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Genetic (RAPD) diversity in *Peromyscus maniculatus* populations in a naturally fragmented landscape

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Abstract

We assessed the effects of long-term habitat fragmentation on genetic (random amplified polymorphic DNA) diversity in 11 *Peromyscus maniculatus* populations in the Lake Superior watershed. We analysed genetic structure at two spatial scales and the effect of island size and isolation on genetic diversity. At the regional scale, island populations differed from mainland populations (F_{ST} = 0.36), but mainland populations did not differ from each other (F_{ST} = 0.01). At the local scale, populations of the main island of Isle Royale differed from adjacent islet populations ($P < 0.001$; Monte Carlo approximation of Fisher's exact test), but not from each other (combined $P = 0.63$). Although geographical distance and genetic distance were positively correlated ($P < 0.01$; Mantel test), cluster analysis revealed some inconsistencies. Finally, genetic diversity was inversely related to isolation ($P = 0.01$), but had an unexpectedly negative relationship with island area ($P = 0.03$). The genetic structure of *P. maniculatus* populations in portions of the Lake Superior watershed appears to have been affected by long-term habitat fragmentation.

Keywords: genetic diversity, habitat fragmentation, *Peromyscus*, RAPD

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Introduction

Anthropogenic fragmentation of habitat continues to alter landscapes at unprecedented rates. More than a third of the species on the U. S. federal endangered species list are endangered by factors associated with habitat fragmentation (Czech & Krausman 1997). Although opportunities to study the short-term effects of anthropogenic fragmentation abound (e.g. Nupp & Swihart 1998; Lynam & Billick 1999), similar opportunities to study the long-term effects of fragmentation are less common (but see Frankham 1997). Although theoretical studies suggest that genetic processes of fragmented populations are complex (e.g. McCauley 1991; Hedrick & Gilpin 1997), empirical data from systems exhibiting long-term effects of fragmentation are limited (but see Saccheri *et al*. 1998).

In this study, we used random amplified polymorphic DNA (RAPD) markers and nonlethal sampling procedures to examine the genetic structure of deer mouse (*Peromyscus maniculatus*) populations in the Lake Superior watershed, where populations have been fragmented since the last glaciation. *P. maniculatus* tend toward promiscuity (Ribble & Millar 1996), which leads to lower effective population sizes (relative to the census size) due to high variances in fecundity (Nunney 1993). *P. maniculatus* also rarely disperse long distances (Bowman *et al*. 1999). Thus, small fragmented populations of *P. maniculatus* should exhibit distinct genetic structuring even over small spatial scales. Thus, we examined population differentiation on regional and local scales, estimated gene flow among populations, assessed the association between geographical distance and genetic distance, and quantified the genetic diversity of each population as influenced by island size and isolation. Beyond yielding potentially general insights, this research may be useful in conserving the 12 *Peromyscus* species and subspecies currently recognized as threatened or endangered (http://www.wcmc.org.uk and http://www.endangered.fws.gov).

Materials and methods

We collected DNA samples from 213 deer mice from 11 populations in the Lake Superior watershed (Fig. 1); 10 from each of two mainland populations (separated by

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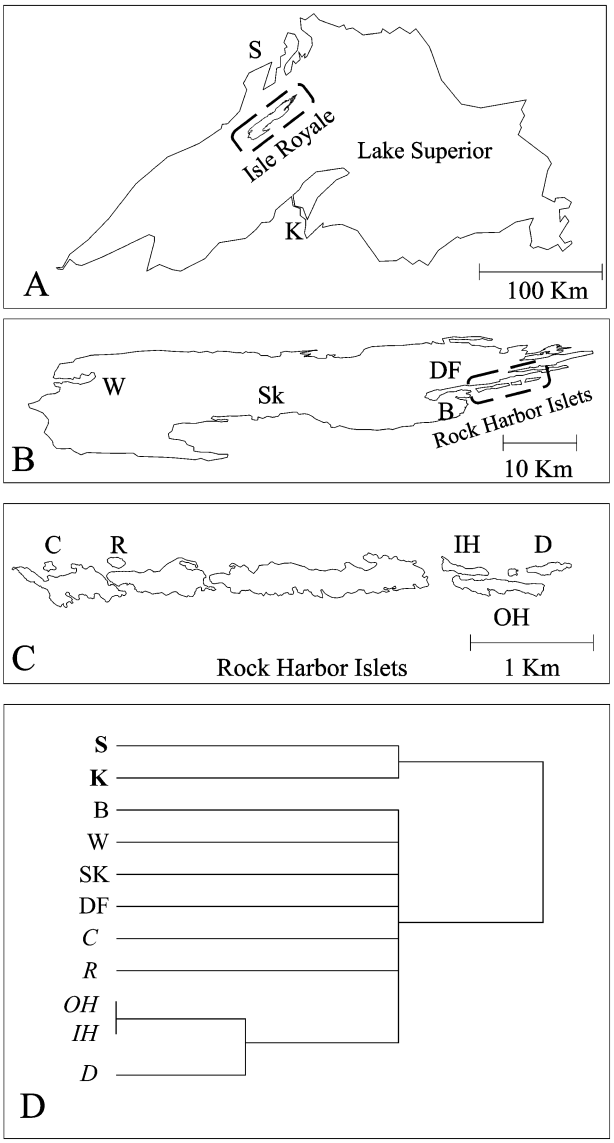

