

**Genetic Structure of a Recolonizing Population
of Fishers (*Martes pennanti*)**

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Abstract

Genetic Structure of a Recolonizing Population of Fishers (*Martes pennanti*)

Denis Carr

We present the results of 769 fisher genotypes (*Martes pennanti*) studied across 35 landscapes in and around Ontario, Canada where fishers were virtually extirpated prior to the 1950s. We hypothesised that a recent expansion and recolonization originated from the historical refuge of Algonquin Provincial Park. Individuals were genotyped at 16 microsatellite loci, and using a Bayesian assignment approach, we identified five discrete inferred genetic populations. Individual assignment data allowed for the quantification of immigration events among the five genetic clusters. We estimated the proportion of immigration events (ppIm) in each landscape and applied an AIC model to test combinations of ecological and environmental variables where the best fit model was average winter snow depth ($F_{(1,31)} = 7.52$; $R^2 = 0.20$, $p < 0.01$). We concluded that there have been multiple areas of recolonization originating from a number of remnant populations and the pattern of migration into deep snow areas is a result of the increased productivity of fishers in areas with less snow.

Keywords: Fisher, *Martes pennanti*, microsatellite, Bayesian, assignment test, migration, landscape genetics, AIC, snow depth, recolonization.

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NN: Mr. Furious

BG: CR, GM, RR, PS, KW, MW, AC, BM, EB, KK, PB, MC, Fiesta Mansion, Rubidge St. Gang, Seven Nation Army, and Clint.

PP: People who nod their heads in agreement when they have no idea what they're agreeing with.

FS: "Your powers are weak old man"

MCM: Work (sleep) = shower, cocktail, PCR (3hrs), pool, sephadex, aliquot, eat (0hrs), add rox, genotype, (3hrs). Repeat for 21 days.

GA: Any one of the few select times my cloning protocol worked

Chapter 1

General Introduction

Study Objectives

The aims of this project were to examine the genetic structure of fishers in southern Ontario and to determine if recent recolonization could be attributed to fishers traveling out of Algonquin Provincial Park. We also wanted to identify factors (ecological and environmental) that may be influencing current fisher migration. Particularly of interest were the fishers in south-eastern Ontario where their presence in an agricultural environment is unusual.

Fisher Population History in Ontario

Fishers (*Martes pennanti*) are mid-sized furbearing carnivores of the weasel family. These animals are endemic to North America and have been an important resource in the fur economy of Ontario. Over the past century there has been considerable concern that fishers might become extremely rare or even extirpated in areas of the province (Seton 1929, de Vos 1952, Douglas and Strickland 1987, and Thompson 2000). The disappearance of fishers in much of Ontario has been attributed to over-harvest, predator control, and habitat fragmentation (de Vos 1952). By 1950, fishers were nearly extirpated from southern Ontario and only occurred in Algonquin Park which likely served as a trapping refuge during fisher population declines (de Vos 1951, de Vos 1952). Over the last decade fishers have been harvested through much of their former range in southern Ontario (Thompson 2000) and recently south-eastern Ontario. The fisher has marked fluctuations in its harvest numbers, indicative of a 9-10 year cycle in abundance

(Bulmer 1974) and 2001, the year the majority of our samples were obtained, represented a peak in this abundance in southern Ontario (Bowman unpubl.).

Fisher Habitat

Fishers are thought to prefer late-seral coniferous and mixed coniferous-deciduous forests (Powell, 1979, Arthur *et al.* 1989b, Krohn *et al.* 1995, Proulx *et al.* 2004) and avoid deep snow (Raine 1983, Arthur *et al.* 1993, Krohn *et al.* 1995, and Krohn *et al.* 1997). For these reasons we investigated forest type and snow depth as ecological and environmental variables that might influence fisher migration patterns.

Landscape Genetics

Animal migration and gene flow often are estimated indirectly from the spatial distribution of genetic variation among populations, but this approach does not distinguish between contemporary and historical gene flow (Berry *et al.* 2004). We employed the use of individual-based assignment tests to overcome this limitation. Assignment tests designate unknown individuals to their population of origin based on the multilocus genotype of an individual and the expected probabilities of that genotype occurring in potential source populations (Manel *et al.* 2005). Assignment tests have typically been applied to populations which are geographically distant, highly differentiated genetically and where recent dispersal is unlikely. We applied an assignment test technique that allowed detection of migration in natural connected populations. Berry *et al.* (2004) combined microsatellite DNA data and long-term mark-recapture records, and provided strong evidence that individual migration can be detected

even in populations with significant ongoing dispersal and detectable population structure.

Landscape genetics merges molecular population genetic techniques, like assignment tests, with landscape ecology. Genetic techniques can be aided greatly by incorporating interactions with spatial patterns and ecological processes. Using this approach we can examine directional relationships and ecological processes driving the assignment test data. The overall objective of a landscape genetic study is to assess the effect of landscape structure on genetic processes. This is particularly relevant in the case of the fisher in southern Ontario where it is necessary to detect not only genetic population boundaries, but also the recent collision of expanding reproductive fronts and hybridization of breeding groups.

Employing genetic techniques rather than conventional methods of gathering population information (eg. radio telemetry and mark-recapture) makes a study involving more than a few sites realistic with regard to cost and effort. Resources can then be allocated to increase the number of study sites in order to expand the study range or detect processes acting at a finer spatial scale.

Chapter 2

Genetic Structure and Diversity of a Recolonizing Population of Fishers (*Martes pennanti*)

Abstract

We present the results of 769 fisher genotypes (*Martes pennanti*) studied across 35 landscapes in and around Ontario, Canada. Fishers were extirpated from much of southern Ontario prior to the 1950s. The recent recolonization of southern Ontario has been highly successful. We hypothesised that this recent expansion and recolonization originated from Algonquin Provincial Park, an area that has historically served as a refuge for fishers. To test this hypothesis, a sampling landscape lattice was created to encompass Algonquin and the surrounding study area. DNA samples were collected mostly from the 2001-2002 fur harvest to examine the spatial dynamics of fishers at a single point in time. Twenty to thirty fishers from each of the 35 landscapes in the lattice were genotyped at 16 microsatellite loci. Using a Bayesian assignment approach, with no *a priori* geographic information, we identified five discrete inferred genetic populations, and used genetic population assignment as a means to cluster landscapes together. The degree of genetic differentiation between these landscape clusters varied ($F_{ST} = 0.019$ to 0.14). This was likely a result of the difference in historical expansion and recent differentiation among breeding groups. Each of the five genetic clusters contained high levels of genetic diversity ($H_E = 0.590$ - 0.682) and had no trace of recent bottlenecks. A regression analysis of geographic distance and F_{ST} values identified a positive relationship when excluding landscapes from the Adirondack cluster ($F_{(1,463)} = 84.27$; $R^2 = 0.154$; $p < 0.001$), and a positive relationship between landscapes in the Adirondack cluster and all other landscapes ($F_{(1,122)Adirondack} = 14.93$; $R^2 = 0.109$; $p < 0.001$). More

over, the F_{ST} values for the Adirondack comparisons showed much more genetic structuring, at any distance, than comparisons within Ontario that excluded Adirondack landscapes ($F_{ST_Adirondack} = 0.122 \pm 0.059$; $F_{ST_Ontario} = 0.054 \pm 0.0312$). We conclude that there have been multiple areas of fisher recolonization in Ontario originating from a number of remnant populations, the most recent being in the southeast as a result of immigration from the Adirondacks in New York State.

Introduction

The reappearance of fishers (*Martes pennanti*) in southern Ontario has been striking, but knowledge of the dynamics behind this recolonization has remained uncertain. Perceived increases in fisher abundance have raised questions regarding increasing harvest quotas, dealing with nuisance animals, and public safety. Fishers are being harvested through much of their former range in Ontario, where until recently they had been absent (de Vos 1952, Thompson 2000). The pre-1950 disappearance of fishers in much of Ontario has been attributed to over-harvest, predator control, and habitat loss (de Vos 1952). Around the time of the commercial trapping ban in 1949, de Vos (1952) identified only a few areas with fishers: near Collingwood, the Quinte Forest District, and in McClure township, but considered them rare outside the reserve of Algonquin Provincial Park. The park acted as a large untrapped reserve area for fishers during the periods of over-harvest. Thompson (2000) reported the eastern part of the Parry Sound District and Pembroke still supported substantial fisher harvest during the late 1950s. He alluded to the existence of a core area of preferred habitat around the southeast shore of Lake Nipissing and in the northern areas of the Ottawa River Valley.

Fisher populations have recently been examined in a number of genetic studies. Kyle et al. (2001) investigated the levels of genetic variation and population genetic structure between thirteen populations across Canada and the north-eastern United States and found relatively high levels of genetic structuring ($F_{ST} = 0.14 \pm 0.05$). On a smaller geographic scale, Wisely et al (2004) found high genetic structure ($F_{ST} = 0.45 \pm 0.07$) between five fragmented populations within a >1600 km narrow strip of forested habitat along the Pacific coast. Both studies involved populations that were relatively isolated from each other, either by geographic distance or unsuitable habitat, and that had little to no exchange of migrants.

Here we attempt to examine the genetic structure between contiguous fisher populations, and identify genetic structure at a single point in time. The geographic scale of our study is comparable to that of Wisely *et al.* (2004), except fisher populations in the current study are in a landscape more interconnected, and potential borders of genetic groups are impossible to discern *a priori*.

Our objective was to examine the current genetic structure of fisher populations in southern Ontario and to test the hypothesis that recolonization resulted from an expansion of fishers out of Algonquin Park.

Materials and methods

Sampling Design

Townships were the principle sampling unit for the study and the smallest resolution possible for individual fisher geographic locations. In a number of cases, two or more townships needed to be grouped together to obtain sufficient sample sizes.

Township sample sites as well as telemetry study locations, and sample areas around Gatineau and the Adirondacks are hereafter referred to as landscapes. In the context of our study, landscapes are defined as geographic sites of comparable size comprised of differing ecological (i.e. land cover type) and environmental parameters.

Sampled landscapes had recently high harvest numbers and were selected to form an ordered lattice across southern Ontario (Fig 2.1, Table 2.1). A spatial distribution was selected with *a priori* knowledge of fisher home ranges and dispersal distance, that best encompassed potential genetic groups. The sampling lattice was also positioned around Algonquin Provincial Park, to detect if fishers were expanding out of the park. Spatial analytical techniques can be used to detect the scale of underlying processes represented in spatial patterns and it was determined that a minimum of thirty landscapes were necessary to achieve satisfactory statistical power for the landscape genetics queries (Legendre and Fortin 1989).

Tissue Sampling and DNA Extraction

Tissue samples from the 2001-2002 fisher harvest in Ontario were collected from the Ontario Ministry of Natural Resources (OMNR) district offices. Manitoulin Island and Adirondack State Park samples from the 2000-2001 trapping season were donated by Dr. Christopher Kyle and were published previously in his 2001 study (Kyle *et al.*, 2001). Samples from the Gatineau park area were provided by Christian Pilon at the Société de la Faune et des Parcs du Québec from their 2001-2002 harvest. Algonquin Provincial Park and Prescott samples were obtained from ongoing fisher telemetry studies started in 2003 (S. Tully and J. Bowman in prep., E. Koen in prep.; respectively). Additionally,

eight historic pre-1950s samples from the Algoma Highlands, Nipissing, and Sudbury areas, were acquired from collections at the Royal Ontario Museum. Small quantities of tissue (hide, hair, blood, or muscle) were sub-sampled for molecular analysis and stored in 1x nucleic acid purified lysis buffer (Applied Biosystems, USA). DNA was extracted from all tissue types using magnetic bead isolation (C. May unpublished) on either 2 cm² of hide, 5-50 hairs, 0.5 g of muscle, or 2 cm² of blood stored on Whatman-FTS paper. Extracted DNA was then quantified by means of Pico Green fluorescence alongside a commercial standard. Basic demographic data (age and sex) was collected for most individuals, while individuals from particular areas came with more detailed information (body length and forest stand type).

Molecular Analysis

We used nineteen microsatellite loci previously developed and shown to be polymorphic on closely related species (Table 2.2). Primers used in this analysis were previously developed from wolverine (Gg007, Gg216, Gg443, Gg454, and Gg101), marten (Ma002, Ma001, and Ma019), mink (Mvi002, Mvi072, Mvi1341, Mvi1354, Mvi1321, Mvi020, Mvi1302, Mvi1342, and Mvi2243), otter (Lut604), and ermine (Mer041). These markers were chosen either because of previous amplification success in fishers (Kyle et al. 2001), or their degree of polymorphism in closely related species.

