

**A GENETIC TEST OF RANGE EXPANSION BY THE  
SOUTHERN FLYING SQUIRREL (*Glaucomys volans*) AT ITS NORTHERN  
RANGE BOUNDARY**

By

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## ABSTRACT

### A genetic test of range expansion by the southern flying squirrel (*Glaucomys volans*) at its northern range boundary

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I present the results of the analysis of 161 southern (*Glaucomys volans*) and northern (*G. sabrinus*) flying squirrel genotypes from nine locations within Ontario, Canada. I hypothesised that if southern flying squirrels were expanding their range and that if the expansion was rapid and recent, then little to no genetic differentiation would be detected. I also did a comparative study looking at the genetic structure of northern flying squirrels within the same region of Ontario. Individuals were genotyped at seven loci and using a Bayesian population clustering approach, I identified one discrete inferred genetic population for each species. Little evidence of isolation by distance was detected for either species. I concluded that each species grouped together and thus exhibited high gene flow among individuals within the study area. The high levels of gene flow found in southern flying squirrels supports the prediction of a recent and rapid range expansion. The high level of gene flow found in northern flying squirrels however was not expected as I predicted that there would be evidence of genetic structure since there has not been any corroborating evidence that this species has been expanding its range in Ontario.

**Keywords:** Southern flying squirrel, *Glaucomys volans*, northern flying squirrel, *Glaucomys sabrinus*, microsatellite, Bayesian clustering, assignment test, isolation by distance, range expansion.

## Table of Contents

	Page
<i>Abstract</i> .....	ii
<i>Table of Contents</i> .....	iii
<i>List of Tables</i> .....	iv
<i>List of Figures</i> .....	v
<i>Acknowledgments</i> .....	vii
<i>Chapter 1</i> .....	1
<b>General Introduction</b>	
<i>Chapter 2</i>	
<b>A genetic test of range expansion by the southern flying squirrel (<i>Glaucomys volans</i>) at its northern range boundary</b>	
<i>Introduction</i> .....	6
<i>Methods and Materials</i> .....	9
<i>Results</i> .....	13
<i>Discussion</i> .....	15
<i>Chapter 3</i> .....	40
<b>General Discussion</b>	
<i>Literature Cited</i> .....	44

## List of Tables

	Page
<i>Table 2.1</i> .....	27
Summary of the number of individual southern and northern flying squirrels profiled at each of the study sites in Ontario, Canada with both mean observed ( $H_O$ ) and mean expected ( $H_E$ ) for each location. Sampling sites in bold font indicate sites for southern flying squirrels and sites in regular font indicate sites for northern flying squirrels.	
<i>Table 2.2</i> .....	28
Pairwise $F_{st}$ values estimated using microsatellite data for southern flying squirrels ( <i>Glaucomys volans</i> ) at five sites within Ontario, Canada. DOR = Dorset, KAW = Kawartha Highlands, HSIX = Hwy 60 in Algonquin Provincial Park, MAT = Mattawa, and RON = Rondeau Provincial Park.	
<i>Table 2.3</i> .....	29
Pairwise $F_{st}$ values estimated using microsatellite data for northern flying squirrels ( <i>Glaucomys sabrinus</i> ) at five sites within Ontario, Canada. AUR = Aurora District, BGC = Bruce/Grey Counties, HSIX = Hwy 60 in Algonquin Provincial Park, PBO = Peterborough County, and TEM = Temagami.	

## List of Figures

	Page
<i>Figure 2.1</i> .....	20
Schematic of possible range expansion scenarios; a) source population breaks up into smaller founding populations and either maintains gene flow with each other and the source population or the founding populations become isolated over time and show a pattern of isolation by distance and b) source population continues to expand outwards where the individuals that are expanding are maintaining gene flow with each other.	
<i>Figure 2.2</i> .....	22
Map of sampling sites for both southern flying squirrels ( <i>Glaucomys volans</i> ) and northern flying squirrels ( <i>Glaucomys sabrinus</i> ) in Ontario, Canada during 2002 to 2004. The sites are Rondeau Provincial Park (Clear Creek Forest) (1), Bruce and Grey Counties (2), Aurora MNR District (3), Peterborough County (4), Kawartha Highlands (5), Leslie M. Frost Centre near Dorset (6), Algonquin Provincial Park (7), near Mattawa (8), and near Temagami (9). Locations shown on map are centroids of sampled areas.	
<i>Figure 2.3</i> .....	24
Factorial correspondence analysis for southern flying squirrels ( <i>Glaucomys volans</i> ) at five sites in Ontario, Canada. Samples were collected during the 2002-2004 field seasons. Outliers include A = ID# 022 Rondeau Provincial Park, B = ID# 1904 Kawartha Highlands, C = ID# 625 Dorset, D = ID# 049 Kawartha Highlands, and E = ID# NA Algonquin Provincial Park. Each location was given a colour and plotted as such in the cloud.	
<i>Figure 2.4</i> .....	26
Factorial correspondence analysis for northern flying squirrels ( <i>Glaucomys sabrinus</i> ) at five sites in Ontario, Canada. Samples were collected during the 2002-2004 field seasons. Outliers include A = ID# GS04E Bruce and Grey County, B = ID# 625 Algonquin Park, and C = ID# 151.287 Algonquin Provincial Park. Each location was given a colour and plotted as such in the cloud.	
<i>Figure 2.5</i> .....	31
Mantel isolation by distance for southern flying squirrels ( <i>Glaucomys volans</i> ) at five sites in Ontario, Canada (Dorset, Kawartha Highlands, Highway 60 in Algonquin Provincial Park, Mattawa, and Rondeau Provincial Park). Samples were collected during the 2002-2004 field seasons.	
<i>Figure 2.6</i> .....	33
Mantel isolation by distance for southern flying squirrels ( <i>Glaucomys volans</i> ) at four sites in Ontario, Canada (Dorset, Kawartha Highlands, Highway 60 in Algonquin Provincial Park, and Mattawa). Samples were collected during the 2002-2004 field seasons.	

<i>Figure 2.7</i> .....	35
Mantel isolation by distance for northern flying squirrels ( <i>Glaucomys sabrinus</i> ) at five sites in Ontario, Canada (Aurora District, Bruce.Grey Counties, Highway 60 in Algonquin Provincial Park, Peterborough County, and Temagami). Samples were collected during the 2002-2004 field seasons.	
<i>Figure 2.8</i> .....	37
Pairwise $F_{ST}$ comparisons between locations in relation to geographic distance for southern flying squirrels ( <i>Glaucomys volans</i> ) at five sites in Ontario, Canada (Dorset, Kawartha Highlands, Highway 60 in Algonquin Provincial Park, Mattawa, and Rondeau Provincial Park). Pairwise comparisons were made between all study sites (excluding Rondeau Provincial Park), which is indicated as Others vs. Others. Pairwise comparisons were made between Rondeau Provincial Park and all other study sites, which is indicated as Others vs. Rondeau. Samples were collected during the 2002-2004 field seasons.	
<i>Figure 2.9</i> .....	39
Pairwise $F_{ST}$ comparisons between locations in relation to geographic distance for northern flying squirrels ( <i>Glaucomys sabrinus</i> ) at five sites in Ontario, Canada (Aurora District, Bruce.Grey Counties, Highway 60 in Algonquin Provincial Park, Peterborough County, and Temagami). Pairwise comparisons were made between all study sites (excluding Bruce and Grey Counties), which is indicated as Others vs. Others. Pairwise comparisons were made between Bruce and Grey Counties and all other study sites, which is indicated as Others vs. Bruce and Grey Counties. Samples were collected during the 2002-2004 field seasons.	