Fig. 1 Locations of and genetic relationships between the study populations in the Lake Superior watershed. (A) Mainland: Sibley (Ontario, Canada), Keweenaw (MI, USA). (B) Main island of Isle Royale National Park (MI): Windigo, Siskiwit, Daisy Farm, Bangsund. (C) Islets of Isle Royale: Cemetery, Rabbit Island, Inner Hill, Outer Hill, Davidson. (D) Genetic relationships among study populations based on Nei's minimum genetic distance (Nei 1972) and the consensus of UPGMA and neighbour-joining methods. Mainland populations are in bold, and islet populations are in italics.

≈750 km), 50 from four locations on the main island (544 km²) of Isle Royale National Park, Michigan (separated by 13–38 km), and 143 from five adjacent islets (1.2–6.24 ha; separated by 50–4200 m). Lung and muscle tissue from snap-trapped mice or a tiny snip of ear tissue from live-trapped mice was minced, preserved in lysis buffer, and stored at room temperature.

Genomic DNA was extracted by either phenol–chloroform–isoamyl alcohol (Bollmer *et al*. 1999) or a QIAamp Tissue Kit (QIAGEN, Inc.). We conducted PCR amplification, electrophoresis and visualization of RAPD markers following standard protocol (see e.g. Joshi & Nguyen 1993). We screened 45 RAPD primers (Operon Technologies) and assessed the reproducibility of bands by running duplicate reactions on different days. We scored 12 clear, polymorphic bands (from three primers) that were highly reproducible (≥ 92%). Although analyses based on 12 highly reproducible bands may raise concern about low statistical power, the influence of number and reproducibility of bands on power are too poorly understood for need of this concern to be patent. Because our analysis is based only on highly reproducible bands and because we observed significant alpha values (see below), a parsimonious interpretation is that our analysis may well represent true underlying patterns.

Statistical analyses were conducted with the assistance of Tools for Population Genetic Analysis (Miller 1997), ARLEQUIN (Schneider *et al*. 1997), and NTSYSPC software (Applied Biostatistics, Inc.). Statistical analyses were performed on the full data set and, to reduce bias, on a data set restricted by the criterion for unbiased RAPD analyses (i.e. marker frequencies less than $[1 - (3/n)]$, where n is sample size, Lynch & Milligan 1994). We estimated the frequency of the recessive allele (Lynch & Milligan 1994): $q = x^{-1/2}(1 - [x(1 - x)/N]/8x^2)$, where x is the proportion in the population without the marker, and N is the number of individuals sampled.

To quantify regional (i.e. mainland vs. island; Fig. 1A) and local (i.e. main island vs. islets; Fig. 1B) genetic structure, we examined genetic variation within and between populations by using analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) and fixation indices. First, we performed AMOVA on RAPD haplotypes. Second, to evaluate the significance of differences in marker frequencies, we calculated a Monte Carlo approximation of Fisher's exact test (Raymond & Rousset 1995). Third, we employed two techniques to calculate fixation indices (F_{ST}). We calculated F_{ST} from heterozygosity based on estimates of allele frequency (Weir & Cockerham 1984) and the Hardy–Weinberg theorem (hereafter, allele frequency-based estimates of fixation). We also used haplotype data directly to derive F_{ST} from the variance components (Cockerham 1973) calculated in the AMOVA (hereafter, haplotype-based estimates of fixation). Finally, we estimated the effective number of migrants per generation: $N_em = (1 - F_{ST})/(4F_{ST})$ (Wright 1951; Vucetich & Waite 2000).