The 19 microsatellite loci were amplified in eight multiplex polymerase chain reactions (PCR) per individual. Amplifications were performed in 10µL volumes containing 5ng of DNA, 1.5mM MgCl, 0.2mM of each dNTP, 1xPCR buffer (Invitrogen), and 0.5Units of Taq polymerase (Invitrogen). PCR conditions were a

modification of the Profiler Plus reaction conditions (T. Fraser, pers. comm. 2005) which involves decreased denaturing and increased final extension times. Multiplex reactions were implemented to reduce the total number of PCR reactions. Multiplexes were created using fluorescently labelled primers and relative concentrations were optimized through trial and error.

Multiplex products were added together in four poolings and desalted by running through sephadex columns. Each pooling was visualized on a MegaBACE 1000 automated genotyper (Amersham Biosciences), with a size standard (ET-Rox 550; Applied Biosystems, USA) run with each sample to determine base pair length. Genotypes, characterized as allele sizes, were scored manually with the Genetic Profiler® v2.2 software package (Amersham Biosciences).

Estimating Populations

Linkage disequilibrium and deviations from Hardy-Weinberg equilibrium were evaluated for each locus using an exact probability test in the GENEPOP 3.3 (Raymond and Rousset, 1995) software program. Tests were adjusted for multiple comparisons using a Bonferonni correction and only accepted loci were used in downstream analyses.

A Bayesian assignment approach using the program STRUCTURE (version 2.1; Pritchard *et al.* 2000) was used to identify genetic structuring and to assign individuals to their likely population of origin. The results generated were based on five independent runs simulating one to ten ($K=1-10$) inferred genetic populations, using a 500,000 burn-in period and 1×10^6 iterations of a Markov chain Monte Carlo simulation. Simulation models were run using no prior information and assuming correlated allele frequencies

and admixture. The number of inferred genetic populations (K) was calculated by comparing the estimated Ln probability values and plotting the posterior probabilities of all possible K and selecting the best-fit (Pritchard *et al.* 2000).

Landscapes were assigned to the inferred genetic population for which the mean of the individual membership values was highest and significantly different than other membership means. In a case where the highest mean was not significantly different, a pair-wise genetic measure of gene flow (F_{ST}) using the program FSTAT (Goudet 2001) was compared to pooled landscapes of known inferred genetic population assignment. Ultimately, all landscapes were grouped into genetic clusters.

After assessing the assignments to inferred genetic populations, STRUCTURE was re-run with the *a posteriori* landscape grouping information. The estimated genetic contribution for each of the inferred genetic populations to each of the 35 landscapes was calculated as a proportion in the software to validate the *a priori* approach.

Descriptive Genetic Statistics

Genetic differentiation between the landscape clusters was estimated by calculating pair-wise F_{ST} values in the program FSTAT (Goudet 2001, Weir and Cockerham 1984). Genetic diversity in each of the landscape clusters was estimated by calculating: allelic richness (A) while compensating for sample size; unbiased heterozygosity (H_E); and the number of unique alleles, in FSTAT. We also performed a regression on pair-wise F_{ST} estimates between all landscapes and the geographic distance between F_{ST} pairs.

We tested for evidence of recent population bottlenecks by identifying an excess of heterozygosity using the program BOTTLENECK (Piry *et al.* 1999). A Wilcoxon sign-rank test was performed since our sample size of loci ($n=16$) was less than the recommended ($n=20$) needed for a standardized differences test. We used a two-phased model of mutation (TPM) in our test. The TPM is intermediate to a stepwise mutation model (SMM) and an infinite allele model (IAM). Most microsatellite data sets better fit the TPM than the SMM or IAM (Di Rienzo *et al.* 1994). We used the TPM recommended by Piry *et al.* (1999) for microsatellites consisting of mostly one-step mutations, with a small percentage (10%) of multi-step changes.

Results

Estimating Population Structure

Each locus was polymorphic and within Hardy-Weinberg equilibrium except for Mvi2243 and Mvi020 respectively. Both were removed prior to subsequent analyses along with locus Mvi1302 which had inconsistent morphology upon examination of electropherograms. The remaining 16 loci were all moderately variable, ranging from six to twelve alleles (8.63 ± 1.63 : $\bar{X} \pm SD$) with heterozygosity values ranging from 0.519 to 0.822 (0.685 ± 0.024 ; Table 2.2) and did not experience linkage disequilibrium.

We detected five inferred genetic populations using a Bayesian assignment approach with the program STRUCTURE . Assigning landscapes to genetic populations produced five landscape clusters, where Manitoulin Island and a mainland group shared one particular assignment (Fig. 2.1, Table 2.3). Each landscape cluster contained between four and twelve sampled landscapes (7 ± 3.32 : $\bar{X} \pm SD$), where each was

assigned to the inferred genetic population where the mean of the individual membership values was highest. Nine landscapes did not have a single mean value that was significantly different in order to confidently assign each of them to a genetic population. Pair-wise measures of F_{ST} between each of the nine unassigned landscapes and pooled landscapes where the inferred genetic populations was known, were used to resolve the unknown genetic population assignment for the nine landscapes (Table 2.4). The majority of cases showed consistent results between the highest mean value and the lowest F_{ST} value for the nine unassigned landscapes; only Ramsay-Huntley and Orillia-Ramara deviated. Both Ramsay-Huntley and Orillia-Ramara landscapes shared greater gene flow to clusters that assigned to a genetic population which they had a lower mean assignment value. The F_{ST} value was used to assign Ramsay-Huntley and Orillia-Ramara landscapes since identifying the cluster sharing the most gene flow, current as well as historic, was the better indicator if a single mean assignment value was not significantly different .

Performing the structure analysis with *a posteriori* landscape groupings produced almost identical results, thereby validating our *a priori* approach. Results from the *a posteriori* analysis are included in Appendix A.

Descriptive Genetic Statistics

Measurements of genetic variability were comparable across landscape clusters (Table 2.5) with a narrow range in expected heterozygosity values (0.590 to 0.682: $\bar{X} \pm SD = 0.649 \pm 0.029$). The main disparity between clusters was in the number of unique

alleles, with the Adirondack cluster having the smallest ($N_{UA} = 2$) and Midhurst-Parry Sound and Bancroft-Manitoulin clusters both having the largest ($N_{UA} = 10$).

The degree of genetic differentiation between landscape clusters varied ($F_{ST} = 0.019$ to 0.146), indicating moderate to high levels of differentiation between some populations and considerable gene flow between others (Table 2.6). F_{ST} estimates were calculated both including and excluding Manitoulin Island due both to its differential capacity to mitigate gene flow and to its translocation history; the separate analyses demonstrated similar results. A high degree of gene flow was found between certain landscape clusters, most notably between the Midhurst-Parry Sound and Central group ($F_{ST} = 0.019$). The Bancroft-Manitoulin cluster had moderate gene flow between both Midhurst-Parry Sound and Central clusters ($F_{ST} = 0.030$ and $F_{ST} = 0.031$). In contrast, the Adirondack cluster had the highest degree of differentiation, highly significant ($F_{ST} > 0.100$) from all clusters other than Gatineau, with which it shared moderate gene flow ($F_{ST} = 0.052$). The Gatineau cluster in fact shared moderate gene flow with all clusters ($F_{ST} = 0.040$ - 0.054).

We found a positive relationship between F_{ST} values and distance between landscapes ($F_{(1,593)} = 130.2$; $R^2 = 0.180$; $p < 0.001$). Further examination into the composition of this relationship identified the Adirondack cluster as having an important effect. When analysed separately both comparisons, landscapes excluding the Adirondack cluster had a better regression fit than landscapes including Adirondack ([All_w/o_Adirondack], $F_{(1,463)} = 84.27$; $R^2 = 0.154$; $p < 0.001$ and [Adirondack] $F_{(1,122)} = 14.93$; $R^2 = 0.109$; $p < 0.001$). Clearly identified were points representing pair-wise F_{ST} values between landscapes in the Adirondack cluster and all other landscapes, where

almost all were exclusively above the 0.1 value at each distance class (Figure 2.2). This demonstrated that the Adirondack cluster is highly differentiated from all other landscapes regardless of distance.

There was no evidence to support either recent or long-term bottleneck events in any of the populations according to the TPM Wilcoxon sign-rank test. Each genetic cluster did not have significant heterozygotic excess ($p < 0.05$).

Discussion

The individual-based assignment test identified five discrete genetic populations in our study area. Mean individual ancestry values for each landscape proved to be an effective method of determining landscape assignment. In the few cases where a particular mean individual assignment value was not significantly different from the others, the F_{ST} values pointed to the intuitive assignment choice of highest mean value. In two locations, Ramsay-Huntley and Orillia-Ramara this was not the case. It appears that in these areas frequent immigration events concealed the historic genetic residents, or these areas have been maintained as high admixture areas.

Manitoulin Island and some core Ontario landscapes were both assigned to the same genetic cluster. This is undoubtedly a result of the translocation of fishers that took place from Bancroft (Berg 1982) to Manitoulin Island during the period of 1979-1982 (Douglas and Strickland 1987, Thompson 2000). It is unclear how the Bancroft district became reproductively isolated, particularly from the surrounding Central group. There is evidence to suggest that there may have been a remnant fisher presence in that area even during the population lows of the 1950s. De Vos (1952) indicated that fishers were still

being trapped in the Quinte Forest District in 1951. Alternatively, this historically productive region for fishers (Thompson 2000) may have originated from a recolonization event or remnant population, but their reproductive success differentiated them genetically through the development and accumulation of unique alleles and allele frequencies produced during mutation events. The absence of equivalent unique alleles in all clusters other than Midhurst-Parry Sound, renders the latter explanation most likely.

The large range of the Central cluster is possibly the result of population spread from Algonquin Park, which is thought to have served historically as a refuge (de Vos 1952). The radiation of fishers from the refuge would be similar to the expansion event of fishers out of the Chapleau Game Reserve during the 1930-1950s (de Vos 1951). A similar proliferation possibly arose from individuals around the Quebec border near Pembroke, spreading into Ontario and forming the Gatineau cluster. Evidence that a substantial harvest existed in the Pembroke area in the late 1950s supports this notion. Gatineau Provincial Park, although trapped historically, may have supplied fishers in and around Gatineau, Quebec.

Refuges of fishers also existed in more northern areas of Ontario, such as the Algoma highlands. Two of the three historical fisher samples from the Algoma highlands assigned to the Midhurst-Parry Sound cluster with >86% assignment. The third sample assigned 50% with the Midhurst-Parry Sound cluster and 50% with the Gatineau cluster. This suggests that the Midhurst-Parry Sound cluster may have originated from individuals moving down from the Algoma Highlands or may have been contiguous with that population at one time. One remaining historical pre-1950s ROM sample, from Nipissing, assigned 80% to the Gatineau cluster. Another from Sudbury, assigned 60%

to the Midhurst-Parry Sound cluster and 30% to the Adirondack cluster. The remaining three historic samples did not amplify.

The recent appearance of fishers in south-eastern Ontario has created some public interest because the agricultural environment is atypical for fishers and some residents perceive fishers to be a potential nuisance. Our results suggest that this population of fishers has recently expanded into Ontario from New York State. Three of the Ontario landscapes assigned highly to the same cluster as samples from around Adirondack State Park. The St. Lawrence river does separate the landscapes, but it is not uncommon for fishers to swim significant distances. Seton (1929) gives an account of a fisher swimming across a lake a mile wide and mentions that fishers are often known to swim rivers and lakes in the Adirondacks. As well, there is recent anecdotal evidence of fishers swimming among the St. Lawrence Islands (OMNR unpublished data).

