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The genetic signature of rapid range expansion by flying squirrels in response to contemporary climate warming

COLIN J. GARROWAY*, JEFF BOWMAN†, GILLIAN L. HOLLOWAY‡, JAY R. MALCOLM§ and PAUL J. WILSON¶

Environmental and Life Sciences Graduate Program, Trent University, Trent University DNA Building, 2140 East Bank Drive, Peterborough, Ontario, Canada K9J 7B8, †Ontario Ministry of Natural Resources, Trent University DNA Building, 2140 East Bank Drive, Peterborough, Ontario, Canada K9J 7B8, ‡Fiera Biological Consulting, Suite 200, 10318-82 Avenue, Edmonton, Alberta, Canada T6E 1Z8, §Faculty of Forestry, University of Toronto, Toronto, Ontario, Canada M5S 3B3, ¶Biology Department, Trent University, Trent University DNA Building, 2140 East Bank Drive, Peterborough, Ontario, Canada K9J 7B8

Abstract

Climate is an important factor limiting species distributions. Historic climate-change related range movements have modified the genetic diversity of species by the merging and splitting of gene pools and by the effects associated with recurrent founder events. These effects are often inferred, either from retrospective analyses of current genetic patterns or from simulations. Rarely has it been possible for the population genetic effects of range expansion to be examined with contemporaneous demographic data. We characterized the genetic signature of rapid range expansion by southern flying squirrels (*Glaucomys volans*) and compared these results to a stationary population of the closely related northern flying squirrel (*Glaucomys sabrinus*) in Ontario, Canada. Samples were taken during an approximately 200 km range expansion by *G. volans* (1994–2003) and genotyped at 6 (*G. sabrinus*) and 8 (*G. volans*) microsatellite loci. For *G. volans*, but not *G. sabrinus*, we found evidence of a latitudinal gradient in allele frequencies and a decrease in allelic richness along the axis of expansion. We found no evidence of isolation-by-distance in either species or of genetic bottlenecks in the area of *G. volans* expansion. These results suggest that serial founder events can cause an immediate reduction in genetic diversity following rapid range expansion with high levels of gene flow giving rise to heterogeneity within what would classically be termed panmixia. Given the pace of anthropogenic climate change, and the increasing incidence of range movements in response, this may be an important, immediate consequence of climate change.

Keywords: climate change, genetic diversity, genetic structure, *Glaucomys sabrinus*, *Glaucomys volans*, range expansion, range movements

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Introduction

Climatic constraints are among the key limiting factors contributing to species range limits (Grinnell, 1917; MacArthur, 1972; Gaston, 2003; Parmesan, 2006). Accordingly, in response to contemporary climate change, many species distributions are shifting, tracking their climatic niche (e.g., Parmesan & Yohe, 2003; Parmesan, 2006). Despite numerous reports of recent climate-change related range shifts, empirical studies of the population genetic consequences of these range shifts are still rare, especially where demographic and genetic responses are observed simultaneously. In contrast, the genetic consequences of historic climate-change related range shifts are comparatively well characterized. Periodic changes in the Earth's orbit have led to recurrent large-scale climate fluctuations (Milankovitch cycles) that vary with periods of 19 and 23 000 years (changes in the annual timing of minimum Earth-Sun distance), 41 000 years (related to the tilt of Earth's axis), and 100 000 years (related to eccentricity of the Earth's orbit) (Berger, 1988; Imbrie *et al*., 1989). The magnitudes of these climate changes are not spatially uniform and their impacts on biodiversity are presumably instructive of the consequences of contemporary climate change. In areas with the highest magnitude climate shifts there has typically been a net loss of biodiversity following population range movements as a result of the merging of gene pools and the extirpation of populations unable to track their climatic niche (Dynesius & Jansson, 2000; Jansson & Dynesius, 2002). Given the additive nature (additional to Milankovitch cycles) and rapid pace of contemporary anthropogenic climate

Correspondence: Colin J. Garroway, tel. +1 705 755 1535, fax +1 705 755 1559, e-mail: colin.garroway@gmail.com

change, we consider it important to understand the population genetic effects of one of its most general consequences, rapid range expansions.