To assess the association between Nei's minimum (Nei 1972) genetic distance and geographical distance (quantified as the logarithm of metres that separate

pairs of populations), we calculated the correlation between the distance matrices (i.e. Mantel test; Sokal & Rohlf 1995). To further examine this relationship, we generated UPGMA (i.e. unweighted pair-group method using an arithmetic average) and neighbour-joining trees based on Nei's minimum distance (Nei 1972), as well as a consensus tree which reports only nodes supported by both trees.

We also quantified how unbiased heterozygosity (Nei 1978) and genetic heterogeneity (Landry & Lapointe 1999) are affected by islet isolation (quantified as the inverse of the distance of each islet to the main island) and islet size (quantified as the logarithm of island area). Island area is a useful surrogate for long-term average population size because almost the entire area of each island comprises suitable *Peromyscus maniculatus* habitat (i.e. complete forest cover). To avoid spurious conclusions due to uneven sampling, we also evaluated the relationship between sample size and each metric of genetic diversity.

Results

Primer OPM-05 yielded bands at 575, 650 and 1200 bp, primer OPM-12 yielded bands at 650, 825, 1000 and 1100 bp, and primer OP26-12 yielded bands at 500, 800, 900, 1200 and 1400 bp. For pooled data, all 12 loci met the Lynch & Milligan (1994) pruning criterion. Because four loci did not meet the criterion for all populations, we created a restricted data set comprising loci meeting the bias criterion for most populations. Because results from the full and restricted data sets were similar, we report results from the restricted data set.

The Monte Carlo approximation of Fisher's exact test failed to detect significant differentiation between the mainland populations ($\chi^2 = 9.1$; $P = 0.91$), despite their being separated by $\approx$750 km (overland route). In contrast, Isle Royale populations differed significantly from both Sibley ($\chi^2 = 97.1$; $P < 0.01$) and Keweenaw ($\chi^2 = 78.0$; $P < 0.01$). The AMOVA of RAPD haplotype data yielded similar results. Here, <1% of the genetic variation on the mainland was due to differences between Keweenaw and Sibley. Moreover, 36.3% of the genetic variation in island and mainland populations was due to differences between those groups ($P = 0.02$; AMOVA). Patterns of F_{ST} between mainland populations (haplotype-based $F_{ST} = 0.01$, $N_em = 25.3$; allele frequency-based $F_{ST} = 0.11$, $N_em = 2.1$) and between Isle Royale populations and mainland populations (haplotype-based $F_{ST} = 0.36$, $N_em = 0.4$; allele frequency-based $F_{ST} = 0.20$, $N_em = 1.0$) were consistent with these results. Isle Royale populations were genetically more similar to Sibley ($D = 0.11$, Nei's minimum genetic distance) than to Keweenaw ($D = 0.16$).

Pairwise comparisons failed to reveal any significant differentiation among the sites on the main island of Isle Royale (Fisher's combined $P = 0.63$ for Monte Carlo approximation of Fisher's exact test). In contrast, significant differentiation was observed between populations of the main island of Isle Royale and adjacent islets ($\chi^2 = 89.4$; $P < 0.001$), and also among the islet populations ($\chi^2 = 97.8$; Fisher's combined $P < 0.001$). AMOVA based on RAPD haplotypes revealed that 9.5% ($P = 0.13$) of the genetic variation in island populations was due to differences between the main island and adjacent islets. Patterns of F_{ST} between the main island and islet populations (haplotype-based $F_{ST} = 0.10$, $N_em = 2.25$; allele frequency-based $F_{ST} = 0.07$, $N_em = 3.60$) were consistent with these results. At the smallest spatial scale (i.e. the islets of Isle Royale, Fig. 1C), haplotype-based F_{ST}s revealed (and allele frequency-based tests supported) that: (i) Davidson differed ($P < 0.01$) from all other islet populations; (ii) Inner Hill differed ($P < 0.001$) from all other islet populations except Outer Hill; and (iii) Cemetery and Rabbit islands differed ($P < 0.001$) from all islet populations except each other.