There were a number of translocation events that occurred since the province-wide low of the 1950s. A transplant of 97 fishers (37M, 60F) during 1956 to 1963 from Algonquin to Parry Sound was reported as “successful,” in that animals were subsequently trapped for several years (Berg , 1982. W. Berg and C. Douglas, pers.comm.1982). Three translocations took place from Bancroft to both Manitoulin Island and the Bruce Peninsula during the years of 1979-1982. Fishers taken from the Bancroft district over the three year period were successfully translocated to Manitoulin Island (n=53; 22M, 31F) and the Bruce Peninsula (28; 14M,14F) (OMNR, unpublished). In order to reach desired quotas for the translocations each year, fishers from Algonquin Park were used as a supplement. Records from the three-year program indicate five fishers from Algonquin Park were used in the translocations, and two of these were

known to be placed on the Bruce Peninsula (OMNR, unpublished). We do not have records for the other three Algonquin fishers. A personal conversation with an OMNR research biologist involved with the translocations (H. Smith pers. comm. 2005) revealed that the majority of fishers translocated to the Bruce Peninsula in the first year of the program were animals from Matchedash township, which is within the Midhurst-Parry Sound landscape cluster.

The Bruce Peninsula is relatively isolated from the rest of the Midhurst-Parry Sound cluster, but assigns completely to the cluster, averaging >0.90 assignment. The large amount of agricultural land and urban development isolating the Peninsula may not be significantly impeding gene flow, but our evidence of this is inconclusive when considering the translocation of Matchedash individuals. If the Matchedash translocated individuals were successful, their similar allelic frequencies could be interpreted as contemporary gene flow between the Peninsula and Midhurst-Parry Sound cluster.

Genetic Diversity and Differentiation

Genetic diversity was high in each cluster with consistent allele numbers, allelic richness, and expected heterozygosity values. Kyle *et al.* (2001) found similar heterozygosity values across their study area ranging from 0.56 to 0.68 (0.62 ± 0.04), but Wisely *et al.* (2004) found much smaller values in the fragmented populations along the Pacific coast, ranging from 0.16 to 0.42 (0.28 ± 0.10). This would indicate that the fisher populations in our study are not experiencing any significant inbreeding pressure.

The most striking difference between the landscape clusters in our study is the number of unique alleles. Both Midhurst-Parry Sound and Bancroft-Manitoulin clusters

have the largest with ten unique alleles each and the Adirondack cluster has the least with only two. The number of unique alleles is an important indicator of genetic distinctiveness in a population, since unique alleles only develop out of mutation within the population and not gene flow into the population.

Genetic differentiation was moderate to weak across almost all landscape clusters when excluding Adirondack ($F_{ST} = 0.019$ to 0.054), but strong between Adirondack and all other clusters ($F_{ST} = 0.054$ to 0.149). This was further demonstrated by the relationship between F_{ST} and geographic distance. The landscapes within the Adirondack cluster were highly genetically differentiated from all other landscapes ($F_{ST} = 0.122 \pm 0.059$) irrespective of geographic distance. This provides evidence that the Adirondack cluster is a recent and genetically distinct arrival into southern Ontario. Furthermore, the comparable F_{ST} score (0.052) between the Adirondack and Gatineau cluster is likely a product of migrant fishers from the Adirondack cluster arriving in the landscapes of the Gatineau cluster and not due to historic gene flow between the two. The moderate levels of gene flow between the other clusters is in all probability a product of the historic relatedness of the populations and the number of cross-assigned migrants within clusters. This is particularly evident between the Central and Bancroft-Manitoulin Island clusters. Kyle *et al.* (2001) found similar F_{ST} scores between select sites within Quebec (0.049) of comparable distance to our study, Ontario and Manitoba (0.028) at a much more sizeable distance, and between Alberta and British Columbia (0.050).

It is unlikely that expansion out of Algonquin Provincial Park originated the five genetic clusters in our study area. There is no genetic signature of a historic expansion out of Algonquin Park that supplied the fishers throughout their current range in Ontario.

Rather, the recolonization appears to originate from multiple sources. Future study of expansion dynamics in each of the five landscape clusters, would identify which of these areas is most productive for fishers.

Summary

Fishers have undergone a recent expansion and recolonization into southern Ontario, which prior to the 1950s had been largely extirpated of fishers. Evidence from our genetic study indicates that the recolonization resolved from separate fronts that now form five inferred genetic populations. The most recent of these fronts has spread into south-eastern Ontario from New York State. The other populations appear to have resulted from expansion and translocation since the 1950s from remnant populations that persisted through the historic lows of that era.

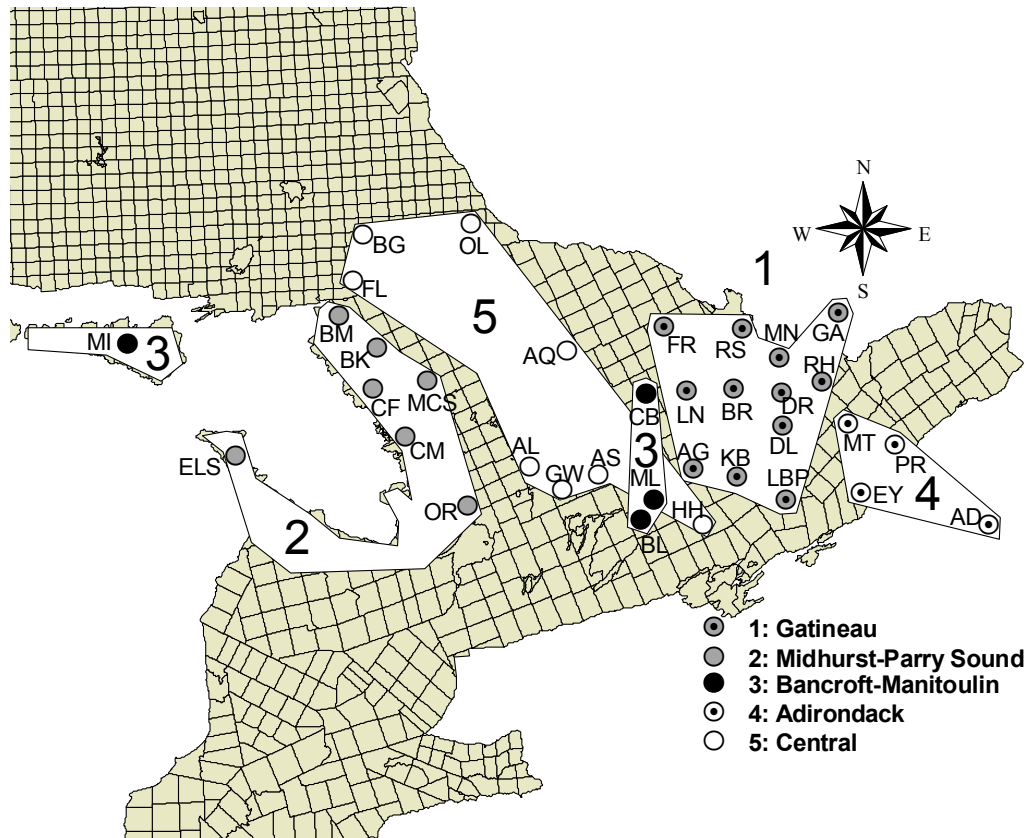


Figure 2.1: Map of landscape sample sites (enclosed points) grouped by landscape cluster (white polygons) in and around southern Ontario. Symbols denote landscape assignment and acronyms indicate landscape names.

Table 2.1: Sample size of fishers and ID labels for each landscape within the landscape clusters.

Landscape Cluster	Landscape	ID	Sample Size
Gatineau			256
	Angelsea-Grimsthorpe	AG	16
	Brougham	BR	23
	Dalhousie	DL	20
	Darling	DR	22
	Fraser-Richards	FR	21
	Gatineau	GA	18
	Kennebec	KB	23
	Loughboroug-Bedford-Portland	LBP	31
	Lyndoch	LN	19
	McNab	MN	24
	Ramsey-Huntley	RH	20
	Ross	RO	19
	Midhurst-Parry Sound		
Blair-Mowat		BM	26
Burton-McKenzie		BK	16
Carling-Ferguson		CF	8
Conger-Freeman		CM	15
Eastnor-Lindsay-StEdmunds		ELS	26
Montieth-Christie-Spence		MCS	26
OrilliaRamarara		OR	17
Bancroft-Manitoulin			99
	Belmont	BL	7
	Carlow-Bangor	CB	20
	Manitoulin Island	MI	40
	Marmora-Lake	ML	32
Adirondack			111
	Adirondack	AD	22
	Escott-Yonge	EY	20
	Montague	MT	21
	Prescott	PR	48
Central			162
	Algonquin	AQ	20
	Anson-Lutterworth	AL	25
	Anstruther	AS	24
	Badgerow	BG	22
	Falconer	FL	22
	Galway	GW	20
	Hungerford-Huntington	HH	14
	Olrig Cluster	OL	15

Table 2.2: Summary statistics for each of the 16 microsatellite loci statistics on 762 fishers from Ontario and surrounding regions. Included are the observed number of alleles (N_A), effective number of alleles (N_E ; Kimura and Crow 1964), observed heterozygosity (H_O) and expected heterozygosity (H_E ; Nei's 1973).

Genetic Marker	Publication	N_A	N_E	H_O	H_E
Mvis002	Fleming et al. 1999	7	2.326	0.497	0.570
Mvis072	Fleming et al. 1999	8	2.358	0.531	0.576
Mer041	Fleming et al. 1999	9	3.266	0.636	0.694
Lut604	Dallas and Piertney 1998	12	5.602	0.695	0.822
Gg443	Walker et al. 2001	9	4.587	0.701	0.782
Gg454	Walker et al. 2001	7	2.814	0.558	0.645
Ggu101	Duffy et al. 1998	9	2.892	0.576	0.654
Ggu216	Duffy et al. 1998	11	4.417	0.619	0.774
Ma1	Davis and Strobeck 1998	8	3.834	0.637	0.739
Ma2	Davis and Strobeck 1998	9	3.797	0.670	0.737
Ma19	Davis and Strobeck 1998	9	4.907	0.675	0.796
Gg007	Davis and Strobeck 1998	8	2.629	0.538	0.620
Mvi1321	Vincent et al. 2002 (unpub)	10	2.080	0.476	0.519
Mvi1341	Vincent et al. 2002 (unpub)	10	4.106	0.638	0.756
Mvi1342	Vincent et al. 2002 (unpub)	6	3.422	0.533	0.708
Mvi1354	Vincent et al. 2002 (unpub)	7	2.278	0.503	0.561
Mean (\pm SD)		8.63 \pm 1.63	3.457 \pm 1.059	0.593 \pm 0.075	0.684 \pm 0.095

Table 2.3: Inferred genetic cluster assignment for each landscape using mean individual membership from the program STRUCTURE (STR) or lowest pair-wise F_{ST} values (F_{ST}).

Inferred Groups	n	Method	1	2	3	4	5
Gatineau	256						
Angelsea-Grimsthorpe (AG)	16	STR	0.340	0.079	0.192	0.158	0.231
Brougham (BR)	23	STR	0.402	0.104	0.190	0.090	0.213
Dalhousie (DL)	20	STR	0.410	0.097	0.138	0.268	0.088
Darling (DR)	22	STR	0.471	0.071	0.167	0.141	0.151
Fraser-Richards (FR)	21	F_{ST}	0.340	0.321	0.143	0.047	0.149
Gatineau (GA)	18	F_{ST}	0.414	0.120	0.079	0.337	0.050
Kennebec (KB)	23	STR	0.640	0.039	0.110	0.161	0.049
Loughboro-Bedford-Portland (LBP)	31	STR	0.631	0.029	0.058	0.253	0.028
Lyndoch (LN)	19	F_{ST}	0.350	0.104	0.265	0.125	0.157
McNab (MN)	24	STR	0.671	0.071	0.065	0.128	0.064
Ramsey-Huntley (RH)	20	F_{ST}	0.341	0.056	0.088	0.428	0.087
Ross (RO)	19	STR	0.467	0.183	0.212	0.053	0.084
Midhurst-Parry Sound	134						
Blair-Mowat (BM)	26	STR	0.081	0.404	0.217	0.103	0.195
Burton-McKenzie (BK)	16	STR	0.048	0.428	0.215	0.085	0.224
Carling-Ferguson (CF)	8	F_{ST}	0.059	0.363	0.144	0.136	0.298
Conger-Freeman (CM)	15	STR	0.035	0.587	0.203	0.014	0.161
Eastnor-Lindsay-StEdmunds (ELS)	26	STR	0.019	0.864	0.068	0.013	0.036
Montieth-Christie-Spence (MCS)	26	STR	0.078	0.472	0.204	0.028	0.218
OrilliaRamarara (OR)	17	F_{ST}	0.103	0.311	0.189	0.072	0.326
Bancroft Manitoulin Island	99						
Belmont (BL)	7	STR	0.122	0.030	0.723	0.013	0.113
Carlow-Bangor (CB)	20	STR	0.109	0.182	0.467	0.036	0.206
Manitoulin Island (MI)	40	STR	0.035	0.126	0.750	0.014	0.075
Marmora-Lake (ML)	32	STR	0.149	0.107	0.529	0.022	0.193
Adirondack	111						
Adirondack (AD)	22	STR	0.120	0.097	0.051	0.665	0.068
Escott-Yonge (EY)	20	STR	0.078	0.017	0.025	0.859	0.021
Montague (MT)	21	STR	0.155	0.026	0.043	0.738	0.038
Prescott (PR)	48	STR	0.024	0.015	0.019	0.923	0.019
Central	162						
Algonquin (AQ)	20	F_{ST}	0.043	0.335	0.194	0.020	0.408
Anson-Lutterworth (AL)	25	F_{ST}	0.033	0.335	0.152	0.020	0.460
Anstruther (AS)	24	STR	0.056	0.139	0.281	0.021	0.503
Badgerow (BR)	22	STR	0.052	0.226	0.132	0.015	0.576
Falconer (FL)	22	STR	0.043	0.145	0.220	0.021	0.571
Galway (GW)	20	STR	0.023	0.041	0.135	0.016	0.785
Hungerford-Huntington (HH)	14	STR	0.213	0.070	0.178	0.066	0.474
Olrig Cluster (OL)	15	F_{ST}	0.074	0.338	0.156	0.029	0.403
ROM*	5		0.383	0.419	0.019	0.141	0.039

*ROM samples represent historic samples (pre-1950) obtained from the Royal Ontario Museum.