There are a number of commonly occurring genetic signatures of historic range expansions (Excoffier *et al*., 2009). First, for neutral alleles we can expect to find allele frequency gradients along the axis of expansion as a result of serial founder effects emanating from the previous range margin (Novembre & Di Rienzo, 2009). Another consequence of historic range expansions is reduced genetic diversity in the newly colonized area (Austerlitz *et al*., 1997; Excoffier *et al*., 2009). Finally there is often differentiation of peripheral populations due to genetic drift and rare allele and mutation surfing at population frontiers (Hallatschek *et al*., 2007; Excoffier *et al*., 2009; Hallatschek & Nelson, 2009).

Recent demographic studies based upon trapping have shown that the southern flying squirrel (*Glaucomys volans*) is limited at its northern range boundary by cold winter temperatures, and has been expanding its range to the north with climate warming (Bowman *et al*., 2005; Myers *et al*., 2009). In Ontario, Canada, a study of the range boundary dynamics of *G. volans* and its parapatric sister species, the northern flying squirrel (*G. sabrinus*) found evidence for a large (>200 km), rapid (1995–2003) northward range expansion of *G. volans* into the range of *G. sabrinus* (Fig. 1; Bowman *et al*., 2005). This range expansion was associated with a series of warm winters and high mast years (Bowman *et al*., 2005). For southern flying squirrels in Ontario oak acorns (*Quercus* spp.) and beechnuts (*Fagus grandifolia*) are the most important mast crops (Stabb, 1988) and the abundance of flying squirrels varies with mast abundance with a 1-year time lag (Bowman *et al*., 2005). Bowman *et al*. (2005) used temperature data to retrospectively estimate that the *G. volans* range expansion began in 1995. Thus, the squirrels are estimated to have spread north 200 km in 9 years (1994–2003). Following a particularly cold winter (2003–2004) that coincided with a mast crop failure, the *G. volans* northern range limit appeared to contract >240 km, returning to its historical range boundary.

During the period of *G. volans* range expansion we also trapped *G. sabrinus*. In some ways these closely related species are ecologically similar. They are both nocturnal, small-bodied flying squirrels that rely on trees for nesting, food, and locomotion. Differences include a tendency to use different forest and food types: *G. volans* typically use hardwood stands and rely primarily on beechnuts and acorns for food whereas *G. sabrinus* typically use softwood stands and rely to a larger extent on fungi for food (Holloway & Malcolm, 2006, 2007a, 2007b; Coombs, 2010). We observed no evidence for *G. sabrinus* range movement; thus, we repeated all analyses on *G. sabrinus* samples, considering that *G. sabrinus* provided an opportunity to compare our findings for *G. volans* range movement to a closely related, ecologically similar species in the same region with no range movement.

We expected that the genetic signature of contemporary range expansions should reflect a combination of both recent and historic processes. We predicted that we should find genetic evidence for the early stages of range expansion in *G. volans* but not in *G. sabrinus*. Specifically, we predicted that we should find a latitudinal allele frequency gradient beginning at the putative point of range expansion extending north into the newly occupied sites for *G. volans*. Second, we predicted that genetic diversity should decrease beginning from the point of expansion through to the new northern range margin for *G. volans*. Subsampling of neutral alleles at the expansion frontier (serial founder effects) can produce clines in both allele frequencies and genetic diversity along the axis of expansion (Novembre & Di Rienzo, 2009). Alternatively, given a stable range margin we would expect alleles to be distributed near randomly in space. Finally, counter to the expected genetic signature of historic range expansion, we expected to find little if any significant genetic differentiation between sample sites toward the new population periphery. We had this expectation because there was likely too large an effective population size and too few generations for genetic drift to have had a strong impact at the range frontier given what was likely a continuous expansion front of large numbers of individuals over long distances with high levels of gene flow (estimated rate of expansion of 22 km per year; Bowman *et al*., 2005). High levels of gene flow will likely not impede differentiation for long time periods (Excoffier & Ray, 2008; Hallatschek & Nelson, 2009) and we would consider the presence of isolation-by-distance as evidence of less recent range expansion or perhaps of expansion from remnant populations above the putative northern range boundary. Thus, we expected to find only weak or no isolation-by-distance if this was a recent range expansion. Because there was no evidence for *G. sabrinus* range movement based upon trapping data we did not predict directional allele frequency gradients or clines in diversity. Trapping data strongly suggested that *G. volans* expanded their range from the previously identified northern range boundary of the contiguous population (Bowman *et al*., 2005). However, an alternative explanation is that *G. volans* expanded from remnant populations above this putative range limit (Bowman *et al*., 2005). Remnant, small populations, isolated from the core population should diverge and differentiate through genetic drift and possibly local adaption. Therefore, if this was an expansion from

