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**Genetic differentiation of mitochondrial DNA at the landscape scale
in patchy populations of *Peromyscus leucopus*.**

by

Etsuko Tsuchiya, B.Sc. (Honours, Biology)

A thesis submitted to the Faculty of Graduate Studies
in partial fulfilment of the requirements for the degree of
Master of Science.

Department of Biology

Carleton University

Ottawa, Ontario

March 9, 1990

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Genetic differentiation of mitochondrial DNA at the landscape scale
in patchy populations of *Peromyscus leucopus*.

submitted by Etsuko Tsuchiya, B.Sc. (Honours, Biology)
in partial fulfilment of the requirements
for the degree of Master of Science

A handwritten signature in black ink, appearing to read "Gray Merrin", written over a horizontal line.

Thesis Supervisor

A handwritten signature in black ink, consisting of stylized initials and a surname, written over a horizontal line.

Chair, Department of Biology

Carleton University

March 12, 1990

ABSTRACT

This research tested the hypothesis that genetic populations of *Peromyscus leucopus* do not have distinct boundaries unless a major geographic barrier is imposed. Previous study of salivary amylase electromorph frequencies showed no evidence of genetic boundaries in mice from forest fragments scattered through an agricultural landscape. Despite demographic isolation sufficient to allow high frequency of local extinctions, there was sufficient gene flow to prevent any significant differentiation in salivary amylase.

To investigate a fuller representation of the genome, this paper reports the results of mitochondrial DNA restriction analysis. This technique was used to determine the degree of genetic change among patch populations of *P. leucopus* over a distance of 100 km from Ottawa, Canada, to the St. Lawrence River. Additional patch populations were sampled in Massena, N.Y., U.S.A., Bainsville, Ontario, and Saint-Anicet, Quebec to test whether the St. Lawrence River acts as a major barrier. These additional sample sites spanned areas of the river where intervening islands were present, and where they were absent.

The mtDNA of *P. leucopus* showed no breaks in gene flow among forest fragments along the transect until the river barrier was encountered. Mice sampled across the river showed distinct differences in mtDNA from those north of the barrier. Islands scattered across the 2 km width of the river did not change the pattern compared to an equal river width without islands.

Although the patchiness of these populations is seasonally important and they do function as a metapopulation demographically, mtDNA analysis confirms that on a

landscape to regional scale, there is sufficient gene flow to prevent genetic differentiation. The longer time base and larger spatial scale of the genetic process required a major barrier to allow genetic patterning.

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TABLE OF CONTENTS

	page
Acceptance Sheet	ii
Abstract	iii
Acknowledgements	v
Table of Contents	vi
List of Tables	vii
List of Figures	viii
List of Symbols and Abbreviations	ix
Introduction	1
Methods and Materials	9
Salivary amylase analysis	9
Mitochondrial DNA isolation	10
Restriction enzyme analysis of mtDNA	12
Results	14
Salivary amylase analysis	14
Qualitative mitochondrial DNA analysis	17
Quantitative mitochondrial DNA analysis	21
Discussion	27
Conclusions	35
Appendix A	54
Appendix B	55
References	56

LIST OF TABLES

TABLE	DESCRIPTION	page
1	Location of study sites.	36
2	Salivary amylase electromorph frequencies of <i>Peromyscus leucopus</i> .	37
3	Nei's distance values based on salivary amylase allele frequencies	38
4	Mitochondrial DNA haplotype frequencies.	39
5	Nucleon diversity values for each study site.	40
6	Overall sequence divergence values of haplotypes.	41
7	Net sequence divergence values for each study site.	42
8	Comparable values from other <i>Peromyscus</i> studies.	43

LIST OF FIGURES

FIGURE	DESCRIPTION	page
1	Map of study sites.	44
2	Schematic diagram of salivary amylase electromorphs of <i>Peromyscus leucopus</i> .	45
3	UPGMA cluster of genetic distances based on salivary amylase allele frequencies.	46
4	Photograph of EtBr stained gel of mtDNA RFLPs observed from use of eight restriction enzymes.	47
5	Schematic diagram of mtDNA RFLPs observed from use of eight restriction enzymes.	48
6	Number of mutation steps between RFLP types for restriction enzymes giving 2 variable fragment patterns.	49
7	Number of mutation steps between RFLP types for restriction enzymes giving greater than 2 variable fragment patterns.	50
8	Phylogenetic network of mtDNA haplotypes.	51
9	Geographic groupings of mtDNA haplotypes types.	52
10	UPGMA cluster of net sequence divergence values.	53

LIST OF SYMBOLS AND ABBREVIATIONS

mtDNA	mitochondrial DNA
RFLPs	restriction fragment length polymorphisms
g	gram
μg	microgram (10^{-6}g)
g	gravitational acceleration
ml	milliliter
μl	microliter (10^{-6}l)
h	hours
M	moles per liter
EDTA	ethylenediaminetetraacetic acid
MBS-EDTA	mannitol borate sucrose-ethylenediaminetetraacetic acid
EtBr	ethidium bromide
KI_3	potassium tri-iodide
SDS	sodium dodecyl sulfate
STE	Tris-EDTA + sodium chloride buffer
TA	Tris-acetate restriction buffer
TAE	Tris-acetate electrophoresis buffer
UPGMA	unweighted pair group analysis using mathematical means
$^{\circ}\text{C}$	degrees Centigrade
ff, fm, mm, ms, ss	homozygous or heterozygous fast (f), medium (m), or slow (s) running salivary amylase electromorphs
bp	nucleotide base pairs
kb	kilobase

INTRODUCTION

In the 'real world' resources are not uniformly distributed; a habitat is usually divided into usable patches (where resources are available) which are separated from one another by unusable patches (where resources are scarce or unavailable). Consequently, populations of organisms also occur in patches. Populations inhabiting these patches are local populations, a community of potentially interbreeding individuals at a given locality (Mayr, 1963). Any two individuals in that local population have equal probabilities of mating with each other and producing offspring, provided that they are of the opposite sex and sexually mature. Ideally, a local population is a panmictic unit (Mayr, 1963). Wright (1969) calls these "more or less completely isolated local populations", demes. I will, however, reserve the term 'deme' for a group of individuals that is distinct from another group of individuals by some measureable quality or quantity due to reduced or lack of gene flow between these groups. Demes operate on a longer temporal scale and a larger spatial scale than local populations (Merriam, 1988).

Unlike the theory of island biogeography where islands are recolonized by individuals from some mainland, in agricultural landscapes, the 'mainland' often does not exist, and recolonization must occur through individuals from other 'islands' (Merriam, 1988). Patches may be viewed as groups of islands with no mainland in the vicinity. However, these 'islands' are perhaps not as isolated as true oceanic islands. Movement between patches can occur and is enhanced by the presence of corridors between patches. Corridors have the effect of reducing patch isolation by facilitating movement (Merriam, 1984). Patch populations that are not connected to other patch populations by corridors have higher probabilities of suffering local extinctions due to lower

population growth rates, resulting from emigration that is not compensated by immigration, and will take longer to repopulate after extinction (Fahrig and Merriam, 1985).

In agricultural landscapes patch populations are characterized by local extinctions and recolonizations. A metapopulation is a demographic unit of patch populations that experience local extinctions, and recolonizations through the exchange of individuals between patch or subpopulations (Levins, 1970). Dispersal is a key element in the dynamics of agricultural landscapes. Connectivity, is the measure of the ease of movement of individuals among patches (Merriam, 1984; Fahrig and Merriam, 1985, Merriam and Lanoue, in press). Increased connectivity, through increased numbers of corridor connections, enhances dispersal and can increase the stability of patch populations and the metapopulation as a whole, in patchy environments (Fahrig and Merriam, 1985, Merriam, 1988).

An example of a patchy system is the agricultural mosaic of eastern Ontario. Originally an area covered by extensive deciduous forest, the area has been fragmented by the clearing of forest for agricultural purposes in the last 200 years since first settlement (Merriam 1984, 1988). Remaining is a patchwork of forest patches in the form of woodlots, some of which are connected to one another by fencerow corridors, surrounded by farmland, roads and buildings.

A common inhabitant of these forest fragments in this agricultural mosaic is *Peromyscus leucopus*. *Peromyscus* is a good organism to study since much is known of its demographic characteristics in agricultural landscapes (Merriam, 1984, 1988, Merriam and Lanoue, in press, Merriam and Weger submitted, Merriam et al., 1989, Fahrig and Merriam, 1985, Middleton and Merriam 1981, Wegner, 1976, Wegner and Merriam,

1979). Populations of these mice in farmland woodlots have been found to undergo and recover from natural and artificially created local extinctions readily (Middleton and Merriam, 1981, Merriam and Wegner, submitted). This is an indication of a moderate amount of movement occurring between patches. Movement of mice between local populations stabilizes the metapopulation demographically (Fahrig and Merriam, 1985); the "rescue effect" (Brown and Kodric-Brown, 1977) has an important role in the maintenance of population numbers.

Although dispersal does not automatically indicate gene flow (Ehrlich and Murphy, 1987), the ability of *Peromyscus* to rapidly recolonize local extinctions and bring low spring population numbers up to autumn highs (Merriam and Wegner, submitted, Middleton and Merriam, 1981) allows for the assumption that dispersal in this case indicates some degree of gene flow. Only when the dispersing individual has successfully bred in the new population does gene flow occur. Gene flow between populations tends to homogenize any differences that may have developed through genetic drift or differential selection pressures.

In a previous genetic study of *Peromyscus leucopus* populations in isolated woodlots (those not effectively connected to other woodlots by fencerow corridors) in this area, a protein which was surveyed nondestructively showed that these woodlot populations were not genetically isolated (Tsuchiya, 1986; Merriam et al., 1989). Salivary amylase allele frequencies revealed that despite the reduction in connectivity there was sufficient gene flow between woodlots to maintain approximate panmixis. Most (98.36%) of the genetic variance found for that locus was common to all populations within the 25 km x 15 km area.