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## **Chapter 1**

### **General Introduction**

In the last half of the 20<sup>th</sup> century there has been a period of rapid global warming that has occurred (Bradshaw and Holzapfel, 2001). Climate change at both global and regional scales is predicted to alter life histories, species distribution, and ecosystem functions (McLaughlin et al., 2002). Evidence suggests that the geographic ranges of many species are shifting due to climate warming (McLaughlin et al., 2002). Global warming also has promoted an earlier arrival of spring and longer growing seasons which can change seasonal patterns and biotic interactions of many species (Bradshaw and Holzapfel, 2001). As a result, it is essential to understand the dispersal patterns of plants and animals, in order to predict the effects that factors such as fragmentation and global climate change will have on natural ecosystems (Gaggiotti et al., 2004).

Understanding the processes and patterns of gene flow and local adaptations requires knowledge of how factors such as climate and landscape structure affect populations (Manel et al., 2003). The field of landscape genetics is a combination of landscape ecology and population genetics and can be used to identify cryptic boundaries, isolation by distance, and metapopulations (Manel et al., 2003). Landscape genetics can also resolve population substructure across different geographical scales and therefore is different from biogeography, which focuses mainly on species diversity patterns at broad temporal and spatial scales (Smouse and Peakall, 1999; Manel et al., 2003).

The type of range boundary shifts predicted from climate change can be considered a case of metapopulation dynamics. This metapopulation approach tends to

view populations as discrete entities by looking at how they interact via migration and gene flow (Hanski and Gaggiotti et al., 2004). It predicts regional persistence if local extinctions are compensated by recolonisation (Schweiger et al., 2004). In the case when geographic ranges are expanding, colonisation exceeds extinction (Gaggiotti et al., 2004). A fundamental process of metapopulation biology is colonisation of migrants into new areas (Gaggiotti et al., 2004). This can be tested a number of ways either using ecological or population genetic approaches. The mark-release-recapture method may be one way to look at metapopulation dynamics, however a potential problem with this approach is that it requires a great deal of time and cost and cannot easily be applied to investigate large extended populations (Gaggiotti et al., 2004). On the other hand population genetic approaches tend to be easier to implement, as they typically require a sampling strategy for collection of samples (i.e., tissue or hair) for DNA extraction and analysis (Gaggiotti et al., 2004). My objective in this chapter is to provide an introduction to genetic techniques used for testing for range expansion.

There are a number of ways a population can expand or contract. In the case of range expansion, a source population may expand into many smaller founding populations where either the founding populations continue to exchange genetic material with each other or the source population or the source population may expand its range where the individuals within the expansion are exchanging genetic material. In these two cases, it would be probable that high levels of gene flow would be maintained. Another case would be if the founding populations became isolated from each other and from their source population which, over time may lead to the process known as isolation by distance (Wright, 1943).

Isolation by distance occurs “when genetic differentiation between individuals (or populations) increases with geographical distance (because gene flow declines at larger distances)” (Manel et al., 2003) or “the accumulation of genetic divergence among populations under geographically restricted dispersal” (Kuchta and Tan, 2004). Some species would be affected by this pattern more than other species. For example, species with slow dispersal rates and slow reproductive rates may be more prone to display patterns of isolation by distance than species with fast dispersal rates and high reproductive rates.

There have been a number of studies that have tested for range expansion and, isolation by distance. Leblois et al. (2000) used seven microsatellite markers to test for isolation by distance in an expanding population of cane toad (*Bufo marinus*). This species was introduced into Australia in 1935 and had spread rapidly (Leblois et al., 2000). They found that the rapid range expansion of the cane toad suggested that the species, as a result of being very mobile, might exhibit large amount of gene flow between its populations thereby reducing the rate of genetic differentiation that may occur in each population (Leblois et al., 2000).

Estoup and Clegg (2003) used Bayesian inference to investigate the recent colonisation history of the bird *Zosterops lateralis lateralis*. Their demographic model had three parameters, which they assumed were the same for each of the four islands observed. They also assumed that new island populations evolved isolated demes after colonisation. For each pair of populations they calculated the mean number of different alleles, the mean genetic diversity, the mean of the variance in repeat number computed across loci in both ancestral and source populations, and  $F_{ST}$ . They concluded that no

single founder event produced any significant reduction in allelic diversity. The Bayesian analysis of the colonisation of this species indicated that a large number of effective founders were involved in establishing three of the four populations, whereas the fourth population was found to have fewer effective founders.

### **Analytical Software and Statistics**

There are a number of computer programs available that use multilocus genotype data to assess population structure. To determine whether there is range expansion, multilocus genotype data need to be analysed using software programs (e.g., STRUCTURE) that will cluster individuals together into populations. Once these populations have been determined, it is possible to see whether individuals from one population are expanding in a particular direction into another population.

The STRUCTURE program of Pritchard et al. (2000) is a model-based clustering method used to infer population structure and to assign individuals to a particular population ( $K$ ) using multilocus genotype data. The applications of this method are (1) to detect the presence of population structure, (2) to assign individuals to a population, (3) to study hybrid zones, and (4) to identify migrants and admixed individuals. This method assumes that the markers being used are unlinked and at linkage equilibrium with one another within populations and that populations are in Hardy-Weinberg Equilibrium (HWE).

There are also a number of statistical methods used in testing for range expansion. A common statistical method is Mantel's test, which can detect the presence of isolation by distance between individuals (Manel et al., 2003). This test measures the association

between two matrices, such as genetic and geographic distance. Also there are Bayesian clustering methods that attempt to group individuals into populations of randomly mating individuals that minimise HWE and gametic disequilibrium (Manel et al., 2003).

### **Purpose of Study**

The purpose of this study was to use genetic techniques to test for the northern range expansion by southern flying squirrels (*Glaucomys volans*) in Ontario using seven microsatellite loci. I also undertook a comparative study of the genetic structure of sympatric northern flying squirrels (*G. sabrinus*). I hypothesised that if the range of southern flying squirrels had expanded rapidly and recently then there would be little genetic differentiation detected among the expanded population. Conversely, as there is no evidence to suggest that northern flying squirrels are expanding their range in Ontario, I expected that this species would show genetic structure due to landscape fragmentation and isolation by distance as a result of restricted dispersal.

## Chapter 2

### **A genetic test of range expansion by the southern flying squirrel (*Glaucomys volans*) at its northern range boundary**

#### **Introduction**

The demography of populations fluctuates over time. Biotic factors such as competition and predation and abiotic factors such as climate or nest availability can cause these fluctuations (MacArthur, 1972; Brown, 1995). Knowledge of processes at a species range boundary can be an important component of conservation and management decisions.

There are a number of ways a species range can expand or contract (Figure 2.1). One scenario involves the source population expanding into smaller founding populations (Figure 2.1a). Once the founding populations have become established several outcomes can occur; (1) the founding populations, although smaller than the source population, still exchange genetic material with each other and with the source population; or (2) some or all of the founding populations may become isolated from each other and from the source population such that a stepping stone pattern occurs and the process of isolation by distance is detected, where the greatest genetic differentiation exists between the source population and the most recent founding population. When a range is expanding not all of the individuals in the population will be expanding with it. As the range increases the result may be isolation by distance where fragmented haplotype structure and evidence of founding events may be evident (DeYoung et al., 2003). An increase in genetic differentiation is expected if the founding populations are not closely connected to the

source population by gene flow and if the sampling of the population includes a large proportion of recently founded individuals (Leblois et al., 2000).

A second scenario is that the source population expands without fragmenting into smaller populations (Figure 2.1b). In this scenario the source population continues to expand its range where there are high levels of gene flow among the individuals within the source population. This scenario may show the process of isolation by distance over time where again the individuals on the front of the expansion may become genetically differentiated from the core of the source population. I suspect that the process of isolation by distance occurring within this scenario would not happen as quickly as the source population is expanding its range instead of founders moving away from the source population.