Table 2.4: Landscape assignment identification through pair-wise F_{ST} estimates to landscape clusters of known assignment. Bolded values indicate probable cluster assignment using the F_{ST} measure.

	Gatineau (1)	Midhurst-P.S. (2)	Bancroft-Man (3)	Adirondack (4)	Central (5)
Algonquin	0.0668	0.0187	0.0371	0.1592	0.0128
Anson-Lutterworth	0.0539	0.0140	0.0343	0.1447	0.0035
Carling-Ferguson	0.0398	0.0087	0.0434	0.1154	0.0326
Fraser-Richards	0.0402	0.0567	0.0673	0.1117	0.0747
Gatineau	0.0132	0.0514	0.0695	0.0297	0.0878
Lyndoch	0.0167	0.0478	0.0288	0.0944	0.0473
Olig-Cluster	0.0517	0.0151	0.0291	0.1483	0.0011
Orillia-Ramara	0.0273	0.0116	0.0239	0.1035	0.0186
Ramsey-Huntley	0.0162	0.0580	0.0762	0.0306	0.0822

Table 2.5: Measurements of genetic variability of fisher landscape clusters: sample size (n), mean observed number of alleles (N_A), allelic richness (A), number of unique alleles (N_{UA}), mean observed heterozygosity (H_O), and mean expected heterozygosity (H_E).

Cluster	n	N_A (\pm SD)	A (\pm SD)	N_{UA}	H_O (\pm SD)	H_E (\pm SD)
Gatineau	256	6.81 \pm 1.60	3.764 \pm 0.734	4	0.6073 \pm 0.0181	0.6593 \pm 0.0282
Midhurst-P.S.	134	6.88 \pm 1.63	3.765 \pm 0.676	10	0.5778 \pm 0.0114	0.6824 \pm 0.0246
Bancroft-Man	99	6.19 \pm 1.38	3.560 \pm 0.699	10	0.5913 \pm 0.0131	0.6541 \pm 0.0257
Adirondack	111	5.69 \pm 1.40	3.336 \pm 0.720	2	0.5339 \pm 0.0127	0.5899 \pm 0.0385
Central	162	6.25 \pm 1.77	3.623 \pm 0.745	6	0.6208 \pm 0.0100	0.6614 \pm 0.0291

Table 2.6: Pair-wise F_{ST} estimates of gene flow between fisher landscape clusters including Manitoulin Island (a), and with Manitoulin island analysed separately (b).

a)

Cluster	Midhurst-P.S.	Bancroft-Man.	Adirondack	Central
Gatineau	0.040	0.045	0.052	0.054
Midhurst-P.S.		0.030	0.112	0.019
Bancroft-Man.			0.139	0.031
Adirondack				0.143

b)

Cluster	Midhurst-P.S.	Bancroft	Adirondack	Central	Man. Isld
Gatineau	0.040	0.044	0.052	0.054	0.062
Midhurst-P.S.		0.032	0.112	0.019	0.041
Bancroft			0.146	0.026	0.027
Adirondack				0.143	0.149
Central					0.052

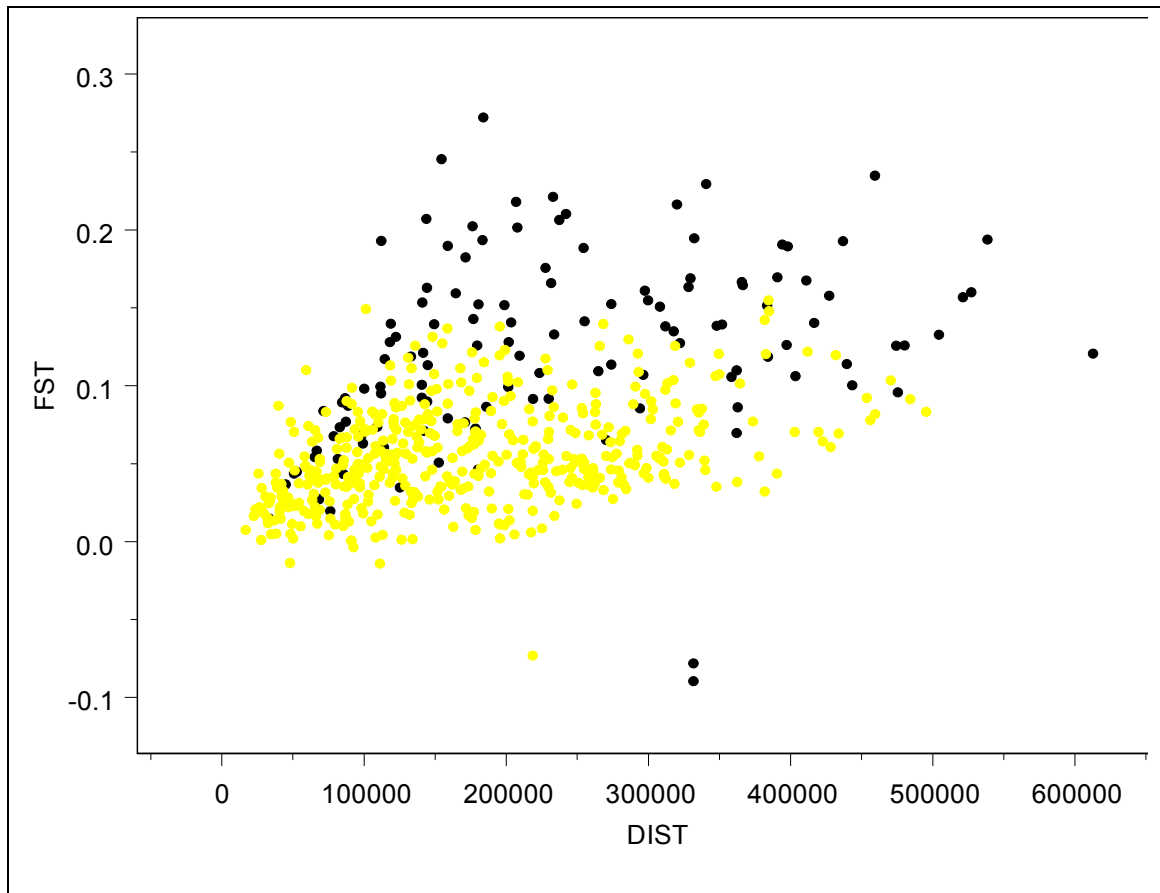


Figure 2.2: Scatter plot of pair-wise fisher landscape F_{ST} measures and geographic distance (m) between all landscapes (black circles = comparisons of all landscapes with landscapes of the Adirondack cluster; grey circles = comparisons between all landscapes other than the Adirondack cluster).

Chapter 3

Landscape Genetics of a Recolonizing Population of Fishers

Abstract

We examined 769 fishers from 35 landscapes in and around Ontario, Canada. Twenty to thirty fishers from each landscape were sampled, most during the 2001-2002 fur harvest. DNA profiles were generated at 16 microsatellite loci and a Bayesian clustering analysis that assumed no *a priori* geographic grouping identified five genetic clusters. Individual assignment data allowed for the quantification of immigration events among the five genetic clusters. This technique served as an effective and contemporary estimator of migration. Additional estimators of migration (Nm), used in equivalent studies, were also calculated for comparison. Conventional indirect estimators of migration produced generally smaller values than those generated by the Bayesian clustering method. Comparative analyses revealed correlations between conventional estimators and our method ($r_s = 0.80 \pm 0.13$, $n = 5$, all $p \leq 0.05$), demonstrating that only the magnitude and not the trend varied between methods. We assumed that landscapes of high quality habitat would be more productive than ones of low quality, and would therefore have a net surplus of emigrants. Conversely, landscapes of low quality should be net receivers of immigrants. We estimated the proportion of immigration events (ppIm) in each landscape and applied an AIC model to test combinations of ecological and environmental variables best representing the variation in the data. The best fit model describing the variation present in the ppIm was average winter snow depth, where we found a significant positive relationship ($F_{(1,31)} = 7.52$; $R^2 = 0.20$, $p < 0.01$). We predicted Algonquin Provincial Park to be a net producer of emigrants; however, the Park had one of the highest proportions of inferred immigration at 0.450 (0.214 ± 0.124). The

Midhurst-Parry Sound and Bancroft-Manitoulin landscape clusters appeared to be the most productive, with the highest emigrant index values ($M_E = 0.35$ and 0.38 ; respectively). Fisher population density has been shown previously to be negatively correlated with deep snow cover; our study suggests a pattern of migration into deep snow areas is a result of the increased productivity of fishers in areas with less snow.

Introduction

Fishers (*Martes pennanti*) were nearly extirpated from southern Ontario by 1950, due to over harvest, predator control, and habitat loss. Since that time, trapping regulations and changing social and environmental conditions have permitted fisher populations to recover. In recent years, fishers have recolonized much of their former range in southern Ontario. It is likely that the sources of this recolonization are remnant populations from trapping refuges, such as Algonquin Provincial Park, where populations persisted through historical lows of the 1950s (de Vos 1952).

The fisher is thought to prefer mixed coniferous habitat where it tends to den in the hollows of dead trees, as well as avoid deep snow unsuitable for travel (Krohn *et al.* 1995, 1997). Krohn *et al.* (1995) suggested that in deep snow environments, fishers would occur at lower densities and would require larger blocks of coniferous forest with closed canopies, and if the connectivity of forest cover was compromised it would make it difficult for fisher migration and survival. Fishers are thought to avoid deep snow because strenuous movement coupled with fasting in dens after snowfall is too energy taxing (Arthur *et al.* 1993). It also has been suggested that deep snow may affect use of habitat and associated hunting success (Raine 1983). It has been found from small scale

maps that forest type was a poor predictor of fisher occurrence (Krohn *et al.* 1997), though there still remains debate on the degree of fisher habitat selection. We hypothesized that stand type and snow depth will have particular influence on fisher migration such that landscapes with adequate coniferous forest and low snow depth will be productive and have a net surplus of fishers. Alternatively, we investigated if the number of fishers removed from landscapes, calculated from annual fur harvest records, was responsible for increased numbers of immigrating fishers.