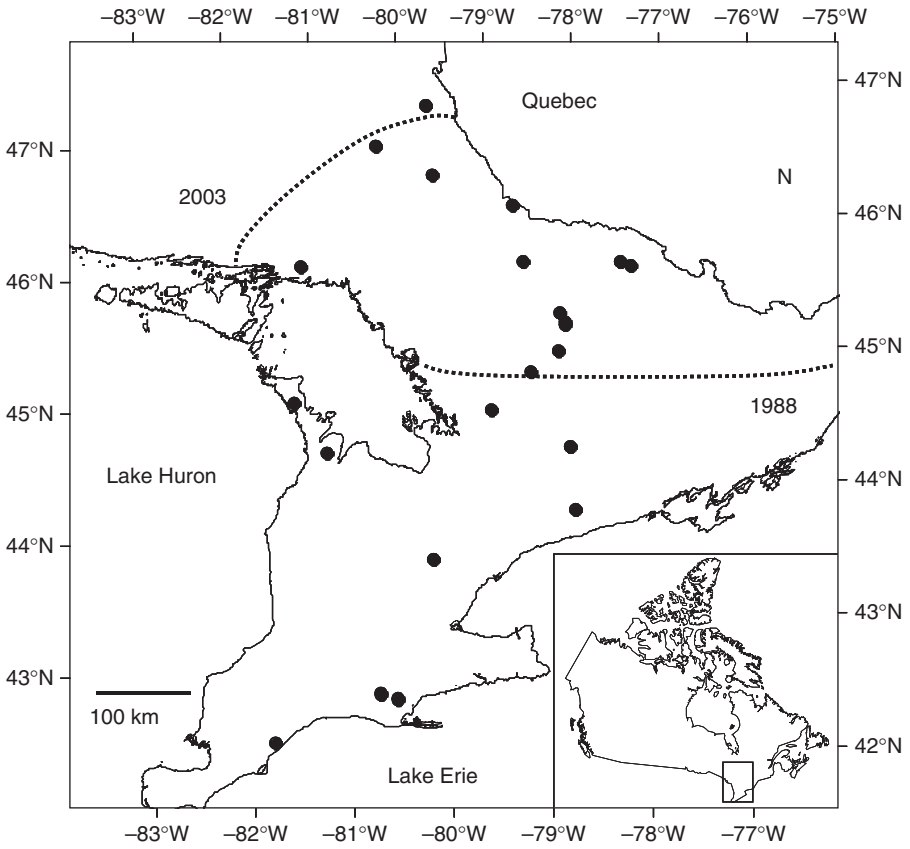

Fig. 1 Map of sample sites for northern (*Glaucomys sabrinus*) and southern (*G. volans*) flying squirrels in Ontario. During the sampling period *G. volans* expanded from their previously delineated 1988 range boundary (Stabb, 1988) >200 km by 2003 as depicted by the dashed lines on the map.

remnant populations we would expect to find: genetic differentiation between samples from the range margin and the southern contiguous populations; isolation-by-distance; and genetic bottlenecks in the area of range expansion. Thus, we tested for the presence of genetically differentiable clusters of individuals, genetic bottlenecks, and isolation-by-distance to address this alternative explanation for the *G. volans* range expansion with genetic data.