There are two species of flying squirrels in Ontario, the northern flying squirrel (*Glaucomys sabrinus*) and the southern flying squirrel (*G. volans*). Both are small, nocturnal, non-hibernating mammals that are morphologically very similar and highly specialised for gliding locomotion (Weigl et al., 1978). Both species are believed to be marginally sympatric in central Ontario (Bowman et al., 2005). It has been observed that home range sizes vary and that males of both species have larger home ranges than female flying squirrels (Martin and Anthony, 1999; Hanski et al., 2000; Taulman and Smith, 2004). Although arboreal, flying squirrels are quite mobile. For example, northern flying squirrels have been documented to move an average of 71 metres between successive daily telemetry locations in various areas in Oregon, USA (Martin and Anthony, 1999). Sonenshine et al. (1979) reported southern flying squirrels in Virginia, USA to travel as much as 560 metres and 792 metres in one night while moving

between study areas. The Siberian flying squirrel (*Petermoys volans*) has been documented to disperse up to 9 km with a dispersal period lasting between one night and a few nights depending on how long it takes the disperser to settle into the new area it will occupy for the next winter (Jokinen, 2000; Selonen and Hanski, 2004). Radio-collared southern flying squirrels returned home within one day following translocations of about 1 km in central Ontario (Jeff Bowman, unpubl. data).

It had been thought that the southern flying squirrel's range is limited to the north by temperature and that based on thermal limitations, the species does not persist above 45° N latitude (Stapp et al., 1991). However, Bowman et al. (2005) found southern flying squirrels occurred north of this latitude, and estimated that their range had expanded by 200 km in nine years (1995 to 2003) in response to recent climate warming. They also observed that their northern range boundary had contracted by approximately 240 km after the winter of 2004, and suggested that this was due to an energetic bottleneck caused by food and weather conditions. There were very cold temperatures in January and February of 2004, along with a failed mast crop in the autumn of 2003 that resulted in an energetic bottleneck, subsequent population crash, and range contraction of southern flying squirrels (Bowman et al., 2005).

The purpose of my study was to use genetic techniques to test for the rapid range expansion by southern flying squirrels within Ontario that was predicted by Bowman et al. (2005). For comparative purposes, I also determined the genetic structure of sympatric northern flying squirrels. Genetic samples were collected from various regions within Ontario along the northern range boundary of southern flying squirrels and within the zone of sympatry between the two *Glaucomys* species. If southern flying squirrels have



undergone a rapid population expansion, with large numbers of migrants colonizing the north of their range during 1995 to 2003, then there should be a lack of genetic structure at the range boundary. In other words, I expected to find a pattern of genetic structure in southern flying squirrels populations consistent with the model in Figure 2.1b where the source population is expanding and the individuals that are expanding with it are exchanging genetic material thereby maintaining gene flow. There has been no evidence in the literature to suggest that the range of northern flying squirrels has recently expanded in Ontario therefore I expected to find some levels of genetic structure for this species influenced by landscape fragmentation and restricted dispersal. Flying squirrels are primarily arboreal and rely on trees for food, travel, and refugia (Taulman and Smith 2004). Therefore, I expected that whereas there may be some movement of individuals between study sites (if forest corridors permitted movement), the number of migrants would not be sufficient to maintain levels of gene flow that would homogenize genetic structure between study sites.

## **Materials and Methods**

### **Sampling Techniques**

Both southern and northern flying squirrels were livetrapped from various locations within Ontario, Canada (Figure 2.2). Livetrapping was undertaken during May to October 2002 to 2004. Most flying squirrels were trapped using Tomahawk 102 live-traps that were baited using a mixture of peanut butter, molasses and oatmeal. Flying squirrels were trapped using either Sherman 13 cm x 13 cm x 38 cm or 8 cm x 9 cm x 23 cm live traps. Traps were baited between 15:00 and 18:00 and checked between 7:00 and

10:00. Trapped squirrels were released into a clear plastic bag and weighed using a Pesola scale. Once weighed, 3 plucks of hair (approximately 5-15 hairs from each pluck) were taken from the tail and put on a hair collection kit provided by the Natural Resources DNA Profiling and Forensics Centre (NRDPFC) at Trent University. Prior to release, an ear tag was put into the ear of the flying squirrel using a National Band and Tag Company 1-g Monel ear tag and the number recorded. Once the ear tag was in place the squirrel was released at the capture site. The ear tag number was recorded on the hair collection kit as well as latitude and longitude values. Either the ear tag number or the trap number was used to identify individuals in this study. Some hair samples were also obtained from a contemporaneous nest box study in Bruce and Grey counties of Ontario (Steve Patterson, unpubl.data).

### **Sample collection and DNA extraction**

Hair samples were collected and profiled from 161 individual southern (N=90) and northern (N=71) flying squirrels from across Ontario during 2002-2004.

DNA was extracted from approximately 20-30 hairs, when available, using a Qiagen DNeasy tissue kit protocol (Qiagen Inc., USA). The hair was suspended in 500 $\mu$ L of 1X lysis buffer (4 M urea, 0.2 M NaCl, 0.5% n-lauroyl sarcosine, 10 mM CDTA (1,2 cyclohexanediamine), 0.1 M Tris-HCL (pH 8.0) (Applied Biosystems Inc., USA), treated with 15 units of proteinase K (Qiagen Inc., USA) and incubated at 37 °C for 12 hours. Samples were then extracted following the DNeasy tissue kit protocol until the final step when the samples were eluted with 70 °C T.E. 0.1 (1.0M Tris-HCl, 0.5M EDTA) rather than the Buffer AE (Qiagen Inc., USA).

### **Genotyping of Microsatellite Loci**

Seven microsatellite loci, GS2, GS4, GS8, GS10, GS13 (Zittlau et al., 2000), SFS03, and SFS14 (Fokidis et al., 2003) were amplified by the polymerase chain reaction (PCR) using fluorescently labelled (NED, 6 FAM, and HEX) primers.

The seven loci were amplified in three multiplex PCRs per individual. Amplifications were performed in 10 $\mu$ L volumes containing 3 $\mu$ L of stock DNA, 0.2mM of each dNTP, 1X Qiagen PCR Buffer, 0.3-0.5  $\mu$ M of forward and reverse primer, and 0.5 units of *Taq* polymerase (Qiagen Inc., USA). All samples were amplified under the following conditions: 94°C for 5 min., 30 cycles of 94°C for 30 sec., 60°C for 45 sec., 74°C for 30 sec., and 65°C for 45 min. Multiplex reactions were implemented to reduce the number of PCR reactions.

Multiplex products were added together in two poolings and desalted through sephadex columns. Each pooling was visualised on a MegaBACE 1000 automated genotyper (Amersham BioSciences Limited, USA), with a size standard (ET-Rox 550; Applied Biosystems, USA) run with each sample to determine base pair length. Genotypes, which were characterised as alleles, were scored manually using the Genetic Profiler® v2.2 software package (Amersham BioSciences).

### **Testing for range expansion**

Deviations from Hardy-Weinberg equilibrium were evaluated for each locus and population using an exact probability test in GENEPOP 3.4 (Raymond and Rousset,

1995). Tests were adjusted for multiple comparisons using a Bonferroni correction ( $\alpha = 0.050/5 \text{ tests} = 0.010$ ) for each species.

Factorial correspondence analysis (FCA)(GENETIX 4.0.3; Belkhir et al., 1999) was used to assess the genetic structure of both southern and northern flying squirrels in the study area. FCA uses contingency tables to find similarities by observing the allele frequencies between individuals and therefore can group individuals whose genotypes are similar regardless of their sampling location.

A Bayesian assignment test (STRUCTURE version 2.1; Pritchard et al., 2000; Falush et al., 2003) was used to identify genetic structure and to assign individuals to their likely population of origin. As with the FCA, STRUCTURE groups individuals without any prior knowledge of their location. The major difference between FCA and STRUCTURE is that FCA strictly looks at allele frequencies without using Hardy-Weinberg assumptions and STRUCTURE uses Hardy-Weinberg assumptions when grouping individuals. The results from STRUCTURE were based on three independent runs simulating one to ten ( $K=1-10$ ) inferred genetic population groups, using a 50,000 burn-in period and 200,000 iterations of a Markov chain Monte Carlo simulation. The simulation models were run with no prior information and correlated allele frequencies were assumed.