Increased genetic distance was significantly associated with increased geographical distance ($r = 0.55$, $P < 0.01$; Mantel test). Additional insights on this relationship are offered by examining the consensus tree (Fig. 1D). First, mainland populations clustered separately from Isle Royale populations. Second, adjacent islet populations clustered closely (Outer Hill, Inner Hill, Davidson). Third, sites across the main island of Isle Royale clustered together (but not exclusively). However, the clustering of Cemetery and Rabbit islands with main island populations is less intuitive, because they are separated by a wide channel of water. UPGMA trees based on Nei's original (Nei 1972), unbiased (Nei 1978) and unbiased minimum, Rogers' (Rogers 1972), and Wright's (Wright 1978) geometric average modification of Rogers' all depict topologies identical to the UPGMA tree based on Nei's minimum distance (Nei 1972).

Among the islet populations (Fig. 1C), isolation is highly and positively correlated with Nei's unbiased heterozygosity (Nei 1978; $r = 0.95$; $P = 0.01$), but not significantly correlated with the natural logarithm of genetic heterogeneity ($r = 0.35$; $P = 0.57$; Fig. 2A). However, area is highly and negatively correlated with heterozygosity ($r = -0.91$; $P = 0.03$), but not significantly correlated with the natural logarithm of genetic heterogeneity ($r = -0.30$; $P = 0.62$; Fig. 2B). Because these indices of genetic diversity were not correlated with sample size ($P = 0.40$ for heterozygosity; $P = 0.79$ for natural logarithm of genetic heterogeneity), these relationships are unlikely to be spuriously associated with sampling effort.

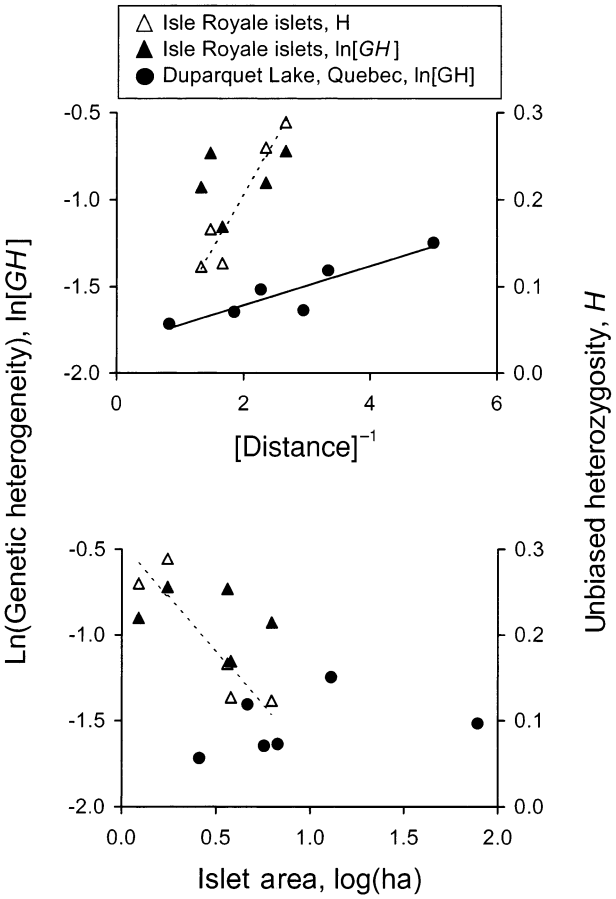

Fig. 2 The influence of isolation (inverse of kilometres of separation from the main island) and islet area (log[ha]) on unbiased heterozygosity (*H*; Nei 1978) and the natural logarithm of genetic heterogenity (ln[*GH*]; Landry & Lapointe 1999). Data include the islets of Isle Royale (△; and ▲; see Fig. 1C) and the islets of Duparquet Lake, Quebec (●; from Landry & Lapointe 1999). Significant relationships are indicated by trend lines (dotted lines for Isle Royale data based on *H* and solid line for Duparquet lake data).

Discussion

Long-term natural habitat fragmentation appears to have influenced genetic processes (according to Fisher's exact test) in *Peromyscus maniculatus* populations at regional and local scales in the Lake Superior basin. At a regional scale, Isle Royale populations differed significantly from mainland populations, but the mainland populations did not differ significantly from one another despite their wide geographical distance (Fig. 1). At a local scale, populations from the main island differed significantly from islet populations. Moreover, populations from the main island were genetically similar despite being separated by tens of kilometres, but samples from many islet populations differed genetically even though separated by less than a few kilometres.