Assignment test based methods have recently been offered as a solution to the problem of estimating contemporary rates of gene flow and dispersal (Waser & Strobeck 1998). Until recently this technique has been limited to studies where populations are distant and highly differentiated genetically, and recent dispersal is unlikely. The accuracy in migrant assignment detection between natural populations with ongoing dispersal was recently addressed by Berry *et al.* (2004) in a comparative genetic and telemetry study of the grand skink (*Oligosoma grande*) in southern New Zealand. They found assignment tests correctly identified the natal population of most individuals (65-100%) even when interpopulation dispersal was common (5-20%); but the accuracy of assignment depended on the stringency applied, the degree of genetic differentiation between populations, and the number of loci used. Levels of heterozygosity present in the loci would also be important to consider; as increased polymorphism adds statistical leverage to discern differentiation. Computer simulations have shown that estimates in the level of genetic differentiation between populations (i.e., F_{ST}) are useful predictors of assignment confidence (Cornuet *et al.* 1999).

We chose a fully Bayesian assignment method because recent studies argued partial Bayesian methods found a lower percentage of individuals that were able to be assigned using high stringency criteria (Berry *et al.* 2004, Manel *et al.* 2002, Maudet *et al.* 2002). Partial Bayesian methods also tended to overestimate the number of dispersers more than the fully Bayesian methods (Berry *et al.* 2004). Berry *et al.* (2004) found the effect of increasing the number of loci was gradual on improving the number of correctly assigning individuals for their sites with low F_{ST} ; conversely the process was much quicker for their higher differentiated sites. Bjørnstad and Røed (2002) found correct assignment to horse breeds was achieved between populations with high differentiation using six loci, while twelve loci were needed in less differentiated populations. Similarly, Berry *et al.* (2004) also found the percentage of correct assignment increased for populations which were more genetically differentiated: where “most likely stringency” reached 100% accuracy for F_{ST} between 0.06 and 0.08. Heterozygosity values were high in the Berry *et al.* (2004) skink study ($H_e = 0.73-0.80$), as were the values in Bjørnstad and Røed’s (2002) horse breed study ($H_e = 0.65 \pm 0.11$), and both are comparable to our study ($H_e = 0.68 \pm 0.10$, Ch.2). Though the populations in our study possessed varying degrees of genetic differentiation ($F_{st} = 0.019$ to 0.14 , Ch.2) assignment confidence should be ample when taking into account the high heterozygosity and polymorphism in the sixteen microsatellites used in the assignment.

Current estimators of migration customarily infer the number of individuals exchanged only between populations per generation. These measures are traditionally generated indirectly from genetic variation and gene flow estimates (F_{ST}). This approach does not distinguish between contemporary and historical gene flow and dispersal,

making comparisons with ecological studies, that use instantaneous rates, problematic (Berry *et al.* 2004). Historic events driving F_{ST} measures may not provide accurate reflections of current migration patterns. Paetkau *et al.* (2004) identified high variance associated with estimates of N_m , while Whitlock and McCauley (1999) found that F_{ST} driven N_m estimates were likely to be correct only within a few orders of magnitude. N_m reflects long-term dispersal rates that would be slow to respond to “real-time” changes in movement patterns.

Assignment tests allow for the identification of migrants in resident populations (Paetkau *et al.* 2004, Berry *et al.* 2004); therefore, in each sample unit we can calculate the proportion of immigration events if the resident population is known. Sample sites were chosen across multiple landscapes permitting the testing of ecological and environmental characteristics with our genetic data. This enables us to apply landscape genetics on a large-scale natural population with ongoing dispersal. We assume in this work that high quality landscapes should be net producers of migrants and low quality landscapes net receivers of migrants. Through this assumption we can test for landscape quality. In this study we examine the effects of ecological and environmental variables on fisher migration to test the hypothesis that snow depth and coniferous forest are important components of fisher landscape quality.

Materials and methods

Samples used in this study were those collected and genotyped in a previous study (Ch.2). We used a Bayesian assignment approach using the program STRUCTURE (version 2.1; Pritchard *et al.* 2000) to assign landscapes to inferred genetic populations a

priori of geographic location. Five inferred genetic populations were identified. Landscapes were assigned to the inferred genetic population for which the mean of the individual membership values was highest and significantly different from other membership means. In cases where the highest mean value was not significantly different, (nine landscapes), a pair-wise genetic measure of gene flow (F_{ST}) using the program FSTAT (Goudet 2001) was used to compared the unassigned landscapes to pooled landscapes where the inferred genetic population assignment was known.

Identifying Migrants

Individuals were assigned to the inferred genetic population for which they had the highest membership value (given as a proportion). Immigration events were identified as fishers assigning to different inferred genetic populations than the landscape where they were sampled. A threshold value of $\geq 60\%$ assignment to an inferred genetic cluster was employed to designate complete membership and individuals not meeting this criterion were deemed of admixed membership. The $\geq 60\%$ criterion identifies fishers that are either currently migrating (F_0) or the first generation offspring (F_1) of successful immigrants. This criterion was employed due to discordant levels of gene flow between landscape-genetic clusters generating an unbalanced distribution of high ($\geq 80\%$) assignment scores. The proportion of immigrants (ppIm) was calculated for each landscape by taking a ratio of the number of immigration events in a landscape over the sum of all individuals in that landscape. This process was repeated for the ppIm in the five landscape-genetic clusters. The ppIm events was recalculated (ppIm_{Admx}) for the nine landscapes where the mean of the individual membership values was not highest or

significantly different from other membership means and an F_{ST} measure was needed to determine assignment. In these cases the landscape was considered to have admixed lineage and individuals with $\geq 60\%$ assignment to any cluster were identified as immigration events.

Identifying the cluster of origin for immigration events enabled us to infer the number of emigrants a particular cluster was producing. By taking a ratio of the number of emigrants a cluster produces over the number of assigned residents, we could estimate an emigration index (M_E) for each landscape cluster. Knowing the cluster of origin also enabled us to count the number of migrants exchanged between two landscape clusters. The distribution of migrants was visualized in ArcView3.3 by plotting assigned individuals around landscape centroids on a map of Ontario.

Several standard estimates of migration also were calculated. The effective number of migrants between any two inferred populations per generation, N_m , was estimated using the method recommended by Cockerham and Weir (1993) which employs F_{ST} values ($F_{ST} = (1 + 4N_e m_e)^{-1}$). The Paetkau *et al.* (2004) method of detecting migrants (L_{Home}/L_{Max}) was calculated at the landscape and cluster level using the default settings recommended in GeneClass v.2.0 (Piry *et al.* 2003) with Paetkau's frequency-based model (Paetkau *et al.* 1995) and probability calculator (Paetkau *et al.* 2003). The microsatellite (Brownian Motion) model with default parameters was used for each simulation in the program MIGRATE (Beerli and Felsenstein 2001), where Theta ($\theta = 4N\mu$) and M (migration rate) were generated from F_{ST} values (Beerli, P. 1997-2002. MIGRATE: documentation and program, part of LAMARC. Version 1.7.6.1).

Relationships between migration estimates were tested using a Spearman rank correlation analysis.

Habitat Variables

Data layers were created for several variables of interest. Snow depth records for the years 1992 through to 2002 were obtained from OMNR monitoring stations and compiled into a data layer of mean weekly snow depth from January to April (Middle unpubl. data). Snow depth values were then calculated at each landscape centroid, for the landscapes within Ontario. Snow depth data was not obtained for the Gatineau or Adirondack New York landscapes so they were removed from the regression. Proportion per km² of dense deciduous, dense coniferous, mixed coniferous and deciduous, and non-forested land cover were assessed within each landscape in Ontario using data from the Ontario 28-class Provincial Landcover Landsat TM Image. Fur harvest information for registered and private trap lines, were obtained from OMNR trapping records for landscapes within Ontario and from New York State Dept. of Environment and Conservation (unpubl. data) for the New York samples. Harvest data was calculated within each landscape, represented as animals/km², for both the 2000-2001 and 2001-2002 trapping seasons. Values for forest cover, snow depth, and annual harvest also were calculated for the five landscape clusters and are represented as an average of their respective landscapes.

Spatial Analysis and AIC Model Selection

Directional correlograms of the landscape attribute ppIm were analysed for spatial autocorrelation in (S-Plus 6). The degree of correlation was calculated for each distance class and a lag was selected that maximized point-pair homogeneity between distances classes. Correlations were analysed for significance using a Bonferonni correction for multiple comparisons (Zar 1999).

We used Akaike's information criterion (AIC), an information theoretic data analysis procedure, to select a linear regression model that best explained the variation in ppIm for landscape and cluster datasets. AIC ranks each suggested model with the goal of generating parsimony between the amount of variation the model explains and squared bias versus the number of variables used in the model (Burnham and Anderson 2000). Models were ranked by AIC score where the lowest score was the most appropriate model.

RESULTS

Identifying Migration

All landscapes were assigned into one of five landscape clusters (Fig. 2.1, Table 2.1). The Adirondack cluster had a lower ppIm than all other clusters with 0.045 (0.186 \pm 0.089). This is understandable, since it contained three of the four landscapes with ≤ 0.10 ppIm values. The Adirondack cluster also was the second lowest producer of emigrants ($M_E = 0.250$; $\bar{X} \pm SD = 0.270 \pm 0.114$). The lowest producer of emigrants was the Gatineau cluster with 0.089, but unlike the Adirondack cluster it had an above average ppIm (0.223). The Central cluster had an average amount of emigration (0.279)

with a larger than average amount of immigration events (0.278). The Midhurst-Parry Sound and Bancroft-Manitoulin groups appeared to be the most productive, with the highest emigrant index values ($M_E = 0.35$ and 0.38 ; respectively). The ppIm for both were in the mid-range (0.224 and 0.162; respectively). Considering that part of the Bancroft-Manitoulin cluster is an island, a separate analysis of both Bancroft and Manitoulin Island was conducted independently, which revealed ppIm values of 0.203 and 0.125 respectively.

Landscape ppIm values ranged from 0.000 to 0.450 (0.214 ± 0.124) with nine landscapes having ≤ 0.10 and three having ≥ 0.40 . No immigration events were found in three of the landscapes: Bruce Peninsula, Prescott, and Escott-Yonge, whereas Algonquin Park and Ramsey-Huntley had the highest ppIm with 0.450.

A visual representation of the distribution of assigned individuals can be seen in Figure 3.1. The figure clearly reflects the M_E values for each genetic cluster, but noteworthy is the pattern of spread. Most prominent was the pattern emanating from the Bancroft-Manitoulin cluster, where relatively equal numbers of assigned individuals were found within the cluster boundaries as the exterior. As well, the emigrating individuals were distributed in a radiating pattern out from the central core in all directions throughout the study site, except for into the Adirondack cluster. The Midhurst-Parry Sound cluster produced emigrants spreading in a gradient from the central core with even a few onto Manitoulin Island.

Conventional estimates of migration between clusters were much smaller on average than those we found using the Bayesian assignment approach (Table 3.1). The estimates however, represent the same trends of migration between clusters as those

demonstrated with the assignment approach, only lesser in magnitude. Each measure was significantly correlated to the ppIm results ($r = 0.80 \pm 0.13$, $n=5$, all $p < 0.05$). Comparisons using assignment values ≥ 0.80 , produced the same scalar quantities as the other N_m measures but the correlation significance decreased. In general the correlation weakened for comparisons between less differentiated genetic clusters (see Appendix B). The largest amounts of migration were found between the Midhurst-Parry Sound/Central, Bancroft-Manitoulin Island/Central, and Gatineau/Adirondack clusters. Very little to no migration was found between Midhurst- Parry Sound/Adirondack, Bancroft-Manitoulin/Adirondack, and Central/Adirondack clusters.

Model and Regression

Examination of the ppIm correlogram showed no significant trend of autocorrelation, further confirmed by a lack of significance in the Bonferonni corrected correlation values. We then tested fifteen alternative models to explain the ppIm, at the landscape scale, and the best fit was average winter snow depth from January to April (Figure 3.2, Table 3.2). A significant positive relationship was identified in the regression ($F_{(1,31)}=7.52$; $R^2=0.20$, $p=.01006$). Regressions also were carried out using the ppIm calculated by cluster and ppIm_{Admix} and identified the same trend ($F_{(1,3)}=35.38$; $R^2=0.92$, $p=.009504$. $F_{(1,31)}=7.57$; $R^2=0.20$, $p=.00983$; respectively). The hypothesis that fur harvest was driving the ppIm was unconvincing, as neither the 2000-2001 nor the 2001-2002 harvest had a significant relationship with ppIm.