Genetic differentiation among individuals was estimated by calculating pairwise  $F_{ST}$  (GENETIX 4.0.3; Belkhir et al., 1999). Potential patterns of isolation by distance were assessed by performing a Mantel test for both species using the software GENALEX 6 (Peakall and Smouse, 2006) for individual-based pairwise comparisons.

The relationship between  $F_{ST}$  and distance was determined using the software and SPAGeDi (Hardy and Vekemans, 2004) for pairwise comparisons among study sites.

## Results

All populations, except Mattawa were in Hardy-Weinberg equilibrium for southern flying squirrels as were all northern flying squirrel populations. The seven loci used were moderately variable ranging from three to twelve alleles for northern flying squirrels and nine to eleven for southern flying squirrels. Table 2.1 summarises the mean heterozygosity ( $H_E$ ) and mean homozygosity ( $H_O$ ) for all study sites along with sample sizes for each study site.

One genetic population was suggested by the factorial correspondence analysis (FCA) for each species (Figures 2.3 and 2.4). For southern flying squirrels five outliers were detected and three outliers were detected for northern flying squirrels. In this study outliers are defined as data points outside of the cloud that have a visual gap between the outlier data point and the rest of the cloud. Outliers were defined visually and without statistical analysis.

The Bayesian assignment approach confirmed the presence of one genetic population ( $K = 1$ ) for each species by observing the mean  $\text{LnP}(D)$  values. STRUCTURE is similar to the factorial correspondence analysis in that it implements a clustering method using multilocus genotype data that infers population structure and groups independently of their sampling location.

Weir and Cockerham's pairwise  $F_{ST}$  was performed using the program GENETIX for both species. The degree of genetic differentiation between sampling locations

ranged from -0.017 to 0.061 for southern flying squirrels (Table 2.2) and -0.022 and 0.090 for northern flying squirrels (Table 2.3), indicating high levels of gene flow among sampling sites for both species. Although a  $F_{ST}$  value of 0.090 indicates a moderate level of genetic structure, this result was found once between Bruce/Grey Counties and Peterborough County. The other  $F_{ST}$  values were quite low indicating high levels of gene flow.

For southern flying squirrels, there were two groups along the geographical axes of the Mantel test (Figure 2.5). The farthest group was due to pairwise comparisons with Rondeau Provincial Park, which was quite far from the other sites. The Mantel test demonstrated some isolation by distance, ( $R^2=0.039$ ,  $p=0.010$ ). I reanalysed this relationship after removing Rondeau samples (Figure 2.6) to determine whether Rondeau Provincial Park was causing the second group and found a lack of isolation by distance ( $R^2=0.000$ ,  $p=0.260$ ). A lack of isolation by distance was also detected for northern flying squirrels ( $R^2=0.003$ ,  $p=0.040$ ) (Figure 2.7).

$F_{ST}$  in relation to geographical distance showed evidence of some genetic differentiation between Rondeau Provincial Park samples and other samples from the study sites for southern flying squirrels (Figure 2.8) based on pairwise comparisons between locations. Although one pairwise comparison between Bruce/Grey Counties and Peterborough indicated genetic structure, overall the data do not indicate that Bruce/Grey Counties showed genetic differentiation from the other central Ontario sites for northern flying squirrels (Figure 2.9).

## Discussion

The individual based assignment test identified one discrete genetic population for each species within the study area. These results were also observed from the factorial correspondence analysis. The five outliers found in the southern flying squirrel grouping and three in the northern flying squirrel grouping were not all from the same study areas and therefore it is difficult to determine the cause of these outliers. It may be that these individuals appear differentiated due to chance, or they may be immigrants from outside the study area.

Pairwise  $F_{ST}$  values for both species indicated that there was little to no genetic differentiation. In other words, there was high gene flow among sampling sites for both species, with  $F_{ST}$  values ranging from -0.017 to 0.061 for southern flying squirrels. A similar result was found by Bednarczuk (2003) who found  $F_{ST}$  values between Norfolk County and the Minden area of Ontario to be 0.010. Pairwise comparisons between Dorset and Rondeau Provincial Park did show some moderate level of genetic differentiation ( $F_{ST} = 0.061$ ), (Hartl and Clark, 1997). This would be expected due to the geographical distance between these two study sites. However it was still unexpected that the rest of the study sites did not show any genetic differentiation from the Rondeau Provincial Park samples.  $F_{ST}$  values ranged from -0.022 to 0.054 for northern flying squirrels. A pairwise  $F_{ST}$  value between Aurora and Temagami was 0.054, indicating that the comparison showed little to moderate genetic differentiation (Hartl and Clark, 1997). I expected  $F_{ST}$  values  $\geq 0.050$  between Bruce/ Grey County samples and all other study sites, indicative of a higher level of genetic differentiation due to the assumed geographical isolation.

The Mantel tests for isolation by distance indicated that neither species showed any genetic structure that would suggest the process of isolation by distance. However, Rondeau Provincial Park may be starting to show some genetic differentiation due to either geographical distance from the other study sites or due to the isolation of Rondeau Provincial Park. Based on pairwise comparisons of individuals across sampling sites, individuals from all sampling sites were genetically very similar to one another, again suggesting high levels of gene flow among sample sites. When Rondeau Provincial Park was kept in the data set for the Mantel test (Figure 2.5), there was a slight positive slope to the regression, however less than one might expect due to the geographical distance. The relationship between  $F_{ST}$  and the distance showed that there were some genetic differences between samples from Rondeau Provincial Park and other sites in our study area for the southern flying squirrel (Figure 2.8). A similar result was detected for one pairwise comparison between Bruce/Grey Counties and Peterborough for northern flying squirrels, however the overall data does not suggest that the northern flying squirrels in Bruce/Grey Counties are showing genetic differentiation compared to other study sites within central Ontario. For both species the range of genetic distance was relatively the same regardless geographic distance (Figures 2.5-2.7).

The northern flying squirrel showed high gene flow and the process of isolation by distance was not detected. This was contrary to my prediction, as I expected to find some evidence of isolation by distance, particularly with the northern flying squirrels in Bruce and Grey Counties due to their geographic distance from the other sampling sites and their potential isolation. The results may indicate one of two things (1) the range boundary of northern flying squirrels is expanding or (2) a lack of isolation by distance



might be due to the northern flying squirrels having a large effective population size, where the population is large enough that genetic drift and founder effects do not shift the population structure away from a neighbouring one even in the absence of gene flow (Kyle and Strobeck, 2003).

Bowman et al. (2005) suggested, based on field observations and mast and temperature data that southern flying squirrels went through a recent range expansion in Ontario. The high levels of gene flow among individual sampling sites supported the prediction that there would be no genetic structure detected during a recent and rapid range expansion. However the data do not support or refute the hypothesis that a range expansion occurred by the southern flying squirrel, given the contradictory findings concerning apparent gene flow of northern flying squirrels.

Assuming that the  $F_{ST}$  and STRUCTURE results are not indicative of only effective population size but also indicative of some level of contemporary gene flow, both species appear to be highly vagile. Not only do southern flying squirrels appear to move quickly, but they also have a high reproductive rate, providing ample opportunity for the species to thrive in their new environment, if suitable. The high level of gene flow noticed in the northern flying squirrel was more surprising as there has been no indication in the literature that Ontario populations of this species have recently expanded, and therefore some genetic differentiation was expected.