Because of the confounding effect of habitat fragmentation, the geographical–genetic distance relationship was weak (Mantel test). An example of how this confound is manifest is that Windigo and Daisy Farm are separated by 37.8 km and a genetic distance (i.e. Nei's minimum) of 0.01, but Davidson and Inner Hill are separated by 0.4 km and a genetic distance of 0.02. Another factor that may contribute to the poor geographical–genetic distance relationship is the simultaneous examination of RAPD genetic structure across multiple spatial scales (Fig. 1). Because RAPD diversity is characterized by high mutation and near neutrality, it should be governed by a mutation–drift balance. Consequently, large populations with low extinction rates (i.e. mainland populations) will be little affected by drift and should accumulate mutations (i.e. diversity). Because high diversity limits the ability to discern differentiation (Nagylaki 1998), comparisons among large populations may yield little differentiation. In contrast, RAPD diversity in small populations with high extinction and recolonization rates will be more influenced by drift and founder events. Consequently, diversity will be low and smaller populations will appear more distinct from one another than larger populations.

The influence of geography also appears to depend on the metric of genetic diversity that is used (Fig. 2). For example, isolation was a significant predictor of heterozygosity, but not genetic heterogeneity. In contrast, isolation was a significant predictor of (the natural logarithm of) genetic heterogeneity ($P < 0.01$) for *P. maniculatus* populations in Duparquet Lake, Québec, which have also been fragmented at a similar spatial scale since the last glaciation (Landry & Lapointe 1999; Fig. 2). Given a sample size of five islets in this study and six islets in the Québec study, it is noteworthy that isolation was a significant predictor of genetic diversity even though other factors are likely to also influence genetic diversity (e.g. colonization history, patterns of human visitation, frequency of ice bridge formation). By the same reasoning, it may not be surprising that islet area was *negatively* correlated with unbiased heterozygosity for this study and uncorrelated to genetic heterogeneity for Isle Royale islets and the Québec populations (Fig. 2B).

Consistent with patterns from previous studies of *Peromyscus* spp. (Frankham 1996, 1997), small islet populations had lower levels of heterozygosity than larger main island populations (mean unbiased heterozygosity was 0.193 for the islets and 0.315 for the main island populations; $P = 0.03$). In contrast to this pattern, populations on the main island of Isle Royale had more genetic diversity than populations on the mainland (mean unbiased heterozygosity was 0.243 for the mainland populations; $P = 0.05$). Another contrast with an earlier *P. maniculatus* study is that genetic heterogeneity was almost twice as great for islet populations of Isle Royale relative to the

islet populations of Duparquet Lake (Landry & Lapointe 1999; Fig. 2). More genetic diversity could result from higher immigration rates and/or effective population sizes. Increased effective population sizes may result from higher densities of *P. maniculatus* on Isle Royale because of reduced interspecific competition. Competition is reduced because Isle Royale is not inhabited by other species of small mammal except for the red squirrel (*Tamiasciurus hudsonicus*) which, because it is much larger than *P. maniculatus* (135–250 g vs. 12–24 g), may compete little. These contrasts highlight the variability that may characterize the effects of habitat fragmentation, even within a single species.

Estimated levels of inbreeding may increase the extinction dynamics of the Isle Royale islet populations. Inbreeding coefficients (F_{ST}) ranged from 0.17 to 0.42 for main island populations and from 0.42 to 0.67 for islet populations. For context, $F_{ST} \leq 0.42$ corresponded to no observed extinctions in laboratory populations of house mice (*Mus musculus*), and $F_{ST} = 0.76$ corresponded to a 50% extinction rate (Eisen & Hanrahan 1974). Moreover, $F_{ST} = 0.73$ corresponded to an 80% extinction rate for populations derived from wild stock (Lynch 1977; Frankham 1998). Because natural populations may be more vulnerable to inbreeding depression than captive populations (Frankham & Ralls 1998), genetic factors may be an important component of extinction dynamics for these islet populations (Vucetich & Waite 1999). If RAPD diversity is a reliable indicator of population viability, then isolation by geography or anthropogenic fragmentation may lead to altered metapopulation dynamics and increased extinction risk.

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This study is part of L. M. Vucetich's Ph.D. research on the genetics and demography of Isle Royale deer mice. C. P. Joshi is a molecular geneticist interested in the application of molecular techniques for determining genetic variation in plants. R. O. Peterson conducts long-term population studies of mammals on Isle Royale. J. A. Vucetich researches the population biology of small populations. T. A. Waite's research interests include the overlap between conservation biology and behavioural ecology.