DISCUSSION

Fisher Dynamics

The fisher is thought to preferentially select coniferous and mixed forest (Arthur *et al.* 1989b), though this is currently a source of debate in the literature. Krohn *et al.* (1995) suggested that study conclusions about fishers using particular forest types might be a consequence of the snow attributes in those areas; climax or mature coniferous forest study areas were located in deep snow environments in the Pacific Northwest and Great Lakes Region, while secondary forests were reported from relatively low snowfall regions of the Northeastern USA. Raine (1983) said fishers preferred coniferous ridges. Arthur *et al.* (1989b) found little evidence that fishers actively selected for particular forest types. They speculated that fishers preferentially rested in the branches of coniferous trees in the spring and summer months, but that during the winter months resting took place within mixed stands in ground burrows. Although in the winter they hunted intensively in coniferous undergrowth opposed to the variety of forest types used in the summer. They concluded that because the fisher has such a diverse diet it would use a variety of forest types.

There has been the suggestion that fishers avoid deep snow (Raine 1983; Krohn *et al.* 1995). Winter denning can become taxing on fishers during areas of heavy snowfall since they apparently fast 3-6 days at a time after snowfalls (Arthur *et al.* 1989). Since fishers appear not to store food in dens it is possible that the number of snowfalls over 6.5 cm is correlated to the number of fasting periods per winter, thus directly affecting body condition, survival, and recruitment (Krohn *et al.* 1995). Fishers also are restricted in

their movements by the soft thick snow cover of midwinter, but not the thin cover of early winter or the crust conditions of late winter (Raine 1983).

We found that snow depth was the best model for proportion of immigrants. Although this may sound contradictory of the established literature on avoiding deep snow cover, the year of our samples was during a fisher population peak (Bowman unpubl. data) and considering that the fisher is territorial, emigrating individuals and dispersing juveniles would be forced to relocate to less optimal habitat. Fishers should also have higher mortality and lower reproductive success in areas with deep snow, according to Krohn *et al.*(1995, 1997). Low snow depth landscapes are more productive and as a net result high snow depth landscapes receive more immigrants.

Identifying Migrants

The individual-based assignment test assigned individuals to their candidate populations with variable scores. Each inferred genetic population contained a high percentage of individuals with >80% assignment. Ideally, an assignment score of >80% would be a suitable cut-off in designating complete assignment to a genetic cluster, but we found this to unfavourably bias areas of high genetic differentiation. High assignment scores should be used to designate complete membership if possible, but in natural populations with ongoing dispersal and potential hybridization at reproductive fronts, this stringent standard becomes unrealistic. The danger of confusing lower assignment percentage individuals with admixed/unknown heritage individuals exists more when the assignment probability is dispersed relatively high across few genetic groups. As the number of inferred genetic populations increases it is likely that in closely related

populations, a lower assignment threshold will be valid for designating complete assignment. For instance in a scenario of questionable assignment to one of five potential genetic populations, an individual with 60% assignment to the first and 10% assignment to the remaining four, is clearly a member of the 60% assignment group.

For this reason the number or percentage of completely assigning individuals was not used to determine the resident genetic population of a landscape, instead the highest and statistically different mean value was chosen (Ch.2). Likewise, identifying immigration events using a high assignment score cut-off would bias immigration events between highly differentiated populations. Values of $\geq 60\%$ were chosen not only to minimize this bias but also to incorporate recent immigration reproductive success, a more accurate reflection of immigration impact than just the identification of migrant movement alone. Using this criterion did not affect the relationship in the regression analysis with $ppIm$, nor did it deviate from the trend identified in the measures of migration.

Individuals could only be designated as immigration events if they were identified in a landscape of contrasting assignment. Therefore landscapes had to be assigned with confidence. In most cases, landscape assignment was clear, but there remains the possibility of a landscape existing as a sink or a hybridization zone, where it is not possible to ascertain the resident population. In our case we chose to use a measure of current and historic gene flow (F_{ST}) to decipher the resident population. Though we are confident in their assignment, we included results in which we interpret these sample sites to be admixed and individuals with $\geq 60\%$ assignment values as immigrants. This classification did not significantly affect the outcome of our regression analyses, but may

become problematic in reciprocal landscape genetic studies where this challenge is represented in the majority of sample sites

Landscapes often appeared to contain immigrants due to proximity to an opposing cluster. There were important exceptions to this; for instance, Algonquin and Olrig landscapes in the Central Cluster both had above average $ppIm$ as a result of immigration events from the Midhurst-Parry Sound cluster. As well, Montague and Escott-Yonge had few to no immigration events though they are adjacent to the Gatineau cluster. Assessing the direction of migration and the characteristics of the clusters involved is essential in the interpretation of immigration events.

Thompson (2000) identified south-eastern Ontario as devoid of fishers as recently as 10 years ago. Emigration out of, with few or no immigration events into the Adirondack cluster lends supports that the area was a recent expansion from New York State. This recent appearance of fishers in south-eastern Ontario has been suggested to be a result of decreased trapping pressure, reduction in predator control, and an increase in forested land (Lancaster *et al.* in prep.) The adjacent Gatineau cluster with average $ppIm$ and the lowest M_E (0.089) is probably a remnant of an expansion front from Quebec that recolonized part of southern Ontario. This cluster is now being out-produced by adjacent clusters which are infringing on its reproductive borders. The Central cluster has the highest $ppIm$ and an average M_E , likely a result of regions of mixed fisher productivity. The remnant of a large expansion front (Ch.2), the Central cluster, possibly contains areas of poor habitat likely being occupied by the expansion of migrant fishers from the productive Midhurst-Parry Sound and contiguous Bancroft-Manitoulin clusters. The Bancroft-Manitoulin cluster, the smallest of the clusters, is either a remnant of a

fisher population from prior to the recolonization of southern Ontario, or a recolonized region which became atypically productive, and therefore genetically distinct, before the translocation to Manitoulin Island. This region of central Ontario has consistently been productive for fishers (de Vos 1952, Thompson 2000) and remains so with little immigration and the highest M_E (0.381). The cluster with the second highest M_E (0.352) was Midhurst-Parry Sound, also a historically productive area. The higher than average $ppIm$ is most likely an effect of the proximity to the Central cluster and a resulting collision of reproductive fronts. Most immigration events in both the Central and Midhurst-Parry Sound clusters were from cross-assigned individuals.

The various measures of migration identified equivalent trends and only the scale of the measures differed. An important difference between the migration estimates is that assignment methods reveal a degree of direction and, in our sample design, can be applied to the smaller scale of the landscape. Assignment methods also are attractive in the current population study because they circumvent the biases prevalent in measures based on F_{ST} , which do not distinguish between historical and contemporary gene flow and dispersal (Berry *et al.* 2004). While these measures identified similar trends at the genetic cluster scale, it was necessary to produce a measure at the landscape scale effective at assignment and detection of ongoing dispersal in a natural population. This method permitted the application of a landscape genetic approach.

Summary

Fishers have recently recovered from extirpation in southern Ontario, where they were nearly extirpated by the 1950s. The current population of fishers in this region is

comprised of five genetically distinct clusters. Each of these clusters have varying immigration and productivity, calculated in this study as proportion of immigration events and identified emigrants. These cluster qualities are a function of the underlying history of the fisher recolonization and a significant relationship exists between the proportion of immigration events and winter snow depth. Fisher population density has been previously shown to be negatively correlated with deep snow cover and our study suggests that the migration into deep snow areas is a result of the increased productivity of fishers in areas with less snow.

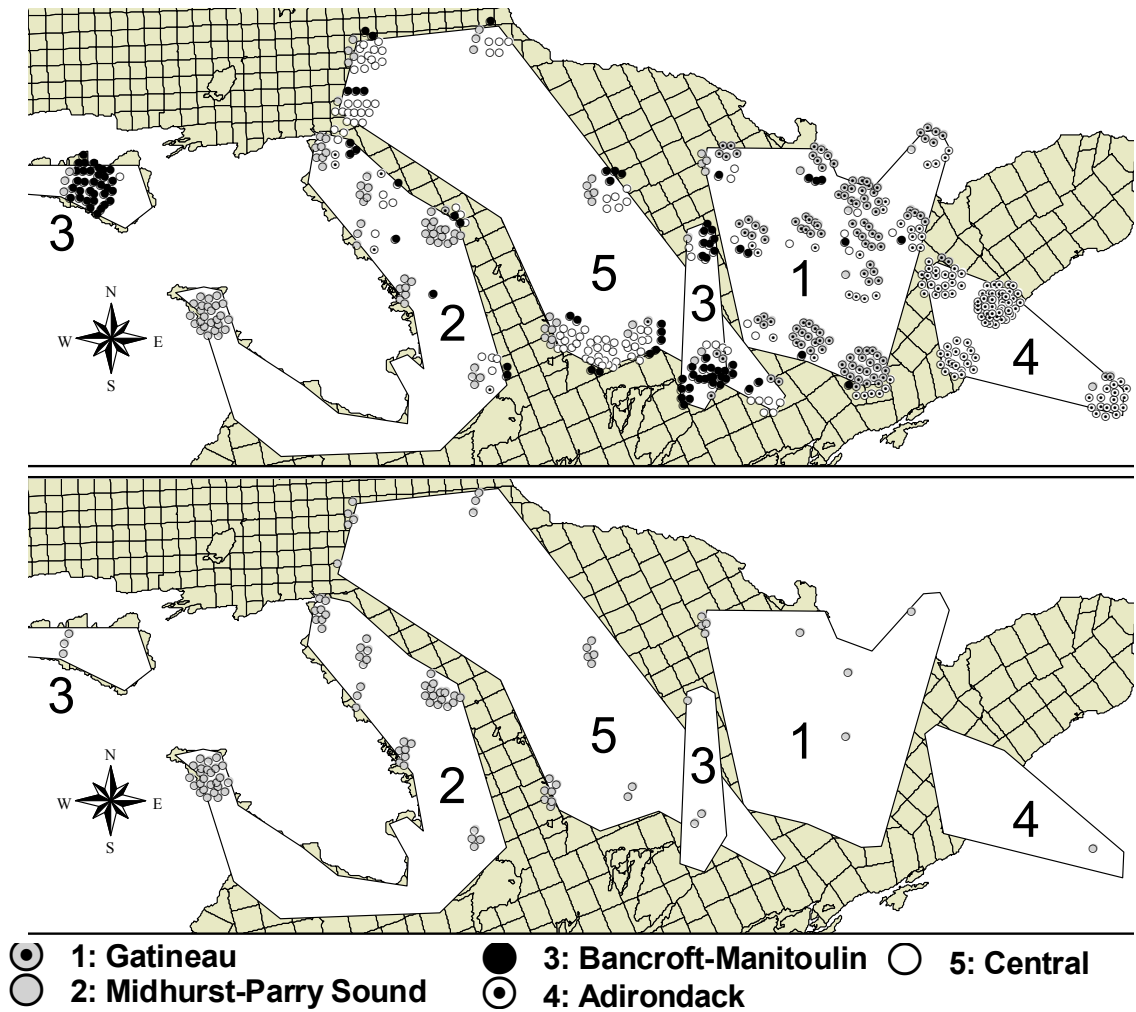


Figure 3.1: Distribution of assigned individual fishers, where symbol represents assignment to an inferred genetic population. Individual symbols are clustered around landscape centroids. White polygons indicate proposed landscape cluster borders.