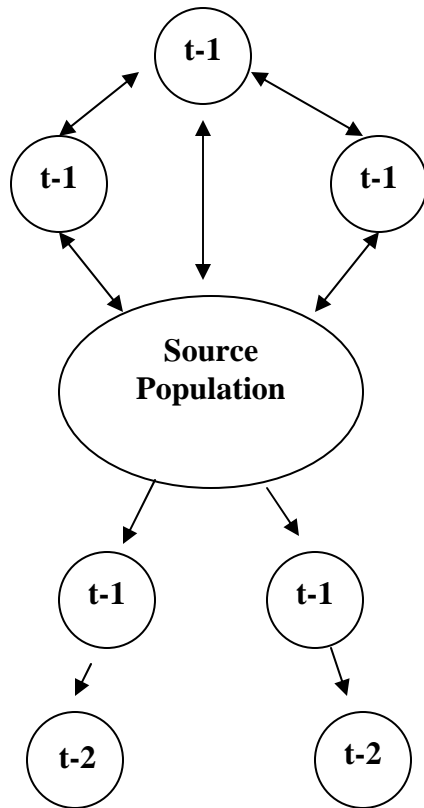
Due to the potentially high effective population size and the reproductive rate of flying squirrels, it is expected that the similar genetic signatures of the flying squirrels in both Rondeau Provincial Park for southern flying squirrels and Bruce and Grey Counties for the northern flying squirrels compared to central Ontario may be due to large effective

population sizes in these locations. If that is true, then it appears that their ecology (i.e., their high reproductive rate and mobility) sustains the high level of genetic diversity despite being geographically isolated. If I were to assume that my results are entirely due to a large effective population size, I can make little inference about contemporary gene flow. Instead I would conclude that effective population sizes in the study sites were large, even in isolated and fragmented landscapes such as those in Bruce and Grey Counties, Rondeau Provincial Park, Aurora District, and Peterborough County.

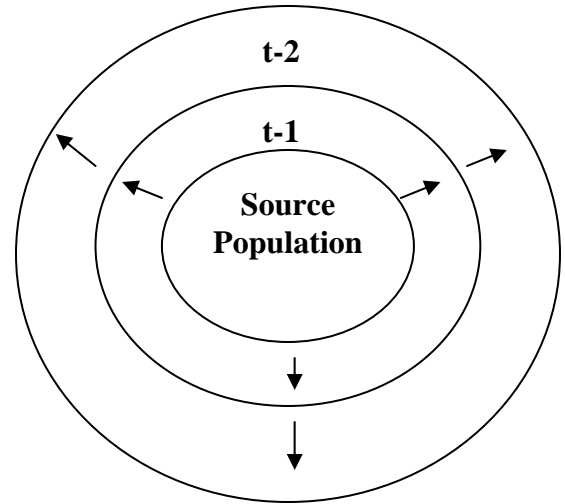
It is most likely that there is some divergence in Rondeau Provincial Park for the southern flying squirrel, which was apparent comparing  $F_{ST}$  and distance (Figure 2.8). This pattern is not as evident for the northern flying squirrels (Figure 2.9). It is likely that this divergence for southern flying squirrels is due to landscape fragmentation and distance. However, it does appear that both species are vagile. In particular, the northern flying squirrel appears more vagile than expected. Perhaps due to their high reproductive rates and in some areas large effective population sizes, flying squirrels are able to maintain high levels of gene diversity in areas that are assumed to be geographically isolated.

Figure 2.1: Schematic of possible range expansion scenarios; a) source population breaks up into smaller founding populations and either maintains gene flow with each other and the source population or the founding populations become isolated over time and show a pattern of isolation by distance and b) source population continues to expand outwards where the individuals that are expanding are maintaining gene flow with each other.

Founding populations maintain gene flow with source population.



Source population expands its range over time.



Founding populations becoming isolated from source population and potentially may show patterns of isolation by distance over time.

a

b

Figure 2.2: Map of sites where southern flying squirrels and northern flying squirrels were sampled in Ontario, Canada during 2002 to 2004. The sites are Rondeau Provincial Park (Clear Creek Forest) (1), Bruce and Grey Counties (2), Aurora MNR District (3), Peterborough (4), Kawartha Highlands (5), Leslie M. Frost Centre near Dorset (6), Algonquin Provincial Park (7), near Mattawa (8), and near Temagami (9). Locations shown on map are centroids of sampled areas.

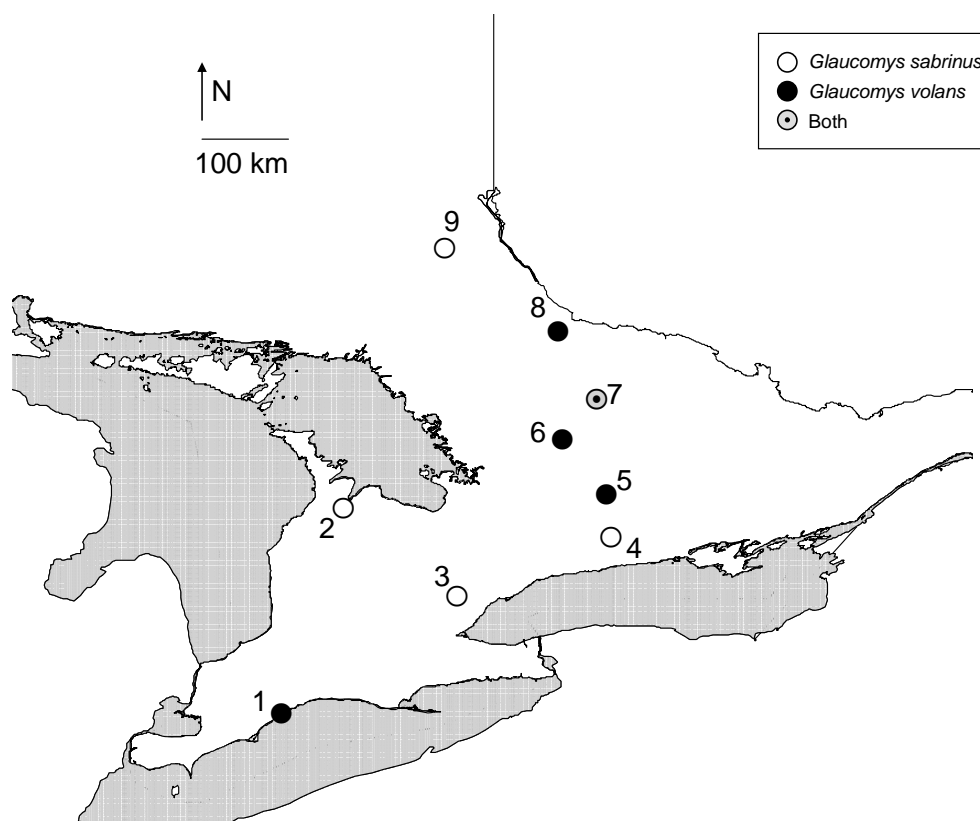


Figure 2.3: Factorial correspondence analysis for southern flying squirrels (*Glaucomys volans*) at five sites in Ontario, Canada. Samples were collected during the 2002-2004 field seasons. Outliers include A = ID# 022 Rondeau Provincial Park, B = ID#1904 Kawartha Highlands, C = ID# 625 Dorset, D = ID# 049 Kawartha Highlands, and E = ID# NA Algonquin Provincial Park. Each location was given a colour and plotted as such in the cloud.