Materials and methods

Live-trapping for flying squirrels in Ontario, Canada took place at 22 sites along an approximate north–south transect from the north shore of Lake Erie (42.52°N latitude) to a site north of Temagami (47.29°N latitude; Fig. 1). Trapping began in 2002 and finished in 2004, the summer after the *G. volans* population crashed (45 771 total trap nights). In 2002 and 2003, southern flying squirrels were captured at distances of up to 200 km farther north than their previously delineated range limit (Stabb, 1988; 24 139 trap nights north of 1988 limit; 788 captures of 506 *G. volans*). In 2004 the northern range limit contracted by approximately 240 km to near the limit delineated in 1988. Five sites north of this 1988 limit were sampled both before (2003) and after (2004) the range contraction. In 2003, there were 485 captures of 309 *G. volans* (13 397 trap nights) and in 2004 no *G. volans* were captured (12 945 trap nights). During the 2002–2004 surveys, we had 376 captures of 232 *G. sabrinus*. Subsets of these captured individuals had hairs plucked for subsequent molecular analyses. Samples from Bowman *et al*. (2005) were supplemented with samples from concurrent studies in southern Ontario (Bednarczuk, 2003). In total, a subset of 171 *G. volans* and 147 *G. sabrinus* had hairs plucked for molecular analysis (See Table 1 for sample sizes per site). Slightly different subsets of eight and six microsatellite loci amplified diagnosable alleles for *G. volans* and *G. sabrinus* respectively. For *G. volans* these were SFS3, SFS15 (Fokidis *et al*., 2003), GS4, GS8, GS10 (Zittlau *et al*., 2000), Pvol41, Pvol74, and PvolE6 (Painter *et al*., 2004). For *G. sabrinus* these were SFS3 (Fokidis *et al*., 2003), GS8, GS10 (Zittlau *et al*., 2000), Pvol41, Pvol74, and PvolE6 (Painter *et al*., 2004). Details of reaction conditions for SFS3, SFS15, GS4, GS8, and GS10 are available in Garroway *et al*. (2010). Briefly, DNA was amplified using the polymerase chain reaction. Multiplex reactions of amplified product were run on an ABI 3730 genetic analyzer (Applied Biosystems, Valancia, CA, USA) and genotypes were scored using Genemarker software (SoftGenetics, State

Table 1 Summary data for northern (*Glaucomys sabrinus*) and southern (*Glaucomys volans*) flying squirrels sampled during a range expansion in Ontario, Canada

	n	H_o	H_e	AR	F_{IS}	Latitude	Longitude
Glaucomys sabrinus							
Achray	5	0.617	0.684	4.41	0.111	45.93	−77.78
Aurora	18	0.637	0.704	4.21	0.1	43.80	−80.01
Bruce County	15	0.657	0.736	4.27	0.12	45.04	−81.38
Grey County	15	0.545	0.656	4.05	0.143	44.65	−81.07
Haliburton	15	0.619	0.694	4.36	0.135	45.31	−78.54
North Algonquin	12	0.677	0.707	4.75	0.049	45.49	−78.44
South Algonquin	20	0.697	0.709	4.28	0.007	45.59	−78.48
Killarney	5	0.55	0.605	4.01	0.05	45.35	−80.23
Mattawa	7	0.706	0.665	4.21	−0.071	42.82	−80.64
Peterborough	11	0.605	0.67	3.94	0.101	44.10	−78.50
Temagami	24	0.702	0.747	4.58	0.044	47.23	−79.77
Glaucomys volans							
Abbot	14	0.685	0.747	5.47	0.094	42.81	−80.64
Dorset	15	0.633	0.765	5.3	0.186	45.16	−78.85
Earl	11	0.769	0.717	5.25	−0.073	42.76	−80.47
Kawartha/Peterborough	19	0.661	0.771	5.69	0.146	44.57	−78.49
Mattawa	11	0.571	0.672	4.42	0.146	46.43	−78.89
Middleton	14	0.687	0.745	5.07	0.081	42.82	−80.64
Minden	17	0.732	0.785	5.62	0.063	44.90	−79.30
North Algonquin/Killarny	15	0.639	0.742	4.85	0.124	45.89	−77.68
Rondeau	8	0.586	0.712	5.18	0.173	42.48	−81.73
South Algonquin/Killbear	15	0.575	0.724	4.99	0.181	45.51	−78.43
Swick	15	0.707	0.714	4.95	−0.006	42.77	−80.47

G. sabrinus were genotyped at 5 and *G. volans* 8 microsatellite loci. Sample size (n), observed (H_o) and expected (H_e) heterozygosity, allelic richness corrected for sample size (AR), and the inbreeding coefficient are presented for each sample site. *P*-values (P) are for tests for genetic bottleneck with one-way Wilcoxon's signed-rank tests for heterozygote excess.