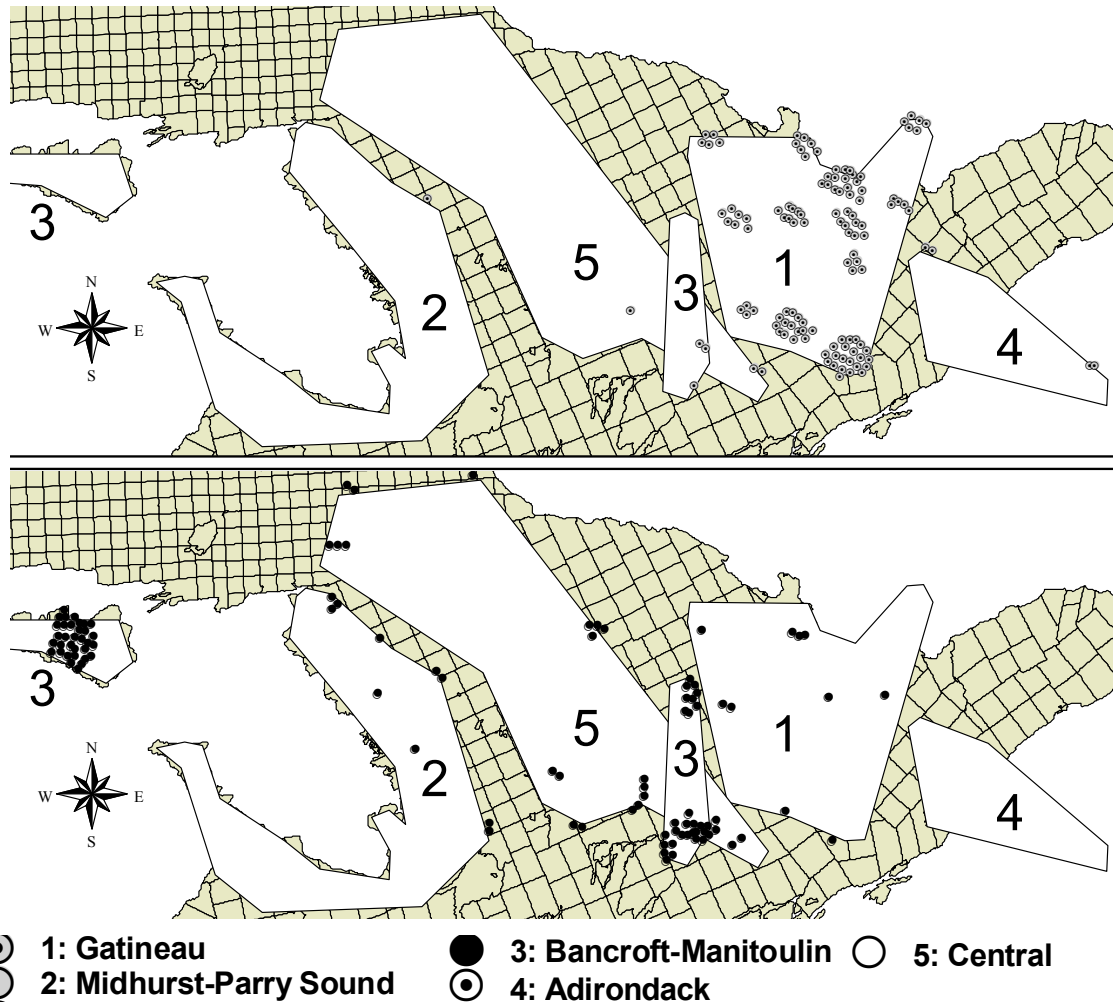


Figure 3.1 continued: Distribution of assigned individual fishers, where symbol represents assignment to an inferred genetic population. Individual symbols are clustered around landscape centroids. White polygons indicate proposed landscape cluster borders.

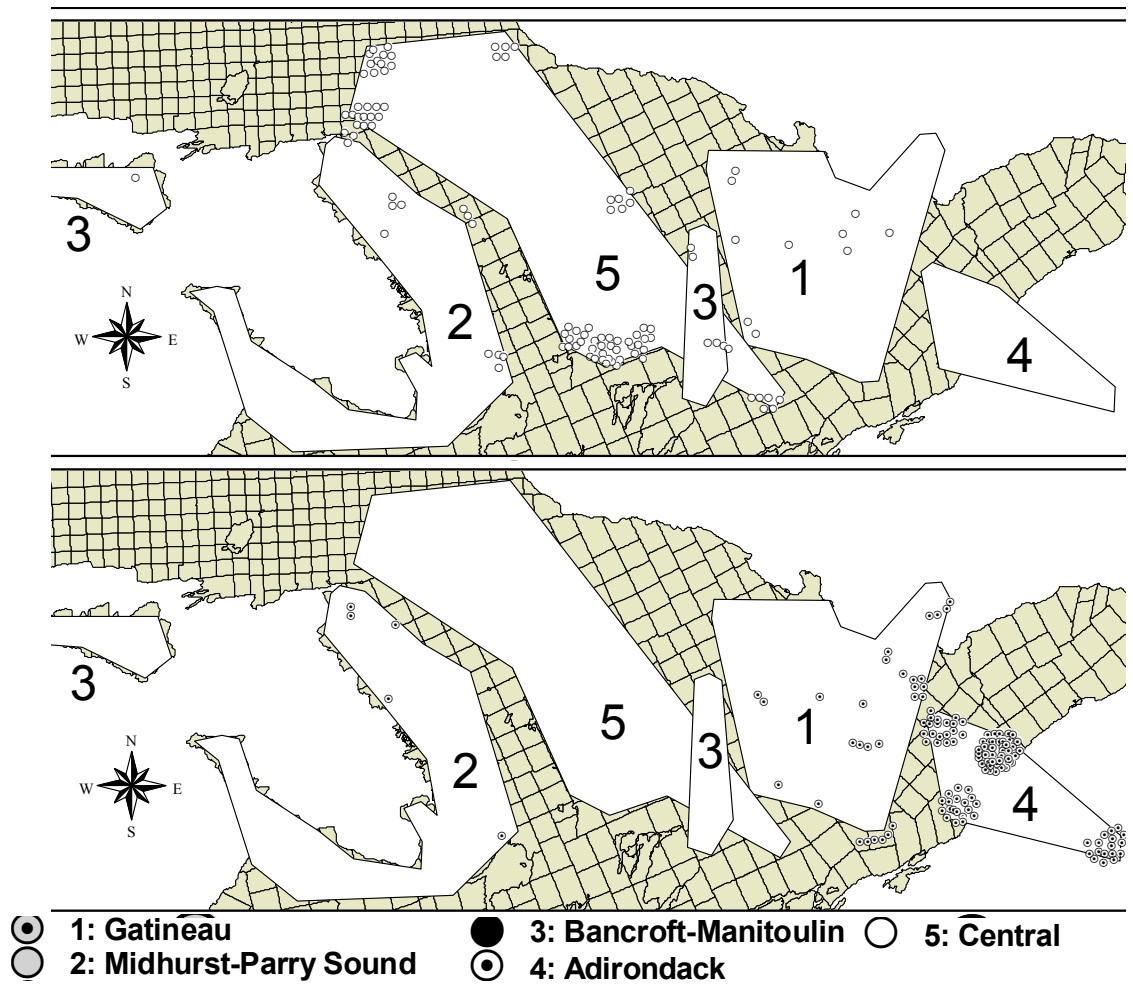


Figure 3.1 continued: Distribution of assigned individual fishers, where symbol represents assignment to an inferred genetic population. Individual symbols are clustered around landscape centroids. White polygons indicate proposed landscape cluster borders.

Table 3.1: Measures of fisher migration (#Im=direct count of individuals found in the cluster with membership ≥ 0.60 to an opposing cluster; #Im80=individuals found with ≥ 0.80 membership to an opposing cluster, Nm= number of effective migrants (Cockerham and Weir 1993); Nm Paetkau=number of first generation migrants using likelihood calculation (Paetkau et al. 2003); and Nm Coalescent = Beerli and Felsenstein 2001).

Cluster:Cluster	#Im	#Im80	Nm	Nm Paetkau	Nm Coalescent
1.2	9	4	6.03	6	18.60
1.3	13	3	5.26	4	11.83
1.4	33	17	4.60	9	16.66
1.5	13	4	4.41	11	19.80
2.3	16	5	7.99	4	11.10
2.4	6	2	1.98	2	4.81
2.5	36	14	12.72	11	19.58
3.4	0	0	1.55	2	3.38
3.5	27	6	7.72	9	16.09
4.5	0	0	1.50	1	5.18
Mean (\pm SD)	15.3 (\pm 12.8)	5.5 (\pm 5.7)	6.03 (\pm 3.49)	5.9 (\pm 3.8)	12.70 (\pm 6.40)

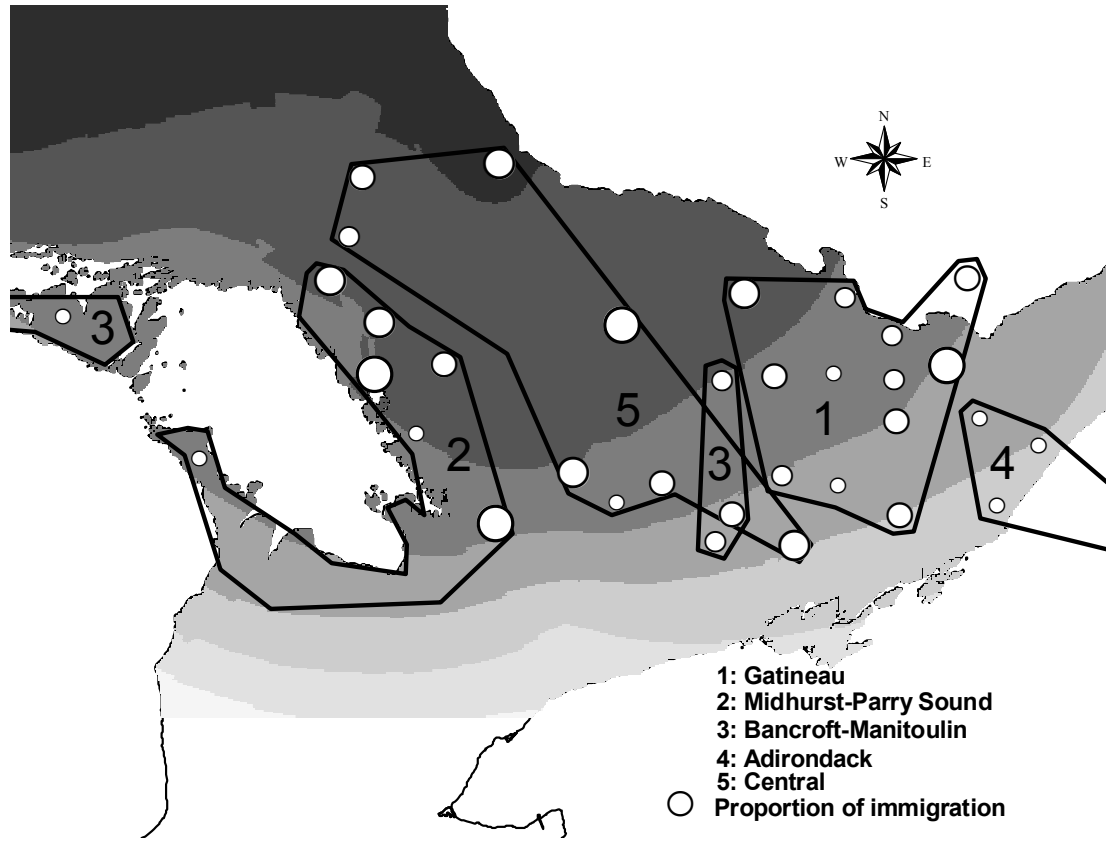


Figure 3.2: Average weekly snow depth from January to April in Ontario (darker shades represent deeper snow) and the proportion of immigration in each landscape (larger circles represent a larger proportion of immigrants).

Table 3.2: AIC scores for habitat variable models (proportions of forest cover per km², mean snow depth from January to April, and annual fisher harvest per km²) used in a regression with ppIm at the landscape scale (k= number of parameters).

Model	K	AIC
snow	1	-45.4691
coniferous+snow	2	-43.9477
deciduous+snow	2	-43.5996
snow+mix_sparse	2	-43.5724
snow+harvest (00/01)	2	-43.48266
snow+harvest (01/02)	2	-43.40222
snow+coniferous+deciduous	3	-42.3372
snow+coniferous+mix_sparse	3	-42.0946
snow+deciduous+mix_sparse	3	-41.6465
deciduous	1	-41.2511
mix+snow+coniferous+deciduous	4	-40.4463
coniferous	1	-40.1704
coniferous+deciduous	2	-39.0838
mix_sparse	1	-39.0208
harvest (00/01)	1	-38.74502
coniferous+mix_sparse	2	-38.5312
harvest (01/02)	1	-38.51620
all_forest	1	-38.45894
coniferous+deciduous+mix_sparse	3	-37.4719
deciduous+mix_sparse	2	-37.24

Chapter 4 General Discussion

Status of fishers in Ontario

The fisher has recovered from near extirpation in southern Ontario. They can be found in the majority of their former range and have recently expanded into south-eastern Ontario, below the Canadian shield. The latest expansion and recolonization from New York state is an unusual one because it is an expansion into an agricultural environment, which is atypical for the fisher.