Electrophoretic analysis of proteins is not the best method of surveying intraspecific populations. However, for interspecific population analysis, it is a commonly used tool. Protein analysis uses nuclear DNA products; it is two steps away from the DNA itself (Ferris and Berg, 1987). Electrophoresis is limited by the detectability of differences in amino acid composition. Changes in composition must result in a net charge change to be detected. Only coding regions of the nuclear genome can be examined and the number of assayable loci is small in comparison to the number coded by nuclear DNA. Perhaps most importantly, because of segregation and recombination events, data from protein surveys tends to be ambiguous. In using allele frequency data, the unit of analysis is the population, not the individual.

Over the last ten years, the use of mitochondrial DNA (mtDNA) as a genetic marker has become a popular and powerful tool in evolutionary and population biology (see reviews by Avise., 1986, Avise et al., 1987, Moritz et al., 1987, Wilson et al., 1985). It appears to be a better method of surveying populations (especially conspecific populations) than the electrophoretic analysis of proteins for several reasons. The mammalian mtDNA genome is small, approximately 16.5 kb (Brown, 1983), highly conserved in terms of gene content and arrangement, yet undergoes evolution at a rate 5 to 10 times faster than single copy nuclear DNA (Brown et al., 1979), and appears to be transmitted clonally through females (Hayashi et al., 1978, Giles et al., 1980, Lansman et al., 1983). The mtDNA genome does not undergo recombination during reproduction. Restriction enzyme analysis of mtDNA surveys the entire genome (both coding and non-coding regions). For analysing genetic differentiation among populations, mtDNA serves as a near-ideal marker. Populations that experience migration rates at which nuclear genes are effectively panmictic, could be effectively subdivided for organelle (mtDNA)

genes (Birky et al., 1983). The individual becomes the unit of analysis, and data can be examined qualitatively and quantitatively.

Cutting mtDNA with various restriction endonucleases, and separating these fragments by size on an agarose gel, can reveal base pair changes in the genome through restriction fragment length polymorphisms (RFLPs). Quantitatively, the proportion of fragments shared by different individuals can be used to get an index of genetic similarity. The frequencies of the various restriction fragment profiles held in each population can be used to get percent mtDNA sequence divergence values. Qualitatively, each RFLP produced by a specific enzyme is given a different code letter, and combining code letters from each enzyme used give each individual sampled an n-lettered character. These characters can be traced through various populations by its presence or absence. By combining these qualitative and quantitative aspects, possible patterns of evolution can be deduced.

The degree of gene flow between populations can be assessed by examining the variety of restriction fragment length polymorphisms found among the populations. If there is free genetic exchange between populations, restriction fragment patterns should be very similar. If gene flow is restricted to some degree, sampling different local populations over a distance should reveal differences in restriction fragment patterns. If the degree of gene flow is fairly even over a distance, fragmentation patterns should remain similar or show a progression of changes. Any major differences in mtDNA restriction fragmentation between populations may indicate a significant barrier to gene flow (Avisé et al., 1987, Ashley and Wills, 1987, MacNeil and Stroheck, 1987). Gene flow may be impeded by geographic barriers such as mountain ranges or large bodies of water, or by differences in physical or behavioural attributes.

Mammalian mtDNA studies have tended to survey macrogeographic areas, such as large sections of the USA and Canada (Avise et al., 1979, Avise et al., 1983, MacNeil and Strobeck, 1987, Thomas and Beckenbach, 1986), entire countries (Avise et al., 1983, Plante et al., 1989a), the entire North American continent (Lansman et al., 1983), and beyond (Ferris et al., 1983). These studies tended to sample few individuals (often varying from one to several individuals per locale) over huge areas. For example, the *Peromyscus maniculatus* study by Lansman et al. (1983) found that five major clonal assemblages existed. These genetic assemblages were separated by at least eight mutation steps, and within each assemblage there was extensive clonal diversity. This was based on 135 mice from 40 locales from across North America, with sample sizes ranging from one to five. Morphological form and mtDNA haplotype, as well as subspecies designation and mtDNA haplotype, did not appear to have any consistent relationship or correspondance. The only macrogeographic study to look at *P. leucopus* did so as part of a study of mtDNA differentiation in the process of speciation in *Peromyscus* (Avise et al., 1983). They looked at 14 individuals from 9 sites across the USA, among which they found 12 different haplotypes which were separated by a minimum of one to five mutational steps.

Relatively few studies have concentrated on mtDNA differentiation at a microgeographic level, that is, sampling populations within a distance of a few hundred rather than thousands of kilometers apart (Ashley and Wills, 1987, Kessler and Avise, 1985, Nelson et al., 1987, Plante et al., 1989b). Only the Nelson et al. study (1987) sampled along a transect; they were interested in *P. leucopus* mtDNA and allozyme variation across a chromosomal hybrid zone. However, their sample sizes for the mtDNA analysis were small, and the sites chosen for this part of the study consisted of

the farthest two locations, the two locations where hybrids were found, and the next two sites away from where the hybrids were found.

Perhaps the best *Peromyscus* mtDNA study to date was the analysis of polymorphisms of *P. maniculatus* on California's Channel Islands by Ashley and Wills (1987). To estimate patterns of colonization among 8 island and 7 mainland populations of mice, mtDNA RFLPs were used as genetic markers; the number of individuals sampled tended to be larger and more consistent than the norm. No haplotype was found in common between mainland and island populations, although all haplotypes were closely related to one another by few mutation steps.

My study looked at populations of *P. leucopus* in 'habitat islands' that are not as isolated as true islands. Dispersal and immigration events among patches occur much more frequently than between real mainland and island populations, yet are probably less than that within one large intact habitat, due to barriers such as roads (Oxley et al., 1974, Merriam et al., 1989). Rather than examining populations scattered throughout the species' range, it concentrated on a microgeographic scale along a transect. It also incorporated a possible barrier to gene flow that was probably less effective than open ocean, yet perhaps more effective than presented by roads and farmland.

The St. Lawrence River may be enough of a barrier to impede dispersal and gene flow. Areas of the river without "stepping-stone" islands may prove to be a more effective barrier to gene flow than areas with these islands. Tegelstrom and Hansson (1987) found that for two small mammals (*Apodemus* spp. and *Sorex araneus*), a frozen body of water (a lake in Sweden) was not a barrier for dispersal and possibly gene flow. Beer et al. (1954) and Lomolino (1984) also stated that winter ice cover provided "highways for dispersal" in non-hibernating species, among islands in a Minnesota lake

and islands in the Thousand Island region of the St. Lawrence River, respectively. Christianson (1977) documented movement of *Peromyscus* across the ice between an island in a lake and the mainland (Minnesota). Sheppe (1965a, 1965b) noted that *P. leucopus* could disperse by swimming between islands in an Ontario lake, and that although not observed directly, they could probably cross over ice during winter. Mice from sites straddling the river where 'stepping-stone' islands are present may have more similar mtDNA haplotypes than sites straddling an island-free part of the river; 'stepping-stone' islands may facilitate dispersal, and permit continuous passage from one side of the river to the other.

This study investigated local populations of *Peromyscus leucopus* along a 100 km transect in an agricultural landscape using mtDNA RFLPs. This information was used to determine whether any genetic patterning was evident among populations. Also, it was used to determine whether the St. Lawrence River was a barrier to gene flow, and if so, whether 'stepping-stone' islands facilitated gene flow.

METHODS

Peromyscus leucopus noveboracensis were live trapped in predominantly deciduous woods found in the agricultural landscape of eastern Ontario, southern Quebec, and northern New York State, during the summer and autumn months of 1988, and the summer of 1989 (Figure 1, Table 1). Sherman traps containing a handful of Terylene bedding were baited with sunflower seeds. Trapping continued until at least 8 specimens of *P. leucopus* were obtained from each study site. Specimens were kept alive in the vivarium until processed since mtDNA yields are higher when extracted from fresh tissue samples (Lansman et al., 1981; Ferris and Berg, 1986).

In eastern Canada and the northeastern United States, *P. leucopus* and *P. maniculatus*, which exhibit overlapping morphological characteristics, are sympatric (Choate, 1973; Klein, 1960; Smith and Speller, 1970). Salivary amylase (α -1,4-glucan 4-glucanohydrolase E.C.3.2.1.1.) samples from all mice were tested to confirm that they were only *P. leucopus*. Accurate species identification was possible by electrophoresis of salivary amylase (Aquadro and Patton, 1980; Feldhamer et al., 1983; Tsuchiya, 1986).

SALIVARY AMYLASE ANALYSIS

Salivary amylase samples were obtained by rinsing the mouth with a few microlitres of distilled water. A 1 ml disposable syringe tipped with a 1 inch 20 gauge needle with the tip ground off was used for sample collection. Saliva samples were diluted 1:1 with 30% glycerol containing 0.1 mg/ml bromophenol blue (Aquadro and Patton, 1980), and stored at -20°C. Gel electrophoresis was carried out on 6%

acrylamide gels in a continuous glycine-sodium glycinate buffer system pH 8.8 (Baker et al., 1975) using a vertical gel electrophoresis cell, model EC 570 as in Carmody et al. (1971). Gels were prerun at 90 mA for at least 30 min. before 10 μ l aliquots of the saliva sample were injected into the wells. Gels were then run for approximately 3 h at 90 mA, after which a starch-agar overlay (Aquadro and Patton, 1980) was poured over the gel and left to incubate for 1 h at room temperature. Following incubation, the overlay was removed and the gel stained with potassium tri-iodide (KI_3) for 2 min. Gels were washed in a mixture of methanol, water and glacial acetic acid (5:5:1), then scored. The most common isozyme of *P. leucopus* was designated Amy-1¹⁰⁰. Amylase isozymes of *P. leucopus* are clearly distinguishable from that of *P. maniculatus* in terms of electrophoretic mobility (Aquadro and Patton, 1980; Feldhamer et al., 1983; Tsuchiya, 1986).