College, PA, USA). Pvol41, Pvol74, and PvolE6 (Painter *et al*., 2004) amplifications were performed in 10 μL volumes containing 3 μL of stock DNA, 0.2 mM of each dNTP, 1X Qiagen PCR Buffer, 0.3–0.5 μM of forward and reverse primer, and 0.5 U of Taq polymerase (Qiagen Inc., Valencia, CA, USA). Samples were amplified under the following conditions (same conditions for each locus except where noted in parentheses): 94 °C for 5 min, 94 °C for 30 s, 53 °C for 1 min (52 °C for Pvol73 and 50 °C for Pvol41), 72 °C for 1 min, 94 °C for 30 s for 34 cycles, and 60 °C for 45 min. Multiplex reactions were implemented to reduce the number of PCR reactions and combinations of multiplex products were then pooled to minimize the number of runs on an ABI 3730 genetic analyzer (Applied Biosystems, Foster city, CA, USA). Amplified product from multiplex reactions were added in equal proportions and 10 μL was ethanol precipitated and re-suspended in 5 μL of ddH20 for loading on the ABI 3730. Genotypes were scored using GeneMarker software (SoftGenetics).

Data Analyses

We used spatially explicit analyses, including both Bayesian and non-Bayesian approaches, to characterize population genetic structure of both flying squirrel species. Specifically, we used Geneland software for Bayesian clustering of individuals incorporating spatial coordinates as model priors (Guillot *et al*., 2005, 2008) and spatial principal components analysis (sPCA), a spatially explicit multivariate method for analysing the spatial variation of allele frequencies including identifying clusters and allele frequency gradients (Jombart *et al*., 2008). Spatial PCA makes no assumptions regarding Hardy-Weinberg or linkage equilibrium (HWE and LE, respectively); thus, it provides a useful compliment to Geneland. Spatial PCA has another important feature, different than Geneland analyses, in that it can explicitly identify spatial clines. This is important because, as noted by Guillot *et al*. (2005) and recently demonstrated via simulation (Frantz *et al*., 2009; Schwartz & McKelvey, 2009), deviations from random mating not caused by hard barriers to gene flow (such as spatial autocorrelation of allele frequencies and isolation-by-distance) and sampling scheme can affect the detection and interpretation of genetic structure.

Geneland clusters individuals such that HWE and LE of multilocus genotypes are maximized. Individual ancestry is then assigned probabilistically to clusters by calculating the probability of an individuals' multilocus genotype given allele frequencies in those populations. Geneland treats the number

of inferred populations (K) as a parameter to be processed in the Markov Chain Monte Carlo (MCMC) algorithm. It can also incorporate potential error in spatial locations by treating true coordinates as unknown parameters to be estimated and can explicitly account for the presence of null alleles within its clustering algorithm. We set parameters for 3 000 000 MCMC iterations, the maximum rate of Poisson's process for 100, and the uncertainty of spatial coordinates for 3 km. We ran the MCMC 25 times allowing K to vary between 1 and 10 to infer K. We then ran the MCMC another 25 times with K fixed at the inferred value from the initial runs to estimate parameters. From this set we selected the run with the highest log posterior probability of population membership for subsequent analyses. The posterior probability of population membership for pixels was computed with a burn-in of 500 000 iterations. The number of pixels was set to 300 along the x-axis and y-axis. Finally, we computed the posterior probability of population membership for pixels and the inferred population membership of individuals to model populations.