High emigration was found out of the Midhurst-Parry Sound and Bancroft-Manitoulin clusters. These areas are currently productive for fishers. The Algonquin landscape proved to be less productive than we initially hypothesized, however it did act as a historical source for some recolonization and most likely remains a functional corridor of connectivity between the northern and southern boundaries of the Central genetic cluster.

The current connectivity of the landscape clusters can be qualitatively expressed in that the Adirondack reproductive front has only recently met the fisher expansion from the north and is marked by a small number of immigrants in opposing clusters and few landscapes containing individuals of mixed ancestry. While the four remaining clusters share admixed borders where hybridized individuals can be found.

Climate conditions may be driving fisher movement. Increases in average winter temperature correlate to decreases in snow depth. This could result in expansion into areas of habitat that were previously unsuitable or low quality for fishers.

Landscape Genetics

Landscape genetics has been mainly used in previous work to identify genetic discontinuities across a landscape and relate these discontinuities to ecological variables such as physical barriers (Manel *et.al.* 2003). We attempted to progress this technique by generating a biological response variable (proportion of immigrants) in many different landscapes, and examining the spatial patterns and ecological process that may be driving fisher population dynamics. We believe this to be a novel and successful application of landscape genetics and one worthy of consideration for future project designs.

Study Critique

Our individual immigrant classification is derived from a contrast between the genetic assignment of the individual and its landscape. Consequently, landscapes must have an assignment otherwise it becomes difficult to identify an immigrant or a resident. In the instances where landscapes could not be assigned we used a measure of gene flow (F_{ST}) to determine with which cluster the landscape shared the most gene flow and we assumed this to be the resident assignment. In our particular study this technique was credible because the clusters have only recently converged so the F_{ST} measure should detect historic gene flow to the resident group. This technique may not be applicable for genetic groups with greater historical admixture.

We assumed that high quality landscapes would be more productive for fishers and generate emigrants, while low quality landscapes should have high amounts of immigration. In order for this assumption to be valid, high quality habitat needs to have been previously occupied by residents so that dispersers have only lower quality habitat

to occupy. There also exists the possibility of complete immigrant dissemination into a landscape. Immigrant fishers in this situation would be recognized as residents by our method and the landscape would be considered to have zero $ppIm$ when actually it should be 100%. This situation is possible for the landscapes of the Adirondack cluster in southeastern Ontario. For these landscapes, we rationalized that since the area was devoid of fishers, the expanding immigrants from New York State are in effect the recolonizing residents.

Manel *et al.* (2005) pose the question in their recent study: how well do clustering methods perform when genetic differentiation is modest ($F_{ST} \leq 0.05$?). We can address this question with regard to the difference between genetic diversity in an inferred genetic population and a landscape cluster. A cluster is based upon the grouping of similarly assigned individuals within a geographic space, while an inferred genetic population is based on the composition of individual allele frequencies independent of space. Ultimately, clusters can share low F_{ST} scores but the individuals within the cluster can still have high assignment values since the overall inferred genetic groups are still distinct. This is the case with most groups in our study, where migration of individuals into opposing clusters was driving the low F_{ST} score, since F_{ST} cannot very well distinguish between historical and contemporary gene flow (Berry *et al.* 2004). This will become problematic though as hybridization between the groups occurs, and as unique alleles and allele frequencies become shared, differentiation will become difficult to discern.

We used a fully Bayesian assignment test method to identify the number of inferred genetic populations and the assignment of individuals to these populations. This

method assumes that all populations are being sampled. In our study this assumption is valid, since the size of our sample lattice encompassed all known remnant populations and the areas between them.

Recommendations for future study

Additional research should focus on examining whether dispersion of fishers throughout our study area is sex and/or age biased. Genetic methods, such as those in the program FSTAT, can identify these trends and would be useful in comparison with field studies like those previously mentioned in Prescott (E. Koen, in prep.) and Algonquin Provincial Park (S. Tully and J. Bowman, in prep.).

It may be useful to sample areas of admixture, where the reproductive fronts have merged, and examine the genetic composition of these individuals at particular intervals. A genetic assessment of the individuals in these areas may give an indication of the expansion status of the reproductive fronts. For example, whether a more productive front is out competing its neighbour. A similar type of analysis is being applied to admixed fish populations to assess the degree of impact historic and stocked sources are having on the genetic attributes of resident fish (Pella and Masuda, 2001).

Exploring the relationship between fisher populations and prey abundance would be an excellent addition to this study. Regrettably, prey abundance data at this scale and for each of our landscapes would be difficult to obtain.

The proportion of individuals that completely assign and those which are admixed in a particular landscape may be interesting variables to test ecological and environmental data with; similar to what was done in this study. The proportion of individuals that

completely assign in a landscape may be representative of the core residents of a landscape cluster and the monopoly they retain on the landscape. It also may be indicative of what represents high quality habitat, since residents would persist in high quality areas preventing the establishment of dispersers. Admixture could be an indication of transition zones but is most likely a product of the proximity of neighbouring clusters. Areas of admixture are likely to occur where reproductive fronts meet. Whether these areas of admixture are also areas of hybridization would be interesting to investigate, since hybridization would indicate that individuals of different ancestry were not only inhabiting the same landscape but reproducing with each other.

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Appendix A:

Table A.1: Landscape assignment based on a STRUCTURE simulation run with a *posteriori* landscape information. Values indicate the posterior probability of a landscape assigning to a particular inferred genetic population.

TWP	n	Cluster	1	2	3	4	5
Gatineau							
Angelsea-Grimsthorpe (AG)	16	1	0.338	0.078	0.190	0.161	0.233
Brougham (BR)	23	1	0.406	0.107	0.187	0.090	0.210
Dalhousie (DL)	20	1	0.410	0.096	0.139	0.268	0.088
Darling (DR)	22	1	0.470	0.074	0.162	0.144	0.151
Fraser-Richards (FR)	21	1	0.346	0.315	0.142	0.046	0.150
Gatineau (GA)	18	1	0.410	0.121	0.080	0.339	0.051
Kennebec (KB)	23	1	0.635	0.040	0.113	0.163	0.050
Loughboro-Bedford-Portland (LBP)	31	1	0.625	0.030	0.059	0.258	0.028
Lyndoch (LN)	19	1	0.349	0.103	0.265	0.126	0.157
McNab (MN)	24	1	0.669	0.071	0.067	0.130	0.063
Ramsey-Huntley (RH)	20	1	0.340	0.056	0.088	0.428	0.088
Ross (RO)	19	1	0.469	0.181	0.211	0.054	0.085
Midhurst-Perry Sound							
Blair-Mowat (BM)	26	2	0.082	0.405	0.217	0.102	0.194
Burton-McKenzie (BK)	16	2	0.050	0.424	0.213	0.086	0.227
Carling-Ferguson (CF)	8	2	0.061	0.362	0.141	0.135	0.300
Conger-Freeman (CM)	15	2	0.035	0.592	0.196	0.014	0.162
Eastnor-Lindsay-StEdmunds (ELS)	26	2	0.020	0.862	0.069	0.013	0.036
Montieth-Christie-Spence (MCS)	26	2	0.078	0.472	0.203	0.029	0.218
OrilliaRamarara (OR)	17	2	0.105	0.310	0.191	0.072	0.322
Bancroft Manitoulin Island							
Belmont (BL)	7	3	0.123	0.030	0.717	0.013	0.117
Carlow-Bangor (CB)	20	3	0.111	0.176	0.473	0.036	0.203
Manitoulin Island (MI)	40	3	0.035	0.125	0.751	0.015	0.075
Marmora-Lake (ML)	32	3	0.148	0.108	0.528	0.023	0.193
Adirondack							
Adirondack (AD)	22	4	0.117	0.101	0.051	0.664	0.067
Escott-Yonge (EY)	20	4	0.077	0.017	0.026	0.859	0.022
Montague (MT)	21	4	0.153	0.027	0.043	0.738	0.039
Prescott (PR)	48	4	0.024	0.015	0.020	0.921	0.020
Central							
Algonquin (AQ)	20	5	0.043	0.333	0.195	0.020	0.410
Anson-Lutterworth (AL)	25	5	0.035	0.330	0.155	0.020	0.460
Anstruther (AS)	24	5	0.057	0.137	0.280	0.022	0.505
Badgerow (BR)	22	5	0.051	0.222	0.134	0.016	0.577
Falconer (FL)	22	5	0.045	0.143	0.222	0.021	0.569
Galway (GW)	20	5	0.024	0.040	0.134	0.017	0.785
Hungerford-Huntington (HH)	14	5	0.212	0.068	0.182	0.067	0.472
Olrig Cluster (OL)	15	5	0.075	0.337	0.157	0.030	0.401

Appendix B

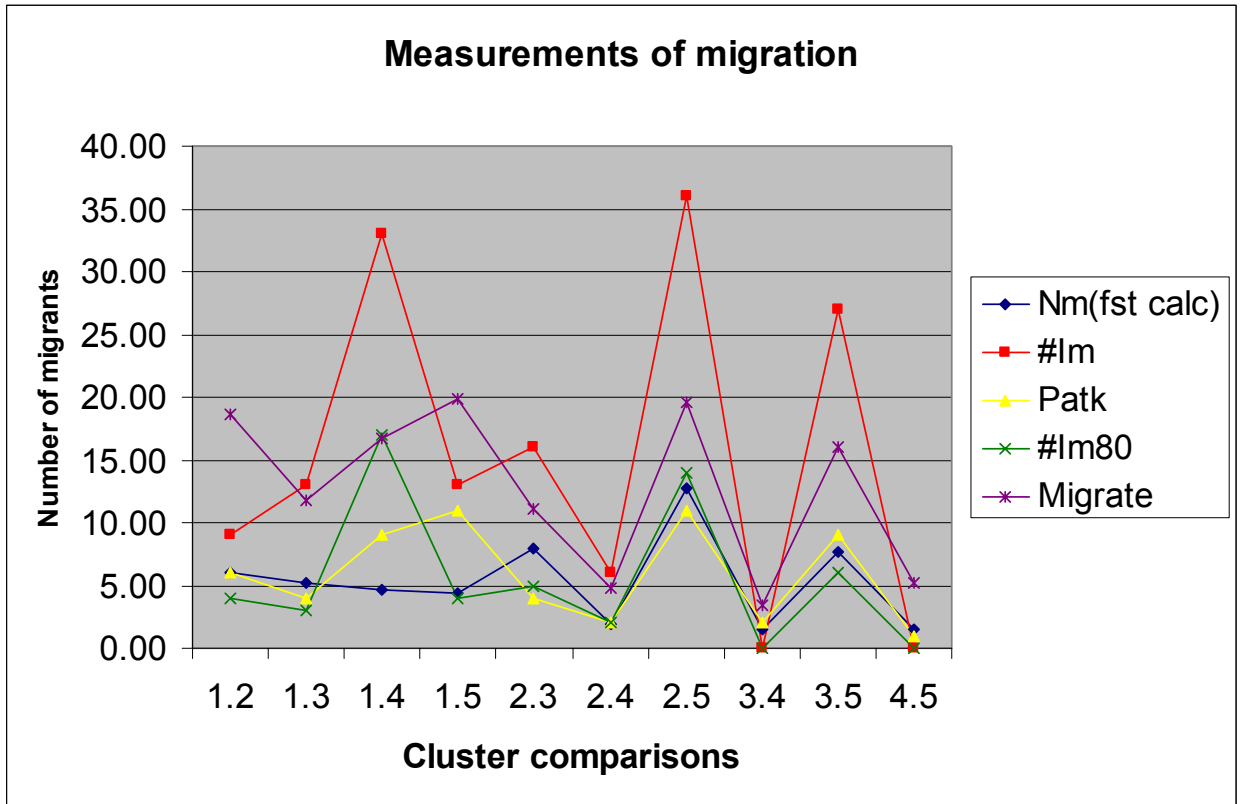


Figure B.1: Comparison of migration measures between genetic clusters. Measures of fisher migration ($\#Im$ =direct count of individuals found in the cluster with membership ≥ 0.60 to an opposing cluster; $\#Im80$ =individuals found with ≥ 0.80 membership to an opposing cluster, Nm = number of effective migrants (Cockerham and Weir 1993); Nm Paetkau=number of first generation migrants using likelihood calculation (Paetkau et al. 2003); and Nm Coalescent = Beerli and Felsenstein 2001).