MITOCHONDRIAL DNA ISOLATION

mtDNA isolation was carried out following the general procedures of Lansman et al., (1981) with modifications by J.C. Avise (pers. comm., Univ. Georgia, Athens, GA). The following modifications of Avise's protocol were adopted. The mice were gassed with CO_2 , then cervically dislocated. Soft tissues consisting of heart, kidneys, liver, and spleen were removed, minced with dissection scissors, and then hand homogenized in 25 ml of MSB-EDTA buffer (pH 7.5) using a glass pestle in a Dounce homogenizer. The mixture was spun for 5 minutes at 4°C in a Sorval centrifuge at 750 rpm (67 g). The crude pellet was retained while the supernatant was spun again at the same speed for an additional 5 minutes. To pellet the mitochondria, the supernatant from this second spin

was balanced with the homogenizing buffer and spun for 20 minutes at 4°C at 14,000 rpm (23,500 g) in the Sorval SS-34 centrifuge head. Crude pellets from the previous slow spins were rehomogenized in 15 ml homogenizing buffer and the entire centrifugation procedure was repeated. Pelleted mitochondria from the high speed spins were resuspended in 15 ml homogenizing buffer and spun one final time at 14,000 rpm for 20 minutes. The resultant pellet was resuspended in 3 ml STE, pH 8.0, (0.1 M NaCl, 0.05 M Tris-HCl, pH 8.0, 0.01 M EDTA, pH 8.0) to which a few drops of 25% SDS was added while the suspension was gently mixed (Lansman et al., 1981). To the lysate solution, 1.1 g CsCl per ml STE was added. The refractive index was checked, and adjusted with either STE or CsCl so that it lay between 1.385 and 1.390. Two hundred microlitres 10 mg/ml EtBr was placed into 5 ml Beckman Quick-seal tubes, followed by the lysate-CsCl solution. Paraffin oil was added to the tubes until the total volume reached the bottom of the Quick-seal tube neck. Tubes were balanced with either paraffin oil or 1.1 g/ml CsCl. After heat sealing, the tubes were inverted to mix the EtBr and CsCl-lysate, then spun at 60,000 rpm (300,000 g) for at least 16 hours in the Vti80 Beckman Ultracentrifuge head.

The mtDNA band which fluoresces at about 0.5 cm below the brightly fluorescing nDNA band, was removed by side puncture using a 21 gauge syringe. EtBr was removed from the mtDNA-CsCl-EtBr solution with CsCl saturated isopropanol washes. This was repeated until the mixture lost all of its original pink/orange colouration. Excess salt was removed by passing the solution through Sephadex spin columns using TE buffer (Maniatis et al., 1982). mtDNA isolated was sufficient for at least 10 digests.

RESTRICTION ENZYME ANALYSIS OF MITOCHONDRIAL DNA

Each specimen's mtDNA sample was digested with 8 different restriction enzymes. 7-, 6- and 4-cutters gave a restriction digest profile for each specimen. The following enzymes were chosen because they were found to reveal variable cleavage sites in *P. leucopus* (Awise et al., 1983; Nelson et al., 1987): BamHI (GGATCC), BstEII (GGTNACC), EcoRI (GAATTC), HincII (GTPyPuAC), HindIII (AAGCTT), HpaII (CCGG), PstI (CTGCAC) and XbaI (TCTAGA). mtDNA samples were restricted in TA buffer (O'Farrell et al., 1980) at the optimal temperature for each enzyme (Maniatis et al., 1982). Samples were then run on agarose gels, whose concentrations ranged from 0.8% to 1.4%, depending on the restriction enzymes used. All gels were run in Tris-acetate buffer (TAE) (Maniatis et al., 1982). PstI and XbaI digests were usually run on lower percentage gels. Fragment lengths were determined by comparison of migration distances with a HindIII cut bacteriophage Lambda DNA molecular weight standard. Gels were stained with EtBr, then illuminated on a Fotodyne Foto-Prep I UV transilluminator and photographed on Polaroid 55P/N or 57P film using a MP4 land camera. To visualize smaller fragments, Southern blots were made from the gels following the procedure in Maniatis et al. (1982). Blots were hybridized with *P. leucopus* mtDNA probes labelled with the Boehringer Mannheim nonradioactive DNA labelling and detection kit. Probes were made from mtDNA isolated from sample *Peromyscus leucopus* offspring born in the vivarium. This mtDNA was first digested with BstEII, repurified by phenol-chloroform washes (Maniatis et al., 1982), then labelled with the kit. mtDNA fragments were detected as directed by the kit. As in other studies (Awise et al., 1979; Brown et al., 1979; MacNeil and Strobeck, 1987; Plante et al., 1989) fragments

under 500 bp in length were not included because of the difficulty in resolving them. For endonucleases that exposed variable restriction sites, each variant was arbitrarily given a letter code. The patterns of fragmentation given by the 8 different restriction enzymes constitute the restriction profile or the composite mtDNA genotype of the specimen. Profiles of specimens from each study area were examined and compared with profiles of specimens from the other study sites. Identical profiles were termed clonal, and a dendrogram linking clonal types was made using the minimal number of mutation steps present between clonal types.

RESULTS

SALIVARY AMYLASE ANALYSIS

All mice trapped at Canadian sites proved to be *Peromyscus leucopus*; of the 23 mice caught at the Massena, New York site, only 8 were found to be *P. leucopus*. Three different salivary amylase alleles were observed, as in the previous study (Tsuchiya, 1986; Merriam et al., 1989); they were characterized as fast (f), medium (m), and slow (s) moving bands. The medium running band corresponded to Amy-1¹⁰⁰, while the fast and slow running bands corresponded to Amy-1¹⁰⁹ and Amy-1⁹⁴, respectively (Aquadro and Patton, 1980). Out of the 6 possible allelic combinations, the heterozygous fast-slow (fs) combination was not observed. Among the 5 amylase electromorphs that were found in various frequencies, the heterozygous fast-medium (fm) electromorph was the most prevalent, while the homozygous fast (ff) and the heterozygous medium-slow (ms) morphs were the least prevalent (Table 2, Figure 2).

Genetic structure of a population usually is the result of different patterns of mating within the population as well as the extent of exchange of genetic material between populations (Eanes and Koehn, 1978). To determine whether the populations from the 8 study sites experienced genetic subdivision because of their patchiness and distance from one another, Wright's F-statistics were calculated (Wright, 1965). F-statistics have been used to describe genetic structure of subdivided populations. There are three levels at which the population in question is analysed. F_{IS} , the inbreeding coefficient, is a measure of the reduction in heterozygosity due to deviation from random mating within the subpopulation (Hartl, 1988). F_{IT} , the overall inbreeding

coefficient of the individual, is the correlation between gametes combining within the individual relative to the entire population (Eanes and Koehn, 1978). F_{ST} , the fixation index, is the reduction in heterozygosity due to random genetic drift (Hartl, 1988); it is also a measure of genetic variability among subpopulations. This estimate is a correlation of inbreeding within the subpopulations relative to the total population. An F_{ST} value of 0 indicates that the subpopulations are identical in allele frequencies and are behaving more or less as one panmictic population. A value of 1 indicates subpopulations that are strongly differentiated.

Since I wanted to determine whether the woodlot populations I sampled were genetically isolated (unlikely, since the previous study indicated sufficient gene flow between woodlot populations (Tsuchiya, 1986, Merriam et al., 1989)) or behaved more like one large panmictic population, the F_{ST} was used. From the allele frequencies in Table 2, F_{ST} was calculated to be 0.0513, based on censusing one locus among eight populations. This indicated that very little (5%) of the variability could be attributed to subpopulation variability; the majority of the variance was common to all subpopulations. Statistically, testing for the null hypothesis ($F_{ST} = 0$, indicating no genetic variability between subpopulations) by using $X^2 = 2N F_{ST}$, where $N = 86$, $X^2 = 8.82$, d.f. = 7, the probability was found to be not significant ($p > 0.25$).

Nei's genetic identity, I , is a measure of the similarity of two populations calculated from the allele frequencies of a given locus in each population (Nei, 1972). It was calculated for each pair of sites (Table 3) using Nei's equation 2 (1972):

$$I = J_{XY} / (J_X J_Y)^{1/2}$$

where J_X and J_Y are the arithmetic means of j_x and j_y , which are the probabilities of identity of two randomly chosen genes in population X and Y ($j_x = \sum x_i^2$, $j_y = \sum y_i^2$; x_i and y_i are the frequencies of the i^{th} alleles in population X and Y, respectively), and J_{XY} is the arithmetic mean of j_{xy} , which is the probability of identity of a gene from population X and a gene from Y ($j_{xy} = \sum x_i y_i$)

Values of genetic identity, I , range from 0 to 1; 0 indicates that two populations have no alleles in common, while 1 indicates that the two populations have the same alleles in identical frequencies. I measures the genetic similarity between populations independently of the variability within populations (Fristrom and Speith, 1980). For this study on the salivary amylase locus, Nei's identity was found to range between 0.8944 and 1.0. Nei's mean genetic identity, $\bar{I}$, was found to be 0.9615, which indicates that the populations are very similar with respect to the Amy-1 locus. Nei's distance measure, D , represents the number of gene or codon substitutions per locus that have occurred after the divergence of two populations. It is a general measure that can be used to compare any two organisms. D is related to I , and is defined as:

$$D = -\ln I \quad (\text{equation 3, Nei, 1972})$$

It was calculated in order to give a value with which I could compare my results, based on only one locus, to other studies that used more than one locus, and to provide the data for cluster analysis. D values ranged from 0 to 0.1115 (Table 3). $\bar{D}$, the mean genetic distance, was 0.0393.

The study sites were clustered using unweighted pair group analysis with arithmetic means, UPGMA (Sneath and Sokal, 1973), using Nei's distance measures based on salivary amylase allele frequencies (Figure 3). The cluster was constructed to determine whether the grouped substructuring had any geographic association. The genetic grouping of study sites does not correlate with geographic location, rather, it appears quite jumbled. For example, one branch of the cluster is populations 1, 5 and 6, of which populations 1 and 6 are the two populations that are geographically the farthest distance apart.

QUALITATIVE MITOCHONDRIAL DNA ANALYSIS

Eight restriction enzymes were used to cleave 86 mtDNA samples from *Peromyscus leucopus*, producing a total of 3383 fragments over 500 bp in size. Fragments under 500 bp in size were not counted because of difficulties in resolving them. The average number of fragments produced per sample (mouse) was 39.34 ± 0.86 . Six out of eight restriction enzymes used gave restriction fragment length polymorphisms (RFLPs). The two enzymes which produced a single fragment profile (morph) each were EcoRI and HindIII. Four enzymes (BamHI, HincII, PstI, and XbaI) each produced two different restriction patterns. BstEII produced 4 different patterns, while the only four-cutter used (HpaII) produced five profiles (see Figure 4, 5). Some of the fragmentation patterns were as observed in Nelson et al., 1987. Probable phylogenetic links could be determined for each restriction enzyme used in the analysis from their respective restriction fragment patterns by determination of the minimum number of mutation steps between morphs produced by each restriction enzyme. Morphs were

connected based on presence and absence of restriction fragments. It was assumed that mutation, in the form of nucleotide substitutions, was the cause of loss or gain of sites/fragments.