Spatial PCA incorporates spatial autocorrelation of allele frequencies into the traditional principal component analysis framework and defines synthetic components that optimize the product of the variance in the data and Moran's I (Moran, 1948, 1950) to summarize spatial patterns of genetic structure (Jombart *et al*., 2008). These components are separated into those that define global (positive eigenvalues) and local (negative eigenvalues) structures. Global scores can identify genetically distinguishable clusters, clines in allele frequencies, and intermediate samples; local scores can detect local differentiation between neighbouring sites. To calculate Moran's I we needed to define neighbouring trap sites. Because our sampling scheme was irregular we chose to define neighbours with an inverse distance neighbouring graph. Thus, all trap sites were connected and spatial weights were proportional to the inverse distance between sites. To help determine whether global or local structures should be interpreted we tested the null hypothesis that scores were distributed randomly on the connection network (Jombart *et al*., 2008). Alternatively, local or global scores from at least one component could display some spatial structure. The null distributions were determined via permutations ($n = 9999$) and the P-values were the relative frequency of permutated values equal to or greater than the test statistics. sPCA was implemented using the Adegenet package (Jombart, 2008) and visualized using the Ade4 (Dray & Dufour, 2007) package in R (The R Core Development Team, 2008).

Typically, populations that undergo a pronounced reduction in size will lose rare alleles and have their heterozygosity reduced; they suffer a genetic bottleneck. These alleles, because they are rare, do not contribute significantly to heterozygosity. Thus, when there is a genetic bottleneck the number of alleles is reduced at a faster rate than heterozygosity leading to the testable prediction that heterozygosity will be greater than the expected equilibrium heterozygosity in populations that have undergone a genetic bottleneck. We tested for genetic bottlenecks with the Bottleneck software (Piry *et al*., 1999). We assumed the 2-phase mutation model and assessed differences with Wilcoxon signed-rank tests as recommended by Piry *et al*. (1999) for microsatellite data sets with <20 individuals per population genotyped.

Standard Genetic Measures

We used the Adegenet package (Jombart, 2008) in R (The R Core Development Team, 2008) to test for deviations from HWE for each locus and sample and to calculate observed (H_o) and expected (H_e) heterozygosities. We calculated allelic richness (A) standardized for sample size via rarefaction (Petit *et al*., 1998) in FSTAT (Goudet, 1995). We calculated D_{est} (Jost, 2008) as a measure of absolute differentiation between sample sites using code written by the us in R (The R Core Development Team, 2008) and features of the Adegenet package (Jombart, 2008) and tested for isolation by distance with Mantel tests. For these summary statistics we pooled *G. volans* samples from Northern Algonquin Park and Killarney as well as Kawartha Highlands and Peterborough due to low sample sizes. We chose these groupings based upon similar latitudes. No locations were pooled for other genetic analyses.

Results

After sequential Bonferroni corrections for multiple testing we found minimal evidence for deviation from HWE at locus-population combinations. For *G. sabrinus* Pvol74 in South Algonquin and Pvol41 in North Algonquin and Peterborough deviated from HWE expectations. For *G. volans* Pvol74 at Middleton and Pvol41 at Earl deviated from HWE expectations. Similarly, there was no evidence for deviation from LE. Inbreeding (F_{IS}) tended to increase with latitude for *G. volans* (Table 1). This relationship was not significant; the Rondeau sample site, the site with fewest samples, was an outlier with higher F_{IS} than other geographically close sites. Differentiation among sample sites was also low for both species (Tables 2 and 3). *Glaucomys sabrinus* sample sites tended to be more differentiated than *G. volans*. There was no evidence of isolation-by-distance for either species (Mantel tests: *G. sabrinus*: $r = 0.04$, $P = 0.23$; *G. volans*: $r = 0.03$, $P = 0.42$).

For both species the posterior density and log-likelihood levels of the 25 replicate runs of Geneland to estimate K stabilized well before the end of the MCMC runs indicating that they had reached convergence. For *G. sabrinus*, replicate MCMC runs converged on $K = 1$ for all 25 runs. For *G. volans* 19 of the 25 runs converged on $K = 1$ with the remaining six runs converging on $K = 2$. The six runs that converged on $K = 2$ all differentiated individuals from the two northeastern-most sample sites. Together these results suggested that there was no detectable genetic clusters among individuals within either species in Ontario with this model based approach.