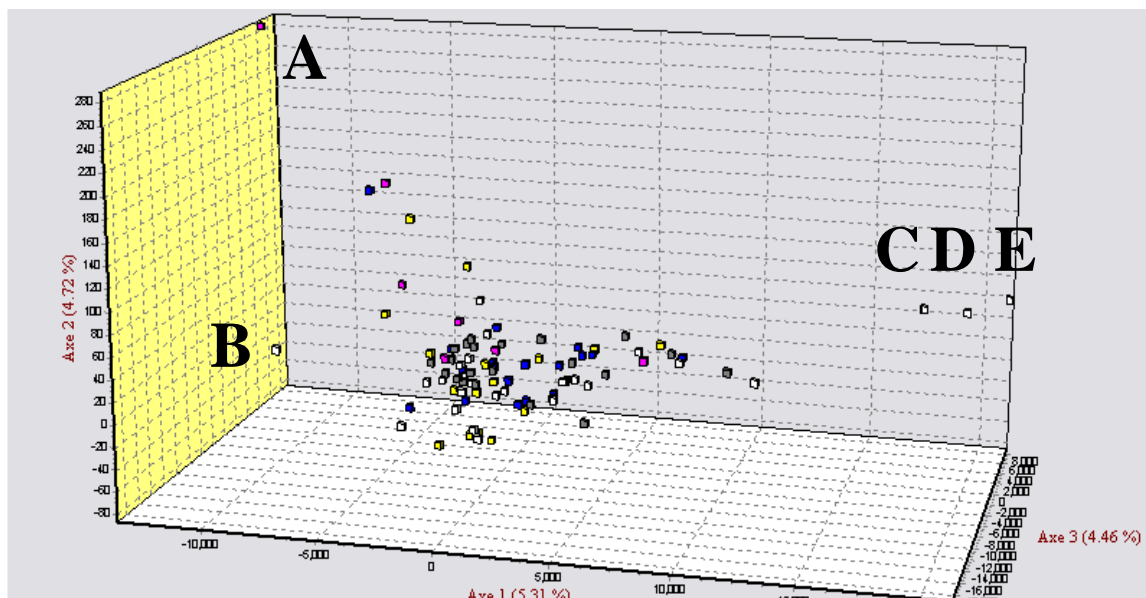




Figure 2.4: Factorial correspondence analysis for northern flying squirrels (*Glaucomys sabrinus*) at five sites in Ontario, Canada. Samples were collected during the 2002-2004 field seasons. Outliers include A = ID#GS04E Bruce/ Grey Counties B = ID#625 Algonquin Provincial Park, and C = ID#151.287 Algonquin Provincial Park. Each location was given a colour and plotted as such in the cloud.

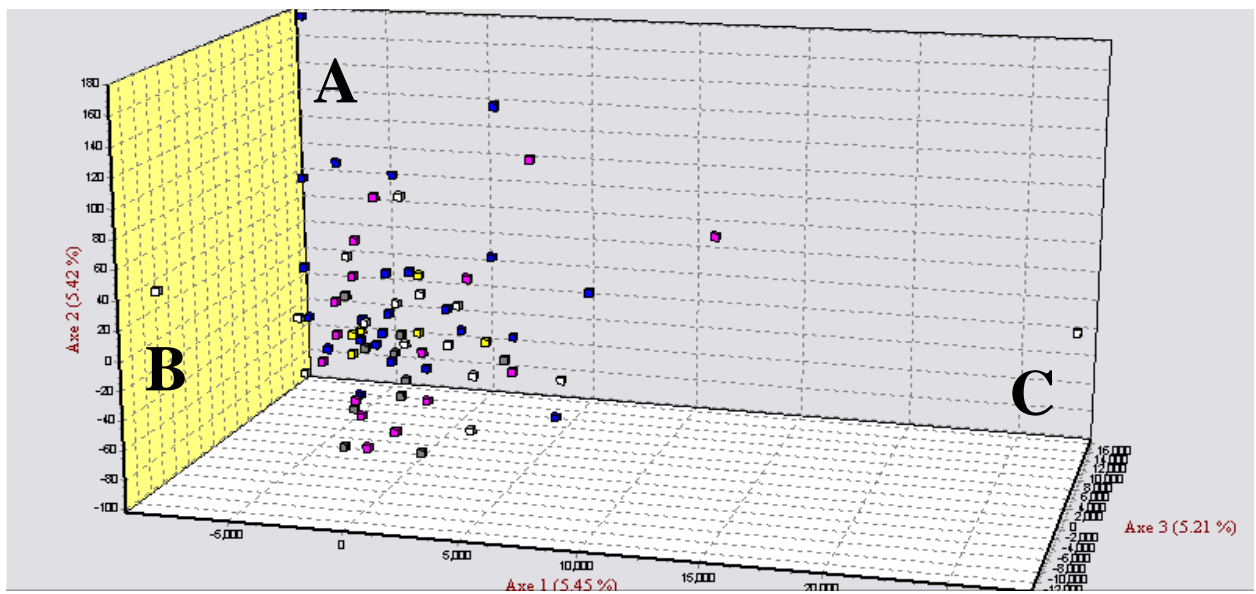


Table 2.1: Summary of the number of individual southern (*Glaucomys volans*) and northern (*Glaucomys sabrinus*) flying squirrels profiled at each of the study sites in Ontario Canada with both mean observed ( $H_O$ ) and mean expected ( $H_E$ ) heterozygosity for each location. Study sites in bold font indicate sites for southern flying squirrel sites in regular font indicate northern flying squirrel sites.

<b>Study Sites</b>	<b>N</b>	<b><math>H_O</math></b>	<b><math>H_E</math></b>
<b>Dorset</b>	<b>17</b>	<b>0.641</b>	<b>0.660</b>
<b>Kawartha Highlands</b>	<b>27</b>	<b>0.701</b>	<b>0.704</b>
<b>Algonquin Provincial Park</b>	<b>18</b>	<b>0.620</b>	<b>0.698</b>
<b>Mattawa</b>	<b>21</b>	<b>0.683</b>	<b>0.665</b>
<b>Rondeau Provincial Park</b>	<b>7</b>	<b>0.553</b>	<b>0.657</b>
Aurora District	6	0.666	0.663
Bruce and Grey County	24	0.750	0.751
Algonquin Provincial Park	15	0.774	0.783
Peterborough County	10	0.717	0.709
Temagami	16	0.750	0.769

Table 2.2: Pairwise  $F_{st}$  values estimated using microsatellite data for southern flying squirrels (*Glaucomys volans*) at five sites within Ontario, Canada. DOR = Dorset, KAW = Kawartha Highlands, HSIX = Hwy 60 in Algonquin Provincial Park, MAT = Mattawa, and RON = Rondeau Provincial Park.

	HSIX	KAW	MAT	RON
DOR	0.010	0.008	0.022	0.061
HSIX		-0.000	-0.017	0.025
KAW			0.001	0.042
MAT				0.030
RON				

Table 2.3: Pairwise  $F_{st}$  values estimated using microsatellite data for northern flying squirrels (*Glaucomys sabrinus*) at five sites within Ontario, Canada. AUR = Aurora District, BGC = Bruce/Grey Counties, HSIX = Hwy 60 in Algonquin Provincial Park, PBO = Peterborough County, and TEM = Temagami.

	BGC	HSIX	PBO	TEM
AUR	0.013	0.006	-0.012	0.054
BGC		0.027	0.090	0.039
HSIX			-0.015	-0.022
PBO				0.026
TEM				

Figure 2.5: Mantel isolation by distance for southern flying squirrels (*Glaucomys volans*) at five sites in Ontario, Canada (Dorset, Kawartha Highlands, Highway 60 in Algonquin Provincial Park, Mattawa, and Rondeau Provincial Park). Samples were collected during the 2002-2004 field seasons.