An overall mtDNA haplotype or composite phenotype was designated for each individual by combining the restriction morphs for each enzyme. Each individual was given a 6 letter haplotype based on the 6 restriction enzymes that gave variable fragment patterns. The order of the restriction enzyme profiles in the composite mtDNA genotype was: BamHI, BstEII, HincII, HpaII, PstI, and XbaI. The enzymes (EcoRI and HindIII) that gave only one fragment pattern each were not included in the haplotype designation since all individuals had the same morphs for these two enzymes. Individuals with the same haplotype were assumed to belong to the same maternal lineage.

Fourteen composite haplotypes were assembled based on the restriction fragment profiles produced by the enzyme set (Table 4). There were 3 to 7 different haplotypes found at each site. Half of the composite phenotypes were represented by at least 5 individuals (greater than 5% of the total number of mice sampled) among the 8 study sites and therefore termed 'common', while the other seven phenotypes, represented by only one or two individuals, were 'rare' (less than 3% of the total number sampled).

All restriction fragment morphs could be linked by the gain or loss of a single restriction site (Figure 6 and 7). Although restriction sites were not mapped, this was determined by examining relative fragment lengths. Fragments larger than 500 bp could be scored, and since the totals of all detectable fragment lengths were sometimes short by lengths greater than 1 kb from the published estimated length of the mammalian mitochondrial genome, 16.5 kb (Brown, 1983), it was likely that the actual number of mutation steps was greater than that estimate.

Restriction morphs for two enzymes appeared to be diagnostic of geographic location of collection sites. Specifically, all individuals caught at sites south of the St. Lawrence River (sites 6 and 6i) had restriction morphs 'B' or 'D' for the enzyme BstEII, and/or morphs 'C' or 'D' for enzyme HpaII, while all individuals caught at sites north of the river (sites 1 - 5, 5i) had restriction morphs 'A' or 'C' for BstEII, and 'A', 'B', or 'E' for HpaII. There were three exceptions to this: one southern-caught individual had a northern-type haplotype, while two northern-caught individuals had southern-type haplotypes. Since this constituted only 3.5% ($\frac{3}{86}$) of the total number of individuals sampled, it was fair to say that these examples were outliers to the norm. The individual caught at site 6 with the northern-type haplotype had a rare haplotype BAAAAA; this was the only example of this haplotype found among all individuals sampled from the study sites. This haplotype is two mutation steps away from the nearest southern-type haplotype, while it is only one step away from two northern-type haplotypes. The two individuals with southern-type haplotypes were caught at site 1 (BBAABA) and site 5 (BBACBA). The latter had southern-type restriction morphs for both enzymes, while the former had one southern-type and one northern-type restriction morph. The former haplotype is one mutation step away from its nearest northern-type haplotype, while the latter is one mutation step from its nearest southern-type haplotype and from the former haplotype. It may be that haplotype BBAABA represents an ancestral mitochondrial genome from which *P. leucopus* in this area have evolved; mice north of the river lost a BstEII restriction site while those south of the river lost a HpaII restriction site.

Phylogenetic trees can be used to hypothesize evolutionary process. Based on the number of mutation steps required between morphs for each restriction enzyme used, the minimum number of mutation steps required between pairs of mtDNA clonal

types was determined by direct count. From this, a possible phylogenetic network was constructed by connection of haplotypes that differed by a minimum number of mutation steps (Figure 8). Clones in this phylogenetic network were separated from their nearest 'relative' by a minimum of one or two mutation steps. Haplotypes found exclusively north of the river differed from one another by only one restriction morph. The only exception to this was one rare haplotype found in one individual at one site (ACAEAA) which differed by two restriction morphs. The differing morphs were also rare, being found only in this one individual. Haplotypes found exclusively south of the river were separated from any exclusively northern haplotype by at least two mutation steps. The exception to this was another rare haplotype found in one individual at one site (BAAAAA); it was separated from two northern haplotypes by one mutation step (two separate restriction morphs). The two mainly southern-type haplotypes found in two individuals caught north of the river were separated from northern-type haplotypes by one and two mutation steps. The BBAABA haplotype, found mainly south of the St. Lawrence River, seems to be the linker joining northern-caught haplotypes with southern-caught haplotypes.

By mapping mtDNA clonal types according to the study sites where they were found, two geographic groups became more evident (Figure 9). Four main (AAAAAA, AAABAA, AABABA, and BAAABA) and three rare (BAAABB, AAAABA, ACAEAA) clonal types were situated exclusively north of the St. Lawrence River while the other three main clonal types (BBACBA, BDBDBA, BBAABA) were mainly situated south of the river. BDBDBA was found exclusively south of the river while for clonal types BBAABA and BBACBA, one individual each was found north of the river ($\frac{1}{5}$ and $\frac{1}{6}$ respectively). The other four rare clonal types were found exclusively at site 6, south of

the river. Overall, seven clonal types were found exclusively north of the river, five exclusively south of the river, and two were found on both sides of the river. Site 6 (Saint-Anicet, Quebec) had the greatest proportion of rare clonal types.

QUANTITATIVE MITCHONDRIAL DNA ANALYSIS

Nucleons, (Nei and Tajima, 1981), are any segment of DNA under study. Since the mitochondrial genome is small relative to the nuclear genome, the entire mtDNA genome can be considered a nucleon. Nucleons are characterized by the number and corresponding lengths of DNA fragments, as used in this study, or by restriction site maps. It follows that nucleon diversity, $\hat{h}$, depends on the mutation rate as well as the numbers and types of restriction enzymes used in the analysis. Nucleomorphs are the different restriction fragment patterns for a given nucleon (Nei and Tajima, 1981). Nucleon diversity is a measure of the genetic variation among individuals (Nei and Tajima, 1981). Values range from 0, indicating fixation of a haplotype, to 1, indicating high variation. Equation 7 from Nei and Tajima (1981) was used to calculate an index of diversity based on mtDNA for all eight sites (Table 5). Values ranged from 0.5557 at site 5 to 0.8667 at site 6. Over all other sites, site 5 had the greatest proportion of a single haplotype (67%), and site 6 had the greatest proportion of rare haplotypes (60%). Nucleon diversity, $\hat{h}$, is measured as:

$$\hat{h} = n (1 - \sum_{i=1}^l x_i^2) / n - 1$$

where n is the number of nucleons drawn from a population, l is the number of nucleomorphs, and x_i is the frequency of the i^{th} nucleomorph.

Since nucleomorphs correspond to alleles at a particular locus, nucleon diversity values are analogous to heterozygosity for nuclear genes (Nei, 1975). Similar to the use of Wright's F-statistics (1969), nucleon diversity values can be analysed for population structure by the use of Nei's G_{ST} (1973), called the coefficient of gene differentiation. This value is calculated from the average overall gene identity within each subpopulation, J_S , and the gene identity of the total population, J_T (equations 7, 9, Nei, 1973). For this study, G_{ST} was calculated to be 0.2983, indicating that approximately 30% of mitochondrial variation in this population of *P. leucopus* could be attributed to inter-study site variation. According to Wright (1978), values of F_{ST} greater than 0.25 indicate very great genetic differentiation. Statistically, using the same X^2 equation as for the salivary amylase analysis, $X^2 = 2 (86) (0.2983) = 51.3076$. With 7 degrees of freedom, this was significant ($p < 0.001$), indicating that chance alone could not likely produce such a large deviation. When the proportion of intrapopulation diversity is high relative to total population diversity, G_{ST} approaches 0. Conversely, when intrapopulation diversity is low relative to total population diversity, G_{ST} approaches 1.

$$J_S = 1/S \sum_{i=1}^S J_i$$

where S is the number of subpopulations,

$$\text{and } J_i = \sum_k x_{ik}^2 \quad (\text{equation 2, Nei, 1973})$$

where x_{ik} is the frequency of the k^{th} nucleomorph (haplotype) in the i^{th} subpopulation.

$$J_T = (\sum_i J_i + \sum_{i \neq j} J_{ij}) / S^2 \quad (\text{equation 5, Nei, 1973})$$

where $J_{ij} = \sum x_{ik} x_{jk}$ is the gene identity between the i^{th} and j^{th} subpopulations.

$$H_T = 1 - J_T, \quad H_S = 1 - J_S$$

$$\text{and } H_T = H_S + D_{ST} \quad (\text{equation 8, Nei, 1973})$$

$$G_{ST} = D_{ST} / H_T \quad (\text{equation 9, Nei, 1973})$$

In comparing frequencies of all haplotypes found at sites north of the river to the frequencies of haplotypes found at sites south of the river, and vice versa using X^2 tests, in both cases, haplotype frequencies were found to be significantly different ($p < 0.001$). In comparing the haplotype frequencies of haplotypes found at sites north of the river, and, at sites south of the river, with the overall haplotype frequencies over all sites using X^2 , the former case was found to be not significant ($p > 0.10$), while the latter was significant ($p < 0.001$).

From the proportion of fragments shared between pairs of composite haplotypes, F , a relative index of genetic similarity was estimated (Nei and Li, 1979, equation 21) (Appendix A, B):

$$F = 2 n_{XY} / (n_X + n_Y)$$

where n_X and n_Y are the numbers of fragments in populations X and Y, and n_{XY} is the number of fragments shared by the two populations.

Values for F may range from 0, indicating no shared fragments, to 1, indicating identity. The more similar the DNA sequences are, the more fragments are shared. Conversely, as mtDNA sequences diverge, the proportion of shared fragments is expected to decrease. From these F values, the sequence divergence, p, the percentage of nucleotides substituted separating any pair of composite types, was estimated using the method of Upholt (1977, equation 6b) (Appendix A, B):

$$p = 1 - [(-F + \{F^2 + 8F\}^{\frac{1}{2}}) / 2]^{1/n}$$

where n is the number of base pairs in the recognition site of the restriction enzyme used.