Clustering with sPCA also suggested that there were no genetically distinguishable units within either species. For *G. sabrinus*, permutation tests assessing the importance of local ($P = 0.45$) and global ($P = 0.23$)

Table 2 Pairwise genetic differentiation (D_{est}; Jost, 2008) between *Glaucomys sabrinus* sample sites in Ontario, Canada

	Aurora	Bruce County	Grey County	Haliburton	North Algonquin	South Algonquin	Killarney	Mattawa	Peterborough	Temagami
Achray	0.104	0.013	0.045	0.003	0.004	0.000	0.135	0.014	0.251	0.028
Aurora	–	0.100	0.119	0.092	0.048	0.064	0.161	0.000	0.121	0.094
Bruce County	–	–	0.102	0.083	0.088	0.041	0.148	0.051	0.205	0.159
Grey County	–	–	–	0.048	0.072	0.051	0.138	0.010	0.100	0.061
Haliburton	–	–	–	–	0.000	0.023	0.109	0.040	0.107	0.065
North Algonquin	–	–	–	–	–	0.009	0.154	0.000	0.100	0.039
South Algonquin	–	–	–	–	–	–	0.090	0.000	0.071	0.028
Killarney	–	–	–	–	–	–	–	0.149	0.014	0.000
Mattawa	–	–	–	–	–	–	–	–	0.062	0.019
Peterborough	–	–	–	–	–	–	–	–	–	0.053

Table 3 Pairwise genetic differentiation (D_{est}; Jost, 2008) between *Glaucomys volans* sample sites in Ontario, Canada

	Dorset	Earl	Kawartha/ Peterborough	Mattawa	Middleton	Minden	North Algonquin/ Killarny	Rondeau	South Algonquin/ Killbear	Swick
Abbot	0.041	0.016	0.010	0.038	0.000	0.001	0.112	0.021	−0.004	0.052
Dorset	–	0.045	0.002	0.001	0.051	0.001	0.035	0.025	0.003	0.025
Earl	–	–	0.054	0.010	0.047	0.032	0.168	0.027	0.002	0.024
Kawartha/Peterborough	–	–	–	0.008	0.056	0.001	0.136	0.075	0.000	0.090
Mattawa	–	–	–	–	0.043	0.041	0.057	0.000	0.013	0.041
Middleton	–	–	–	–	–	0.051	0.074	0.007	0.028	0.086
Minden	–	–	–	–	–	–	0.129	0.038	0.000	0.040
North Algonquin/Killarny	–	–	–	–	–	–	–	0.041	0.118	0.073
Rondeau	–	–	–	–	–	–	–	–	0.045	0.052
South Algonquin/Killbear	–	–	–	–	–	–	–	–	–	0.040

components suggested that there was no significant genetic structure (Fig. 2). Permutation tests for *G. volans* suggested that there was no important local structure ($P = 0.21$) but likely important global structure ($P = 0.046$; Fig. 3). Plots of scores from the first global component suggest that this structure was related to south–north spatial autocorrelation of allele frequencies at sample sites in the region of range expansion (Fig. 3).

For *G. sabrinus*, allelic richness (corrected for sample size with rarefaction) was highest at the northernmost sample site and decreased, but not significantly ($F = 2.75$; df = 1, 9; $P = 0.13$; $R^2 = 0.15$), as sample sites progressed farther south (Fig. 4). For *G. volans* the northern-most sample site was the least diverse and allelic richness increased the farther south the sample sites were. The trend across all *G. volans* sample sites was not significant ($F = 1.21$; df = 1, 9; $P = 0.29$; $R^2 = 0.02$). However, when we examined allelic richness at sites beginning at the point of expansion, through to the new northern limit there was a distinct, significant decrease ($F = 196.7$; df = 1, 4; $P < 0.0001$; $R^2 = 0.97$; Fig. 5); that is, allelic richness of *G. volans* declined towards the new, expanded northern range boundary. We found no evidence of genetic bottlenecks at any *G. volans* sample site. There was evidence for a genetic bottleneck however, at the Temagami sample site for *G. sabrinus* (Table 1).