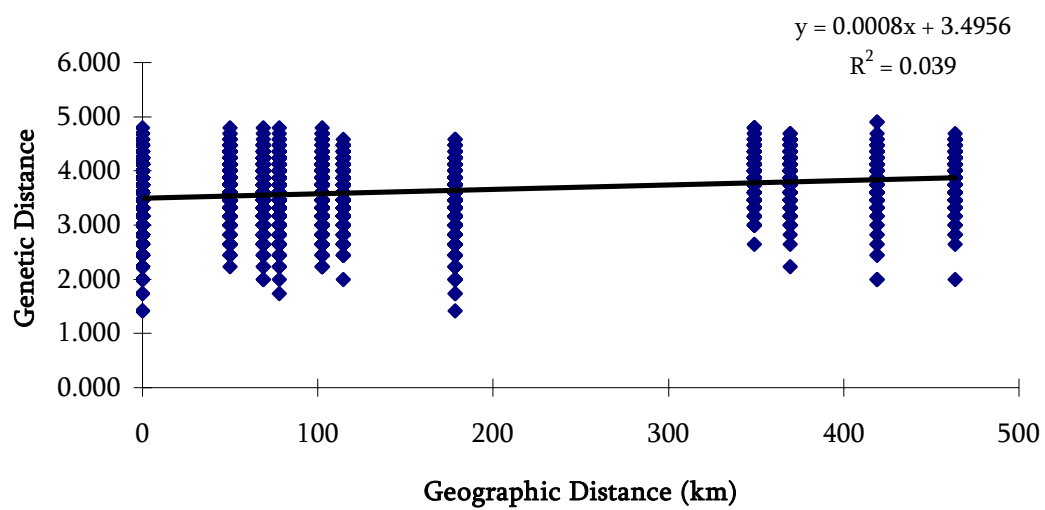


Figure 2.6: Mantel isolation by distance for southern flying squirrels (*Glaucomys volans*) at four sites in Ontario, Canada (Dorset, Kawartha Highlands, Highway 60 in Algonquin Provincial Park, and Mattawa). Samples were collected during the 2002-2004 field seasons.



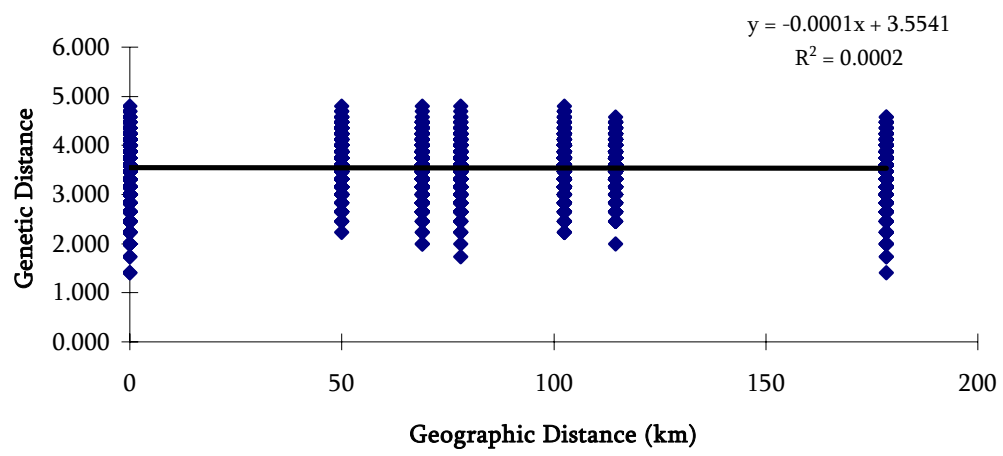


Figure 2.7: Mantel isolation by distance for northern flying squirrels (*Glaucomys sabrinus*) at five sites in Ontario, Canada (Aurora District, Bruce.Grey Counties, Highway 60 in Algonquin Provincial Park, Peterborough County, and Temagami). Samples were collected during the 2002-2004 field seasons.

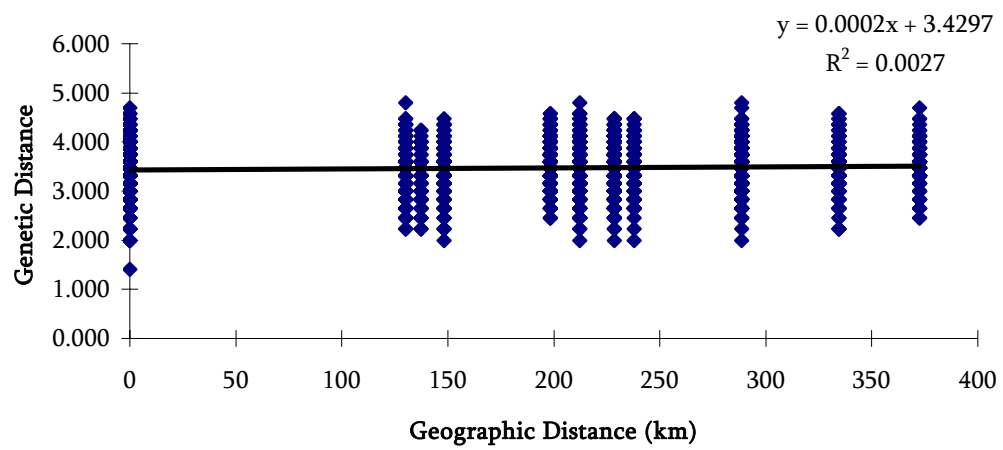


Figure 2.8: Pairwise  $F_{ST}$  comparisons between locations in relation to geographic distance for southern flying squirrels (*Glaucomys volans*) at five sites in Ontario, Canada (Dorset, Kawartha Highlands, Algonquin Provincial Park, Mattawa, and Rondeau Provincial Park). Pairwise comparisons were made between all study sites (excluding Rondeau Provincial Park), which is indicated as Others vs. Others. Pairwise comparisons were made between Rondeau Provincial Park and all other study sites, which are indicated as Others vs. Rondeau. Samples were collected during the 2002-2004 field seasons.