Large p values indicate a longer length of time since the compared pair of haplotypes last shared a common female ancestor. F and p values had to be calculated separately for enzymes recognizing 6 bp, 5 bp and 4 bp sequences (Appendix A, B, C). Enzymes recognizing 6 bp sequences were BamHI, BstEII, EcoRI, HindIII, PstI and XbaI. Although BstEII recognizes a 7 bp sequence, the central base pair can consist of any combination of bases, therefore it can be considered as being equivalent to a hexanuclease. HincII is officially a 6-cutter, but since the two central base pairs in the recognition sequence recognize a purine-pyrimidine pair, then a pyrimidine-purine pair, it is equivalent to a five-cutter. HpaII was the only enzyme used that recognized a 4 bp

sequence. The magnitude of difference in p values based on 4-, 5- and 6- cutters is due to the number of possible substitutions ($4^4 = 256$ versus $4^5 = 1024$ versus $4^6 = 4096$). An overall distance matrix was calculated from p values weighted by the number of nucleotides each restriction enzyme group recognized (Table 6). Overall mean sequence divergence, $\bar{p}$, for *Peromyscus leucopus* in the study area was 0.0083 (range 0.0014 - 0.0178), or less than 1% sequence divergence.

Overall sequence divergence values and the frequencies of haplotypes were used to calculate sequence divergence of haplotypes found within each study site as well as between study sites. In order to focus on the divergence of haplotypes between populations and eliminate the divergence of haplotypes within populations, the net sequence divergence between populations was calculated. To get a net sequence divergence value between pairs of study sites, the following equation was used:

$$\text{Net } p = \sum_{i \neq j} (X_i Y_j p_{ij}) - \frac{1}{2} (X_i X_j p_{ij} + Y_i Y_j p_{ij})$$

where X_i , X_j are the frequencies of the i^{th} and j^{th} haplotypes in population X,
 Y_j is the frequency of the j^{th} haplotype in population Y,
 and p_{ij} is the overall sequence divergence between haplotype i and j
 (Nei and Tajima, 1981).

The matrix of net sequence divergence of all pairs of study sites (Table 7) was used in UPGMA cluster analysis (Figure 10). Although there is very little overall net sequence divergence (range of 0.0014 to 0.0135), the cluster shows separation of study sites north

of the St. Lawrence River from those south of the river at an net sequence divergence of 0.0088 or 0.88%.

DISCUSSION

The use of salivary amylase as a protein genetic marker has again failed to show any genetic patterning among the local populations sampled. As in the previous study of *Peromyscus leucopus* (Tsuchiya, 1986, Merriam et al., 1989), the local populations sampled in this study appeared to be behaving as part of one large panmictic population. The small F_{ST} value of 0.0513 is not significantly different from 0, which indicates a lack of population subdivision. F_{ST} values were almost identical to that found in the previous study (0.0552), despite the fact that woodlot isolation was not a criterion for choosing study sites in this study. Nei's mean genetic identity and mean genetic distance show that differentiation was low and there was a lack of genetic isolation. Values for F_{ST} , genetic identity, and genetic distance found in this study were within range of those found in other studies, despite the differences in the number of loci and the size of samples used (Zimmerman et al., 1978, for local populations of *Peromyscus* $I = 0.971$, $D = 0.030$; Fristrom and Spieth, 1980, for within species of *Peromyscus* $I = 0.97$).

Clustering of genetic distances by UPGMA did not show concordance with the geographic location of sample sites; there does not appear to be any genetic patterning with respect to the salivary amylase locus. Sufficient gene flow tends to homogenize allele frequencies. The presence of a potential barrier to gene flow did not appear to have any discernable effect on genetic patterning. Protein markers, perhaps because of segregation and recombination, do not seem to be sensitive enough to detect genetic population structure; for this locus coded by nuclear DNA, the St. Lawrence River apparently posed no barrier to gene flow.

Mitochondrial DNA analysis proved to be much more sensitive to population structuring than protein analysis. Despite sequence divergence values of less than 1% indicating little differentiation of mtDNA haplotypes, clustering net sequence divergence of each local population separated all sites north of the St. Lawrence River from sites south of the river at a net sequence divergence of 0.0088.

Based on the 8 enzymes used in this study, genetic patterning was evident. Quantitatively, the G_{ST} indicated that 30% of the variation in mtDNA was due to interpopulation variation. This indicates that gene flow was somewhat hindered between the patch populations. However, qualitatively, the populations could not be grouped finer than two assemblages, one which encompassed the populations north of the river, and one south of the river.

In grouping mtDNA haplotypes geographically, there were three instances of haplotype overlap. Separate restriction morphs were common to mice depending on geographic location of capture. This formed 'northern-' and 'southern-' type mtDNA haplotypes, and could predict location of capture with an accuracy of 96.5% (83 out of 86 mice sampled). Considering how little all 14 haplotypes differed from one another in sequence divergence, I felt these could be accepted as outliers to the general pattern. Based on the possible phylogenetic 'map' in Figure 7, the haplotype that seemed to link northern haplotypes to southern ones was BBAABA, a haplotype found on both sides of the river. Presence of this mainly south-of-the-river haplotype north of the river may represent a remnant ancestral type, or an example of convergent evolution back to the ancestral type, or a possible example of 'man-mediated dispersal'. The other southern haplotype found north of the river could also be an example of any of the three possibilities; it is however two mutation steps away from any 'northern haplotype' and

one step away from the possible ancestral type. The presence of major thoroughfares both upstream and downstream from sites 5 and 6 (the International Bridge at Cornwall, Ontario, and Montreal Island, Quebec) may explain the presence of that southern-type haplotype north of the river and a northern-type haplotype south of the river. For the latter haplotype, man-mediated dispersal seems to be the most probable explanation. It is more closely related to northern-type haplotypes than southern ones; it is one mutation step away from two different northern-type haplotypes, but at least four steps away from the nearest related southern-type haplotype. In terms of common clonal types, there were only two instances of northern-caught individuals with clonal types that were commonly found south of the river (see above). Mice with clonal types common to northern mice were not found south of the river.

Although use of a greater number of restriction enzymes may have revealed finer patterns of differentiation (by surveying a greater proportion of the genome), and at the same time solved the problem of possible convergent evolution, this was limited by the amount of mtDNA available and the fact that there comes a point where every individual has a unique haplotype. Sampling a greater number of individuals is limited by the nature of the study areas sampled; population sizes are usually fairly small in these woodlots (sample sizes were approximately 30% of estimated populations) (Merriam et al., 1989). The numbers of individuals sampled and the number of restriction enzymes used was believed to be a good balance among all factors.

Comparing the macrogeographic study by Avise et al. (1983) to this one is somewhat difficult because of their small overall and per locale sample sizes (Table 8). The range of sequence divergence found in this microgeographic study was less than 2% while for theirs was twice that. Most clones could be interconnected by one mutation

step, with the exception of 2 individuals from the western part of the *P. leucopus* range, and one individual from the central portion, while all of the clones found in this study could be interconnected by one or two mutation steps. What was somewhat surprising was the fact that some individuals from states as far as Ohio and Georgia, and New Hampshire and Georgia or North Carolina were more closely related to each other than to another individual from Virginia. Fourteen haplotypes were found in this study in comparison to 12; seven haplotypes were common (4 north of the river, 3 south of the river) in frequency and geographic distribution in my study while no such haplotypes was found in their study. With the vast area from which they sampled, one would expect to see a greater variety of haplotypes, at both local and regional levels. Therefore, due to low sample sizes, many haplotypes were probably missed.

Nelson et al. (1987) looked at the two end points and four middle points of a continuous gradient through a hybrid zone. There were several similarities in the results of their study and the results of this study in spite of the differences in overall and per locale sample size, and sampling method. In their study of a chromosomal hybrid zone they found 7 closely related haplotypes, half of what was found in this one. They were able to separate a southwestern haplotype from 6 northeastern haplotypes by restriction profiles from 2 restriction enzymes. However, two individuals from the most northeastern site had southwestern haplotypes. They hypothesized that these were a result of either convergent evolution (loss of a single restriction site from a common northeastern restriction morph) or maintenance of an ancestral lineage. All six individuals sampled from the two sites west of the hybrid zone had the same southwestern haplotype, but no haplotype predominated the northeastern sites; in my study there were several haplotypes common to either side of the river. Northeastern

haplotypes were separated by one or two mutation steps from either southwestern haplotype, which was the situation in my study. Their sequence divergence values ranged within the values found in this study.

Probably the most useful comparison in terms of genetic patterning is with the study of *P. maniculatus* by Ashley and Wills (1987). It was the only comparable study, in terms of the numbers of samples and samples per site. There is apparently little effective gene flow in terms of mtDNA between the eight California Channel Islands and the mainland since no haplotype was found in common between any island and any mainland population sampled. The authors note that the presence of the same haplotypes on some of the islands probably reflects the more recent separation of these islands in geological history. Their calculation of sequence divergence ($p \leq 1\%$) for the most common haplotype found at each study site, matched what was found in my study. Their mean value of nucleon diversity, $\hat{h}$, for mainland mice was 0.79, slightly higher than my value of 0.70.

In another California Channel Islands study, Ashley and Wills (1989) found that unlike studies of mainland populations (Avise et al., 1979; Lansman et al., 1983), protein and mtDNA differentiation was correlated. Gene flow seems to be sufficiently restricted in this case so that the island populations have indeed become subdivided for nuclear genes and mtDNA. Variability of both mtDNA and nuclear-DNA coded allozymes were reduced in island populations in comparison to mainland populations, although the degree of reduction was much more pronounced for mtDNA (Ashley and Wills, 1987). They hypothesized that the differences in transmission genetics of mtDNA and nuclear genes was the reason behind this finding.

