus maniculatus gracilis*. (A) Pairwise  $F_{ST}$  values for all Great Lakes populations (Mantel:  $r = -0.29$ ;  $P = 0.04$ ). (B) Pairwise  $F_{ST}$  values for UP populations as a function of terrestrial Euclidean distance (Mantel:  $r = 0.19$ ;  $P = 0.27$ ). (C) Pairwise  $F_{ST}$  values arranged by the presence or absence of Lake Michigan water between populations (Mantel:  $r = 0.63$ ;  $P < 0.0001$ ).

population sizes (Wright 1931). Significant population fluctuations on the islands are indicated by the limited published trapping data, for example, a 2-fold variation in trapping success between years on High Island (Meagher 1999; Lalor 2010). Finally, diversity patterns on the islands provide additional support for a strong role of drift in island population structure, as discussed below.

Our finding that island microsatellite diversity is low and correlated with island size (Table 1) is consistent with our first hypothesis, which was that genetic diversity would be lower on the islands than on the UP. Thus, these results are consistent with studies of mtDNA (Taylor and Hoffman 2010) and of allozymes from *P. m. gracilis* (Meagher 1999) on the same islands. Taken together with the high levels of differentiation we observed among island populations, this evidence indicates that drift has promoted the genetic distinctiveness of island populations compared with those on the mainland.

As with the Lake Michigan islands, we expected little genetic diversity in LP populations of mice. Instead, we found levels of genetic diversity comparable to those on the UP (Table 1), contrasting markedly with the reduced mitochondrial haplotype diversity previously observed on the LP (Taylor and Hoffman 2010). This distinction may be important because LP populations of *P. m. gracilis* have long been in decline (Myers et al. 2009), and low genetic diversity has been associated with reduced survivorship, depressed growth rates, and increased parasite load in *Peromyscus* species (Brewer et al. 1990; Jimenez et al. 1994; Meagher 1999). The differences between mitochondrial and nuclear genetic diversity here could indicate a reduction in effective population size that has reduced mitochondrial diversity but has not yet been severe enough to affect microsatellite diversity, which has a 4-fold higher effective population size than mtDNA and should therefore be less sensitive to population decline. Male-biased dispersal in the LP may also have restored microsatellite genetic diversity while leaving mitochondrial diversity unaffected.

### Population Genetic Processes

Our results indicate that individual populations are close to HWE with respect to the loci studied, resulting in few significant deviations from HWE and in similar values for  $H_O$  and  $H_E$  (Table 1). The loci selected do not exhibit linkage disequilibrium, as is expected for independently segregating loci representing different segments of the *P. m. gracilis* genome. The overall genetic diversity reported here is comparable to levels reported in studies of other *Peromyscus* species (Mossman and Waser 2001; Yang and Kenagy 2009; Anderson and Meikle 2010). We did find some evidence for null alleles in the form of possible null homozygotes (ungentyped individuals) and heterozygote deficiencies that were restricted to specific loci rather than distributed throughout the genome, as would be expected for population-wide deviations from HWE. Null alleles were observed at some of the same loci in a study of *P. leucopus* (Anderson and Meikle 2010), but interspecific differences complicate direct comparisons between the 2 studies.

### Microsatellite Genetic Structure and the Phylogeographic Context

The genetic structure we have described using nuclear microsatellite markers differs from the previously reported mitochondrial patterns in more ways than those detailed

above. First, the differentiation between mice on the 2 peninsulas is more pronounced in the present study. Second, and more dramatically, the east–west contact zone observed on the UP in mitochondrial studies (Figure 1; Lansman et al. 1983; Dragoo et al. 2006; Taylor and Hoffman 2010) is absent from the present microsatellite study. The lack of any statistical correlation between  $F_{ST}$  values from the 2 kinds of analyses underscores this point. Such cytonuclear discordance has been observed in a number of studies at both the interspecific and intraspecific levels of organization (Lindell et al. 2008; Yang and Kenagy 2009). One possible explanation for the results of the present study is that genetic admixture occurs more slowly for mitochondrial loci because smaller effective populations can sustain less diversity and are less likely to maintain introduced alleles, whereas male-biased dispersal speeds dissemination of nuclear alleles relative to mtDNA (Yang and Kenagy 2009). An alternative explanation is that the relative lack of recombination at mitochondrial loci allows mitochondrial lineages to persist despite the presence of different lineages in a single population (Avice 2000). Although subtle spatial structure occurring within populations might be missed by the population-level distance analyses described here (Coulon et al. 2006), we consider this unlikely because the maximum distance across each of our trapping sites is typically much smaller (<0.5 km) than the distances between sites (2.7–440 km) and well within the dispersal capabilities of an individual mouse. Still, analytical methods incorporating individually georeferenced genotypes are a promising route for the exploration of fine-scale genetic structure in similar systems (McRae et al. 2005; Coulon et al. 2006).

However, unlike prior studies documenting intraspecific cytonuclear discordance (Lindell et al. 2008; Yang and Kenagy 2009), we here describe a system in which landscape fragmentation and geographical range limits apparently restrict nuclear gene flow. In this case, the results paint 2 temporally distinct pictures of the development of genetic structure in Great Lakes populations of *P. m. gracilis*. Prior studies describe the postglacial colonization of Ontario, the Lake Michigan islands, the LP of Michigan, and the eastern UP by a single mitochondrial lineage (Lansman et al. 1983; Dragoo et al. 2006; Taylor and Hoffman 2010), with a second lineage in the western UP. The picture based on microsatellite data describes the postcolonization differentiation of deer mouse populations on the isolated peninsulas and islands of the Great Lakes region. The distinctiveness of these 2 pictures suggests that the functional properties of the relevant barriers are important. For instance, small numbers of cross-barrier colonization events over hundreds or even thousands of years would suffice to distribute a mitochondrial lineage across the Great Lakes, but higher levels of migration are presumably necessary to prevent genetic divergence of populations. The differences we have observed suggest that the gene flow rates permitted by the Great Lakes fall somewhere in between, resulting in different barriers to gene flow for nuclear and mitochondrial markers.

## Supplementary Material

Supplementary material can be found at <http://www.jhered.oxfordjournals.org/>.

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