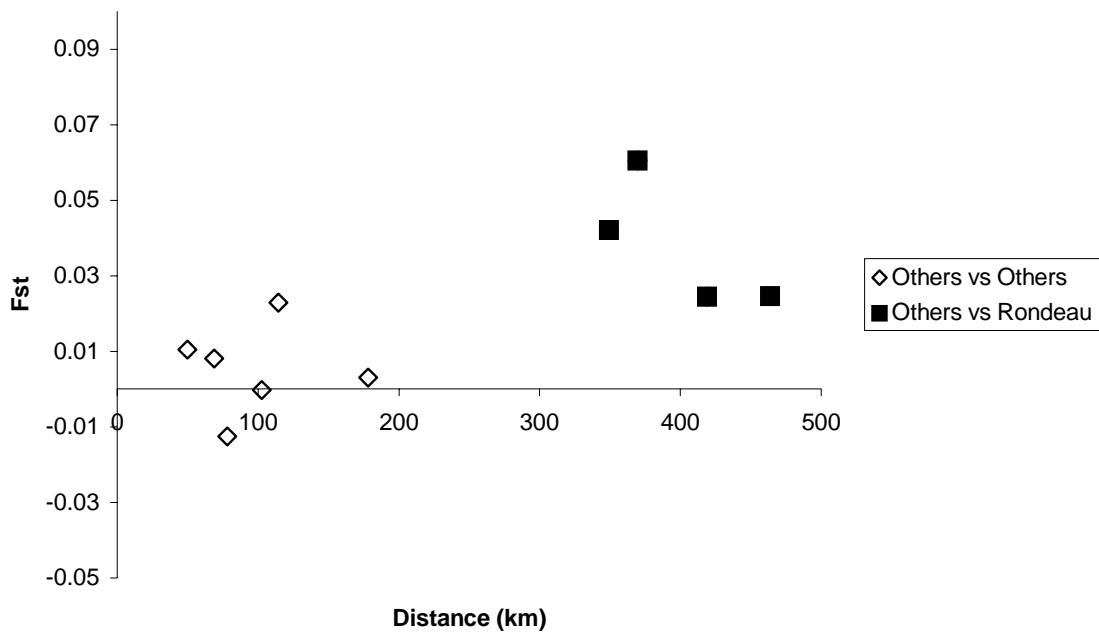
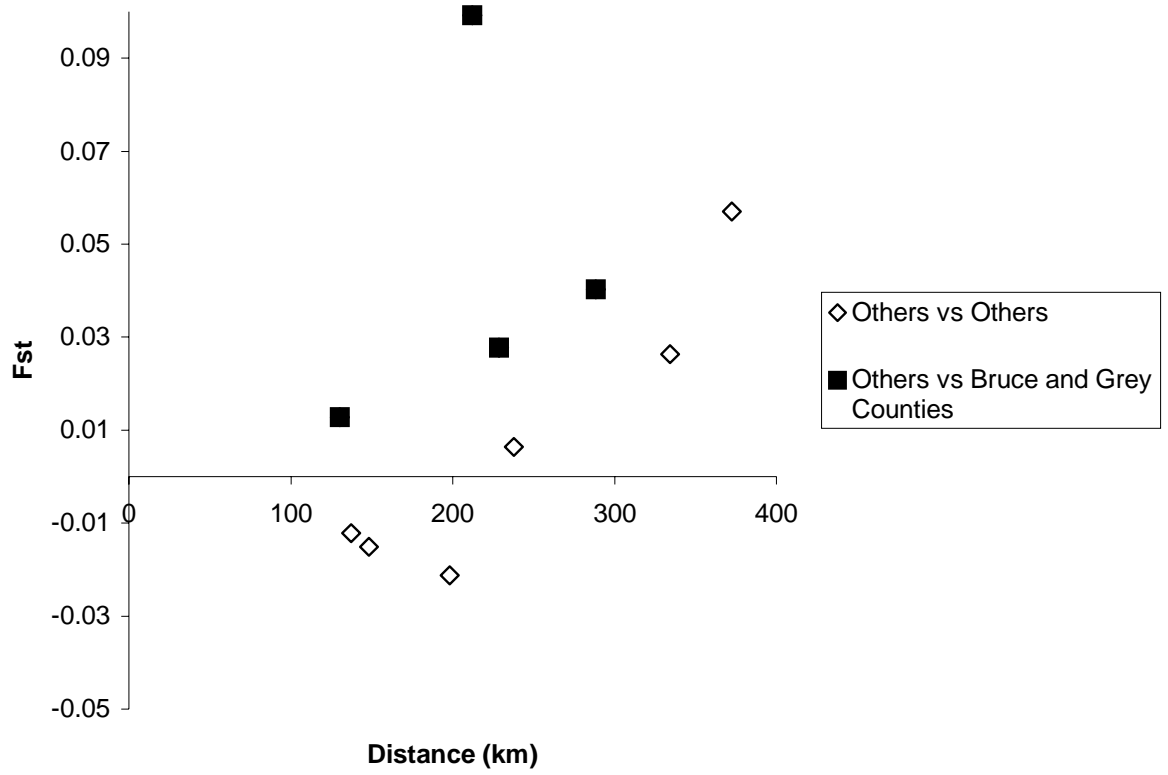


Figure 2.9: Pairwise  $F_{ST}$  comparisons between locations in relation to geographic distance for northern flying squirrels (*Glaucomys sabrinus*) at five sites in Ontario, Canada (Aurora District, Bruce and Grey Counties, Highway 60 in Algonquin Provincial Park, Peterborough County, and Temagami). Pairwise comparisons were made between all study sites (excluding Bruce and Grey Counties), which is indicated as Others vs. Others. Pairwise comparisons were made between Bruce and Grey Counties and all other study sites, which is indicated as Others vs. Bruce and Grey Counties. Samples were collected during the 2002-2004 field seasons.



### Chapter 3 General Discussion

Both southern and northern flying squirrels had very similar genetic structure such that there were high levels of gene flow between sample sites and each species grouped together into a single inferred genetic population ( $K = 1$ ). There was evidence of weak isolation by distance for southern flying squirrels, when Rondeau Provincial Park samples were in the data set, and a lack of isolation by distance for northern flying squirrels. Some genetic differentiation was detected between Rondeau Provincial Park samples and the other southern flying squirrel samples when using pairwise  $F_{ST}$  comparisons between study locations.

It was surprising that northern flying squirrels showed genetic structure similar to southern flying squirrels. There has not been any documentation suggesting that northern flying squirrels have been expanding their range in Ontario. It was assumed that some of the northern expansion of southern flying squirrels would be into northern flying squirrel habitat and therefore I thought that there might have been the potential for range contraction in northern flying squirrels in some areas in Ontario, particularly due to competition of resources or the intestinal parasite (*Strongyloides robustus*) that southern flying squirrels carry which can be deleterious to northern flying squirrels (Wetzel and Weigl, 1994). I expected that the northern flying squirrels in Bruce and Grey Counties would have shown some genetic differentiation from other sampling areas within Ontario. The landscape south of the sample areas in Bruce and Grey Counties is heavily fragmented due to agriculture and as such this site was somewhat isolated from the other sample sites. I assumed that there was a lack of functional connectivity for northern flying squirrels between the Bruce and Grey County populations and those found



elsewhere. It is most likely that the ecology of both the southern and northern flying squirrel helps maintain the high levels of genetic diversity due to high effective population sizes in localised areas and high reproductive rates.

Future recommendations for similar studies would be to incorporate more microsatellite markers as well as to increase the amount of hair plucks to allow for more hair to be extracted and therefore to allow for potentially higher quantities of genomic DNA. As both species show a panmictic population in southern and central Ontario it would be reasonable to assume that these two species are highly mobile. The use of both mitochondrial DNA and Y chromosome markers may be useful to look at dispersal patterns of female and male flying squirrels respectively.  $F_{ST}$  is a valuable index to determine whether there is gene flow occurring between subpopulations or in the case of this study between sampling sites, but it cannot indicate which sex is moving.

Due to the mobility of flying squirrels it appears that the southern flying squirrels are able to expand their range rapidly. This combined with their high reproductive rate leads to a high amount of gene flow between the study locations. Although some levels of genetic structure appear to be occurring in Rondeau Provincial Park, it was surprising that southern flying squirrels in such a localised area appears to maintain levels of genetic diversity similar to the other study sites in central Ontario despite the assumption that this area is geographically isolated. It is surprising that the northern flying squirrels do not appear to be showing the same levels of genetic structure in Bruce/Grey Counties. Like Rondeau Provincial Park, Bruce/Grey Counties was assumed to be geographically isolated from the other flying squirrel study sites.

With regard to the study by Bowman et al. (2005) it appears that climate may have a part in the range expansion of southern flying squirrels. If this is the case then I believe that the expansion and persistence of southern flying squirrels in their expanded range will continue to be limited by both temperature and mast production. Flying squirrel dispersal appears to be related to habitat exploration such that long-distance dispersers tend to move in the direction where movement is most facilitated (i.e., in directions of preferred tree species) (Selonen and Hanski, 2006). Flying squirrels have also been observed to abandon their current habitat quickly or adjust home ranges after periods of disturbance or poor mast seasons (Taulman and Smith, 2004). Therefore, during periods of warm winters I expect that southern flying squirrels will continue to move northwards into new habitat until a period of cold temperatures and low mast production occurs that will most likely result in another energetic bottleneck.

The results of this study provided insight into these cryptic species that may not have been known otherwise. Although flying squirrels are thought to be mobile, it appears that as long as there is adequate means of dispersal southern flying squirrels are capable of expanding their range northwards if conditions are favourable. It also appears that within the study sites that were thought to be geographically isolated, the effective population size must be large enough to have maintained high levels of gene flow. However, a detectable level of genetic differentiation appears evident in the Rondeau Provincial Park area.

When this study started, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) listed southern flying squirrels as a species of Special Concern in Ontario. During the course of this study, southern flying squirrels were de-listed. Based

on previous data submitted to COSEWIC, the overall trend in habitat availability is stable and their population size and range appears to be greater than expected (Adams and Bednarczuk, 2005) which led to the species re-designation. Although southern flying squirrels appear to be limited by temperature and mast production at their northern boundary (Bowman et al., 2005), due to their apparent high dispersal and reproductive rate, I believe that southern flying squirrels in Ontario will be able to persist after energetic bottlenecks.

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