In this study, the sample populations did not appear subdivided with respect to salivary amylase allozymes, but did with mtDNA. When migration rates are enough to keep nuclear genes panmictic, populations will be subdivided for mtDNA (Ashley and Wills, 1987). As few as one effective disperser per generation between patches may be sufficient to maintain effective panmixia and thereby prevent differentiation on the landscape scale (Wright, 1951, Maruyama, 1970, Spieth, 1974, Slatkin, 1985). Because the effective population size for nuclear genes is four times that of mtDNA (when sex ratios are 1:1), time to fixation for mtDNA will be half as long. Theoretically, if there is mtDNA gene flow between populations, there should be a subset of the same haplotypes present among these populations. In the sites located on either side of the St. Lawrence River, this is so, but rarely does a haplotype present on one side of the river appear on the other. The presence of 'stepping-stone' islands did not appear to facilitate mtDNA gene flow; there were no haplotypes in common found at sites 5i and 6i. Curiously, one haplotype did appear at both sites 5 and 6, sites that straddled a section of the St. Lawrence River where islands were absent. This could be accounted for by any of three reasons, ranging from convergent evolution, to man-assisted dispersal. Because this was the only instance of haplotype overlap at either pair of sites, mtDNA gene flow across the river is probably a rare event.

Avise et al., (1987) categorized the theoretical outcomes of overlaying intraspecific phylogenies with geographic maps. The five phylogeographic categories vary in terms of genetic divergence pattern and geographic distribution of mtDNA assemblages. Category I and II deal with genetically distinct assemblages that occupy separate geographic areas, or co-occur (respectively) within a species' range. Categories III and IV deal with continuous genetic assemblages that are either spatially disjunct or

overlapping. The last category deals with spatially nested continuous genetic assemblages. The data from this study seem to fall into categories I and V: there is a discontinuous genetic divergence pattern at the St. Lawrence river barrier while north and south of the river there are nested continuous genetic assemblages. Avise et al. (1987) hypothesizes that the likely evolutionary circumstances for category I are long term barriers to gene flow and/or extinction of intermediate genotypes in species with limited gene flow, while for category V, it is intermediate gene flow in a species not subdivided by long-term zoogeographic barriers. The rare presence of predominantly southern clonal types north of the river, which are more closely related to northern clonal types, may reflect possible extinction of those types over a wide area north of the river.

By surveying both the salivary amylase locus and mitochondrial DNA, I attempted to determine whether eight patch populations in the agricultural landscape of eastern Ontario, southwestern Quebec, and upstate New York displayed any sort of genetic patterning. This study concurs with a previous study (Tsuchiya, 1986, Merriam et al., 1989) in suggesting that there is no evidence of genetic boundaries among *Peromyscus leucopus* populations in forest fragments, with respect to allozyme analysis. The data from mtDNA analysis in this study also agree with this. Although patchiness of these populations is seasonally important and they do function as a metapopulation demographically, mtDNA analysis confirms that on a landscape to regional scale, there is sufficient gene flow to prevent significant genetic differentiation.

The presence of the St. Lawrence River as a barrier to dispersal did not appear to hinder gene flow in terms of the amylase locus, but did appear to restrict mtDNA flow. A low fixation index, high genetic identity and low genetic distance supports the

lack of genetic substructuring among the populations for gene coded by nuclear DNA. Separation of overall net sequence divergence of populations north of the river and those south of the river, and low frequency of haplotype overlap between the two assemblages, and significant X^2 differences in haplotype frequencies between sites north and south of the river, support restricted and perhaps lack of mtDNA gene flow between the two assemblages. The differences observed between the results of allozyme and mtDNA analysis may reflect the different dispersal abilities between male and female *Peromyscus*, or the differences in transmission genetics of nuclear and mitochondrial DNA.

According to the mtDNA findings, a major barrier to gene flow, such as the St. Lawrence River, is required to allow noticeable genetic differentiation on the landscape to regional scale. Such a barrier may be one way to bound a deme.

CONCLUSIONS

1. Populations of *Peromyscus leucopus* in patches of forest fragments in agricultural landscapes do not appear to be subdivided according to restriction analysis of mitochondrial DNA.
2. Genetic differentiation of populations was observed only when a major barrier to dispersal and gene flow, such as the St. Lawrence River, was present.
3. The presence of stepping stone-islands did not appear to facilitate mitochondrial DNA gene flow.
4. Geographic barriers on the scale of the St. Lawrence River do not appear to limit gene flow for genes coded by nuclear DNA.
5. Mitochondrial DNA analysis is more sensitive to genetic differentiation of subpopulations than allozyme analysis of proteins.

Table 1. Location of study sites.

map number*	location	abbreviation	co-ordinates
31G/4 ed. 7	Manotick, Ontario	1	N45°15' - W75°41'
31G/3 ed. 5	Spring Hill, Ontario	2	N45°12' - W75°28'
31G/3 ed. 5	Winchester, Ontario	3	N45°08' - W75°15'
31G/3 ed. 5	North Valley, Ontario	4	N45°03' - W75°03'
31G/2 31B/15 ed. 6	Ingleside, Ontario	5i	N45°08' - W75°15'
	Massena, New York, USA	6i	N49°75' - W75°01'
31G/1 ed. 8	Bainville, Ontario	5	N45°10' - W74°25'
31G/1 ed. 8	St. Anicet, Quebec	6	N45°08' - W74°22'

5i, 6i Denotes sites on opposite sides of the St. Lawrence River where intervening islands are present.

5, 6 Denotes sites on opposite sides of the St. Lawrence River where islands are absent.

* All maps are issued by Energy, Mines and Resources, Canada, with the exception of the Massena, N.Y. map, which is issued by the U.S. Geological Survey.

Table 2. Salivary amylase electromorph frequencies and allele frequencies for *Peromyscus leucopus* from eight study sites in eastern Ontario, southern Quebec and upstate New York.

site	N	phenotype frequencies*					Allele frequencies		
		fl	fm	mm	ms	ss	f	m	s
1	10	0	4	6	0	0	0.20	0.80	0
2	15	1	6	7	0	1	0.27	0.67	0.06
3	10	0	8	2	0	0	0.40	0.60	0
4	10	0	5	4	0	1	0.25	0.65	0.10
5i	11	0	9	1	1	0	0.41	0.55	0.04
6i	8	0	7	1	0	0	0.44	0.56	0
5	12	0	4	8	0	0	0.17	0.83	0
6	10	0	4	6	0	0	0.20	0.80	0
Total	86	1	47	35	1	2	0.28	0.69	0.03

* The fs phenotype was not observed in this study.

Table 3. Nei's genetic identity, I, and genetic distance, D, calculated from salivary amylase allele frequency data. Genetic identities above the diagonal; genetic distances below the diagonal. Sites: 1 - Manotick, Ont.; 2 - Spring Hill, Ont.; 3 - Winchester, Ont.; 4 - North Valley, Ont.; 5i - Ingleside, Ont.; 6i - Massena, N.Y.; 5 - Bainsville, Ont.; 6 - Saint-Anicet, P.Q.

site	1	2	3	4	5i	6i	5	6
1	---	0.9866	0.9417	0.9825	0.9196	0.9147	0.9989	1.0000
2	0.0135	---	0.9744	0.9987	0.9655	0.9568	0.9791	0.9866
3	0.0600	0.0260	---	0.9658	0.9963	0.9973	0.9247	0.9417
4	0.0177	0.0013	0.0348	---	0.9597	0.9474	0.9756	0.9824
5i	0.0838	0.0351	0.0038	0.0411	---	0.9976	0.9002	0.9196
6i	0.0892	0.0442	0.0027	0.0540	0.0024	---	0.8944	0.9147
5	0.0011	0.0212	0.0783	0.0247	0.1051	0.1115	---	0.9989
6	0	0.0135	0.0600	0.0177	0.0838	0.0892	0.0011	---

Table 4. Frequencies of mtDNA composite genotypes (based on 8 restriction enzymes) observed in eight study sites.

clone	1 (10)	2 (15)	3 (10)	4 (10)	5i (11)	5 (12)	6i (8)	6 (10)	overall frequency
AAAAAA	0.40	0.53	0.50	0.60	0.09	0	0	0	0.2791
AAABAA	0	0.20	0.20	0	0.27	0.67	0	0	0.1860
AABABA	0.30	0	0.20	0.30	0.55	0.17	0	0	0.1860
BAAABA	0.10	0.20	0	0.10	0	0.08	0	0	0.0698
BAAABB	0.10	0	0.10	0	0	0	0	0	0.0233
ACAEAA	0	0	0	0	0.09	0	0	0	0.0116
AAABABA	0	0.07	0	0	0	0	0	0	0.0116
BBAABA	0.10	0	0	0	0	0	0.375	0.10	0.0581
BBACBA	0	0	0	0	0	0.08	0.50	0.10	0.0698
BDBDBA	0	0	0	0	0	0	0.125	0.40	0.0581
BAAAAA	0	0	0	0	0	0	0	0.10	0.0116
BDADBA	0	0	0	0	0	0	0	0.10	0.0116
ABACBA	0	0	0	0	0	0	0	0.10	0.0116
BBBDBA	0	0	0	0	0	0	0	0.10	0.0116

* Order of restriction enzymes in composite phenotype is BamHI, BstEII, HincII, HpaII, PstI, XbaI. Enzymes EcoRI and HindIII each gave a non-variable fragment pattern and therefore were not included in the composite.

Table 5. Observed nucleon diversity, $\hat{h}$, of *Peromyscus leucopus* mtDNA from eight sites in eastern Ontario, southern Quebec and upstate New York.

	site							
	1	2	3	4	5i	6i	5	6
$\hat{h}$	0.8000	0.6795	0.7333	0.6000	0.6692	0.6786	0.5557	0.8667

Table 6. Overall pairwise sequence divergence, p , calculated from all restriction enzymes used.

[illegible]

Table 7. Matrix of overall net sequence divergence. Listed values are 10^2 of actual values. Sites as listed in Table 1.

site	1	2	3	4	5i	6i	5	6
1	---							
2	0.1614	---						
3	0.2350	0.1578	---					
4	0.2052	0.1395	0.1716	---				
5i	0.2791	0.2825	0.2544	0.2156	---			
6i	0.6413	0.7017	1.3540	0.7703	0.9083	---		
5	0.2906	0.1883	0.2151	0.2601	0.2844	0.7524	---	
6	0.8196	1.0010	0.9674	0.9229	0.9007	0.4104	0.9162	---

Table 8. Comparable values from other *Peromyscus* studies.

species	N	scale	number of locales	number of RE used	number of clones	sequence divergence, p (range) mean	reference
<i>P. leucopus</i>	86	~ 100 km	8 sites	8	14	(0.0014-0.0178) 0.0083	this study
<i>P. leucopus</i>	14	> 1000 km	9 counties	8	12	(0.003-0.026) 0.014	Awise et al., 1983
<i>P. leucopus</i>	18	~ 263 km	6 sites	8	7	(0.0020-0.0109) 0.004	Nelson et al., 1987
<i>P. maniculatus</i>	87 44	~ 400 km overall	8 island 7 mainland	9 9	10 16	(0.0032-0.0095) 0.004	Ashley and Wills, 1987
<i>P. maniculatus</i>	135	> 1000 km	35 locales	8	61	(0.03-0.05)	Lansman et al., 1983

Figure 1. Geographic location of study sites.

- 1 - Manotick, Ontario;
- 2 - Spring Hill, Ontario;
- 3 - Winchester, Ontario;
- 4 - North Valley, Ontario;
- 5i - Ingleside, Ontario;
- 6i - Massena, New York;
- 5 - Bainsville, Ontario;
- 6 - Saint-Anicet, Quebec.

Figure 2. Schematic diagram of salivary amylase electromorphs observed among eight study sites in eastern Ontario, southern Quebec, and upstate New York.

ff

fm

mm

ms

ss

Pmg

Peromyscus leucopus electromorphs

P. maniculatus

marker

Figure 3. Cluster analysis of study sites using unweighted pair group method using arithmetic means (UPGMA) for salivary amylase data.

Figure 4. Photograph of ethidium bromide stained gel of mtDNA restriction profiles for *P. leucopus*. Lanes 1 and 20 (left to right) are molecular weight standards using HindIII cut Lambda DNA (lengths listed to the left in kb). Lane 2: EcoRI; lane 3: HindIII; lanes 4 and 5: PstI 'A' and 'B'; lane 6 and 7: XbaI 'A' and 'B'; lanes 8 and 9: BamHI 'A' and 'B'; lanes 10 and 11: HincII 'A' and 'B'; lanes 12 to 14: BstEII 'A', 'B', and 'C'; lanes 15 to 19: HpaII 'A', 'B', 'C', 'D', and 'E'. Note that enzymes EcoRI and HindIII each gave a single fragment pattern. Missing is BstEII profile 'D'. Extra fragments (in comparison to Figure 5) indicate incomplete digestion of mtDNA by restriction enzyme.

Figure 5. Schematic diagram of mtDNA restriction fragment length polymorphisms observed when using eight restriction enzymes, based on Figure 4 and various southern blots. Lane 1 is the molecular weight standards using HindIII cut Lambda DNA. Lane 2: EcoRI; lane 3: HindIII; lanes 4 and 5: PstI 'A' and 'B'; lane 6 and 7: XbaI 'A' and 'B'; lanes 8 and 9: BamHI 'A' and 'B'; lanes 10 and 11: HincII 'A' and 'B'; lanes 12 to 15: BstEII 'A', 'B', 'C', and 'D'; lanes 16 to 20: HpaII 'A', 'B', 'C', 'D', and 'E'. Note that enzymes EcoRI and HindIII each gave a single fragment pattern.

Figure 6.

Minimum number of mutation steps required between restriction fragment pattern morphs for enzymes giving variable fragment patterns. Lines connecting profiles indicate phylogenetic links. Arrows indicate direction of loss of fragment, but do not necessarily indicate direction of evolution. Slashes across arrows indicate minimum number of mutation steps required between fragment patterns. Positioning of tick marks on the mtDNA genome show relative size of fragments rather than placement of restriction sites.

BamHI

HincII

PstI

XbaI

Figure 7. Minimum number of mutation steps required between profile types for enzymes producing multiple profiles. Lines connecting profiles indicate phylogenetic links. Arrows indicate direction of loss of fragment, but do not necessarily indicate direction of evolution. Slashes across arrows indicate minimum number of mutation steps required between fragment patterns. Positioning of tick marks on the mtDNA genome show relative size of fragments rather than placement of restriction sites.

BstEII

HpaII

Figure 8. Phylogenetic network for fourteen mtDNA haplotypes found in eastern Ontario, southern Quebec and upstate New York. Slashes indicate the minimum number of mutations required between clonal types. The sites where each haplotype was found is indicated in brackets. Boldface font indicates haplotypes found exclusively north of the St. Lawrence River. Italics indicate haplotypes found exclusively south of the St. Lawrence River. Boxed haplotypes indicate clonal types found on both sides of the river.

Figure 9. Geographic grouping of *P. leucopus* mtDNA clonal types. Clonal types as indicated below.

Major clonal types:	AAAAAA	————	BBACBA
	AAABAA	- - - - -	BDBDBA
	AABABA	- - - - -	BBAABA	
	BAAABA			
Minor clonal types:	* BAAABB	\$ BBBDBA	+ BAAAAA	
	# AAAABA	& ABACBA		
	@ ACAEAA	% BDADBA

Figure 10. UPGMA cluster analysis of study sites (numbered 1 through 6, listed in Table 1) using overall net sequence divergence.

Appendix A. Pairwise sequence divergence, p, and proportion of fragments shared, F, estimated for 6 enzymes recognizing a 6 bp sequence. Enzymes used were BamHI, BstEII, EcoRI, HindIII, PstI, and XbaI. Composite genotypes differing in the HincII and HpaII profiles are presented in Appendix B. p estimates are given above the diagonal. F values given below the diagonal. AAAAAA and AAABAA / AABABA and AAAABA and AAABAA / BBAABA BBACBA and BBBDBA and BDADBA have identical values.

	AAAAAA	AABABA	AAAAABA	BAAABA	BAAABB	ACAEAA	BBBDBA	BDBDBA	BAAAAA	ABACBA
AAAAAA	-----	0.0031	0.0053	0.0087	0.0033	0.0087	0.0126	0.0064	0.0021	0.0064
AABABA	0.9455	-----	0.0020	0.0051	0.0066	0.0051	0.0087	0.0030	0.0053	0.0030
BAAABA	0.9091	0.9643	-----	0.0030	0.0090	0.0030	0.0064	0.0051	0.0031	0.0051
BAAABB	0.8571	0.9123	0.9474	-----	0.0126	0.0061	0.0097	0.0083	0.0064	0.0083
ACAEAA	0.9434	0.8889	0.8519	0.8000	-----	0.0126	0.0090	0.0101	0.0055	0.0101
BBAABA	0.8571	0.9123	0.9474	0.8966	0.8000	-----	0.0051	0.0020	0.0064	0.0020
BDBDBA	0.8000	0.8571	0.8929	0.8421	0.8519	0.9123	-----	0.0074	0.0101	0.0074
BAAAAA	0.9630	0.9091	0.9455	0.8929	0.9057	0.8929	0.8364	-----	0.0087	0.0087
ABACBA	0.8929	0.9474	0.9123	0.8621	0.8364	0.9655	0.8772	0.8571	-----	-----

Appendix B. Pairwise sequence divergence, p , and proportion of fragments shared, F , estimated for the restriction enzymes HincII and HpaII, recognizing a 5 bp and a 4 bp sequence, respectively. Letters indicate restriction fragmentation pattern. p values above the diagonal; F values below.

HincII (5-cutter): HincII A versus HincII B $F = 0.6667$, $p = 0.0278$

HpaII (4-cutter):

	A	B	C	D	E
A	---	0.0154	0.0154	0.0523	0.0271
B	0.8333	---	0.0346	0.0523	0.0271
C	0.8333	0.6667	---	0.0271	0.0523
D	0.5455	0.5455	0.7273	---	0.0189
E	0.7273	0.7273	0.5455	0.8000	---

REFERENCES

- Aquadro, C.F., and Patton, J.C. 1980. Salivary amylase variation in *Peromyscus*: use in species identification. *J. Mamm.* 61:703-707.
- Ashley, M.V., and Wills, C. 1987. Analysis of mitochondrial DNA polymorphisms among Channel Island deer mice. *Evol.* 41:854-864.
- Ashley, M.V., and Wills, C. 1989. Mitochondrial-DNA and allozyme divergence patterns are correlated among island deer mice. *Evol.* 43:646-650.
- Avice, J.C. 1986. Mitochondrial DNA and the evolutionary genetics of higher animals. *Phil. Trans. R. Soc. Lond. B* 312:523-342.
- Avice, J.C., Giblin-Davidson, C., Laerm, J., Patton, J.C., Lansman, R.A. 1979. Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, *Geomys pinetis*. *Proc. Natl. Acad. Sci. USA* 76:6694-6698.
- Avice, J.C., Shapira, J.F., Daniel, S.W., Aquadro, C.F., Lansman, R.A. 1983. Mitochondrial DNA differentiation during the speciation process in *Peromyscus*. *Mol. Biol. Evol.* 1:38-56.

- Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A.,
Saunders, N.C. 1987. Intraspecific phylogeography: the mitochondrial DNA
bridge between population genetics and systematics. *Ann. Rev. Ecol. Syst.*
18:489-522.
- Baker, J., Smith, J.M., Strobeck, C. 1975. Genetic polymorphism in the Bladder
Campion, *Silene maritima*. *Biochem. Genetics* 13:393-410.
- Beer, J.R., Lukens, P., Olson, D. 1954. Small mammal populations on the islands of
Basswood Lake, Minnesota. *Ecol.* 35:437-445.
- Birky, C.W., Maruyama, T., Fuerst, P. 1983. An approach to population and
evolutionary genetic theory for genes in mitochondria and chloroplasts, and some
results. *Genetics* 103:513-527.
- Brown, W.M. 1983. Evolution of animal mitochondrial DNA. In *Evolution of genes
and proteins*, pp. 62-88. Edited by M. Nei and R.K. Koehn. Sinauer Associates,
Inc., Sunderland, Mass.
- Brown, W.M., George, M., Wilson, A.C. 1979. Rapid evolution of animal mitochondrial
DNA. *Proc. Natl. Acad. Sci. USA* 76:1967-1971.
- Brown, J.H., and Kodric-Brown, A. 1977. Turnover rates in insular biogeography: effect
of immigration on extinction. *Ecol.* 58:445-449.

- Carmody, G.R., Fenton, M.B., Lee, D.S.K. 1971. Variation of body weight and proteins in three Ontario populations of hibernating *Myotis lucifugus lucifugus* (LeConte) (Chiroptera: Vespertilionidae). Can. J. Zool. 49:1535-1540.
- Choate, J.R. 1973. Identification and recent distribution of white-footed mice (*Peromyscus*) in New England. J. Mamm. 54:41-49.
- Christianson, L. 1977. Winter movements of *Peromyscus* across a lake in northern Minnesota. J. Mamm. 58:244.
- Eanes, W.F., and Koehn, R.K. 1978. An analysis of genetic structuring in the monarch butterfly, *Danaus plexippus* L. Evol. 32:784-797.
- Ehrlich, P.R., and Murphy, D.D. 1987. Conservation lessons from long-term studies of checkerspot butterflies. Conserv. Biol. 1:122-131.
- Fahrig, L., and Merriam, G. 1985. Habitat patch connectivity and population survival. Ecol. 66:1762-1768.
- Feldhamer, G.A., Gates, J.F., Howard, J.H. 1983. Field identification of *Peromyscus maniculatus* and *P. leucopus* in Maryland: reliability of morphological characteristics. Acta Theriol. 28:417-423.

- Sheppe, W. 1965b. Dispersal by swimming in *Peromyscus leucopus*. J. Mamm. 46:336-337.
- Slatkin, M. 1985. Gene flow in natural populations. Ann. Rev. Ecol. Syst. 16:393-430.
- Smith, D.A., and Speller, S.W. 1970. The distribution and behavior of *Peromyscus maniculatus gracilis* and *Peromyscus leucopus noveboracensis* (Rodentia: Cricetidae) in a southeastern Ontario woodlot. Can. J. Zool. 48:1187-1199.
- Sneath, P.H.A., and Sokal, R.R. 1973. *Numerical Taxonomy*. W.H. Freeman and Company, San Francisco.
- Speth, P.T. 1974. Gene flow and genetic differentiation. Genetics 78:961-965.
- Telegston, H., and Hansson, L. 1987. Evidence of long distance dispersal in the common shrew (*Sorex araneus*). Z. Saugetierkunde 52:52-54.
- Thomas, W.K., and Beckenbach, A.T. 1986. Mitochondrial DNA restriction site variation in the Townsend's vole, *Microtus townsendii*. Can. J. Zool. 64:2750-2756.
- Tsuchiya, E. 1986. A salivary amylase isozyme study of two sympatric species populations (*Peromyscus leucopus noveboracensis* and *P. maniculatus gracilis*). Undergraduate thesis. Queen's University, Kingston, Ontario, Canada.

- Klein, H.G. 1960. Ecological relationships of *Peromyscus leucopus noveboracensis* and *P. maniculatus gracilis* in central New York. *Ecol. Mono.* 30:387-407.
- Lansman, R.A., Shade, R.O., Shapira, J.F., Avise, J.C. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. *J. Mol. Evol.* 17:214-226.
- Lansman, R.A., Avise, J.C., Huettel, M.D. 1983. Critical experimental test of the possibility of "paternal leakage" of mitochondrial DNA. *Proc. Natl. Acad. Sci. USA* 80:1969-1971.
- Lansman, R.A., Avise, J.C., Aquadro, C.F., Shapira, J.F., Daniel, S.W. 1983. Extensive genetic variation in mitochondrial DNA's among geographic populations of the deer mouse, *Peromyscus maniculatus*. *Evol.* 37:1-16.
- Levins, R. 1970. Extinction. In *Lectures on mathematics in the life sciences. Some mathematical questions in biology*, Vol. 2, pp. 77-107. Editor M. Gerstenhaber. American mathematical society, Providence, Rhode Island.
- Lomolino, M.V. 1984. Immigrant selection, predation, and the distributions of *Microtus pennsylvanicus* and *Blarina brevicauda* on islands. *Am. Nat.* 123:468-483.

- MacNeil, D., and Strobeck, C. 1987. Evolutionary relationships among colonies of colombian ground squirrels as shown by mitochondrial DNA. *Evol.* 41:873-881.
- Maniatis, T., Fritsch, E.F., Sambrook, J. 1982. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory, U.S.A.
- Maruyama, T. 1970. On the rate of heterozygosity in circular stepping stone models of populations. *Theor. Pop. Biol.* 1:101-119.
- Mayr, E. 1963. *Animal species and evolution*. Belknap Press of Harvard University Press, Cambridge Mass.
- Merriam, G. 1984. Connectivity: A fundamental ecological characteristic of landscape pattern. In *Proc. First Int. Sem. Meth. Landscape Ecol. Res. Plann. Theme 1*. pp. 5-15. Edited by J. Brandt and P. Agger. Roskilde, Denmark.
- Merriam, G. 1988. Landscape dynamics in farmland. *Trends in Ecol. and Evol.* 3:16-20.
- Merriam, G., and Lanoue, A. Corridor use by small mammals: field measurements for three experimental types of *Peromyscus leucopus*. In press, *Landscape Ecology*.

- Merriam, G., and Wegner, J. Local extinctions, habitat fragmentation and ecotones.
Submitted to Proceedings of SCOPE workshop on ecotones. Paris, December
1988.
- Merriam, G., Kozakiewicz, M., Tsuchiya, E., Hawley, K. 1989. Barriers as boundaries
for metapopulations and demes of *Peromyscus leucopus* in farm landscapes.
Landscape Ecol. 2:227-235.
- Middleton, J., and Merriam, G. 1981. Woodland mice in a farmland mosaic. J. Appl.
Ecol. 18:703-710.
- Moritz, C., Dowling, T.E., Brown, W.M. 1987. Evolution of animal mitochondrial
DNA: Relevance for population biology and systematics. Ann. Rev. Ecol. Syst.
18:269-292.
- Nei, M. 1972. Genetic distance between populations. Am. Nat. 106:283-292.
- Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Nat. Acad.
Sci. USA. 70:3321-3323.
- Nei, M. 1975. *Molecular population genetics and evolution*. North-Holland Research
Monographs: Frontiers of Biology, Vol. 40 (eds. A. Neuberger and E.L. Tatum)
North-Holland Publishing Company, Amsterdam.

- Nei, M., and Li, W. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA* 76:5269-5273.
- Nei, M., and Tajima, F. 1981. DNA polymorphism detectable by restriction endonucleases. *Genetics* 97:145-163.
- Nelson, K., Baker, R.J., Honeycutt, R.L. 1987. Mitochondrial DNA and protein differentiation between hybridizing cytotypes of the white-footed mouse, *Peromyscus leucopus*. *Evol.* 41:864-872.
- Oxley, D.J., Fenton, M.B., Carmody, G.C. 1974. The effects of roads on populations of small mammals. *J. Appl. Ecol.* 11:51-59.
- Plante, Y., Boag, P.T., White, B.N. 1989a. Macrogeographic variation in mitochondrial DNA of meadow voles (*Microtus pennsylvanicus*). *Can. J. Zool.* 67:158-167.
- Plante, Y., Boag, P.T., White, B.N. 1989b. Microgeographic variation in mitochondrial DNA of meadow voles (*Microtus pennsylvanicus*) in relation to population density. *Evol.* 43:1522-1537.
- Sheppe, W. 1965a. Island populations and gene flow in the deer mouse *Peromyscus leucopus*. *Evol.* 19:480-495.

- Sheppe, W. 1965b. Dispersal by swimming in *Peromyscus leucopus*. J. Mamm. 46:336-337.
- Slatkin, M. 1985. Gene flow in natural populations. Ann. Rev. Ecol. Syst. 16:393-430.
- Smith, D.A., and Speller, S.W. 1970. The distribution and behavior of *Peromyscus maniculatus gracilis* and *Peromyscus leucopus noveboracensis* (Rodentia: Cricetidae) in a southeastern Ontario woodlot. Can. J. Zool. 48:1187-1199.
- Sneath, P.H.A., and Sokal, R.R. 1973. *Numerical Taxonomy*. W.H. Freeman and Company, San Francisco.
- Speith, P.T. 1974. Gene flow and genetic differentiation. Genetics 78:961-965.
- Telegstom, H., and Hansson, L. 1987. Evidence of long distance dispersal in the common shrew (*Sorex araneus*). Z. Säugetierkunde 52:52-54.
- Thomas, W.K., and Beckenbach, A.T. 1986. Mitochondrial DNA restriction site variation in the Townsend's vole, *Microtus townsendii*. Can. J. Zool. 64:2750-2756.
- Tsuchiya, E. 1986. A salivary amylase isozyme study of two sympatric species populations (*Peromyscus leucopus noveboracensis* and *P. maniculatus gracilis*). Undergraduate thesis. Queen's University, Kingston, Ontario, Canada.

- Upholt, W.B. 1977. Estimation of DNA sequence divergence from comparison of restriction endonuclease digests. *Nucl. Acids Res.* 4:1257-1265.
- Wegner, J.F. 1976. Corridors for birds and small mammals in hedgerows adjoining a beech-maple farm woodlot. M.Sc. thesis. Carleton University, Ottawa, Ontario, Canada.
- Wegner, J.F., and Merriam, G. 1979. Movements of birds and small mammals between a wood and adjoining farmland habitats. *J. Appl. Ecol.* 16:349-357.
- Wilson, A.C., Cann, R.L., Carr, S.M., George, M., Gyllenstein, U.B., Helm-Bychowski, K.M., Higuchi, R.G., Palumbi, S.R., Prager, E.M., Sage, R.D., Stoneking, M. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. *Biol. J. Linn. Soc.* 26:375-400.
- Wright, S. 1951. The genetical structure of populations. *Ann. Eugenics* 15:323-354.
- Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. *Evol.* 19:395-420.
- Wright, S. 1969. *Evolution and the genetics of populations, Vol. II: the theory of gene frequencies.* University of Chicago Press, Chicago.

Wright, S. 1978. *Evolution and the genetics of populations, Vol. IV: Variability within and among natural populations.* University of Chicago Press, Chicago.

Zimmerman, E.G., Kilpatrick, C.W., Hart, B.J. 1978. The genetics of speciation in the rodent genus *Peromyscus*. *Evol.* 32:565-579.

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