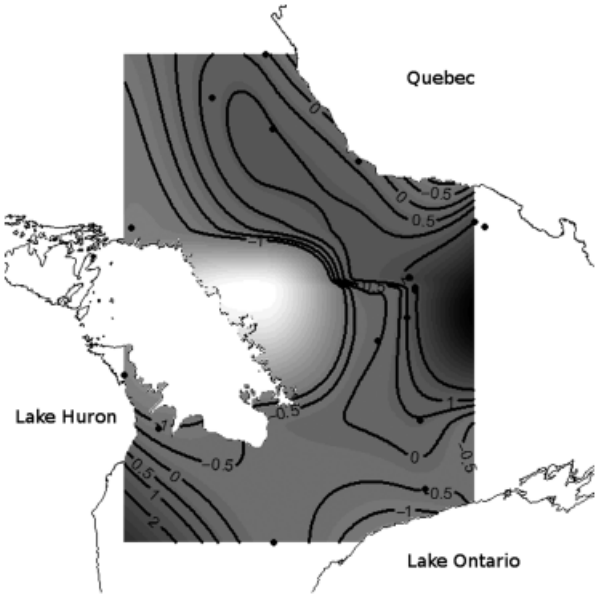

Fig. 2 Component 1 scores from a spatial principal components analysis of *G. sabrinus* microsatellite genotypes plotted on a map of Ontario. Contours were interpolated with a LOESS regression. Randomization tests suggest that these patterns were not significant.

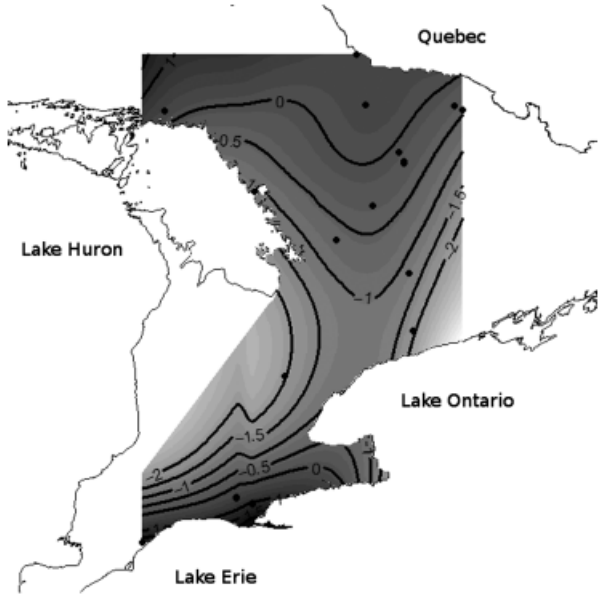

Fig. 3 Component 1 scores from a spatial principal components analysis of *G. volans* microsatellite genotypes plotted on a map of Ontario. Contours were interpolated with a LOESS regression. Randomization tests suggest that there is significant genetic structure associated with component one. This is a north–south allele frequency cline in the area of range expansion.

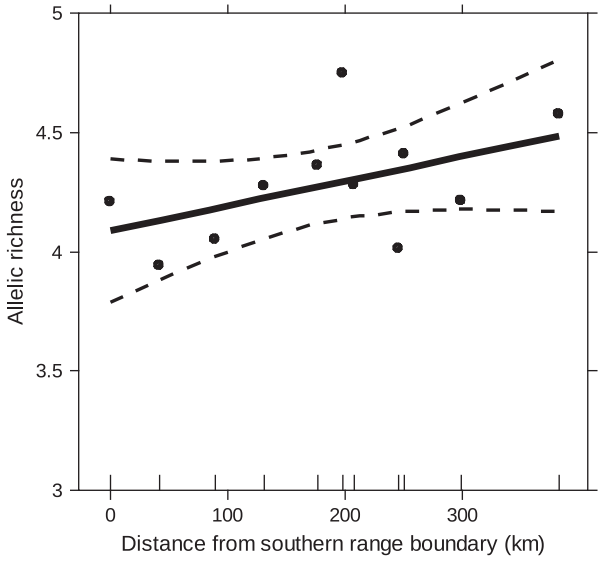

Fig. 4 A plot of *G. sabrinus* allelic richness corrected for sample size vs. the distance from their southern contiguous range limit.

Discussion

The genetic structure of populations is a function of selection, gene flow, and genetic drift, each of which are influenced by the spatial distribution of individuals and

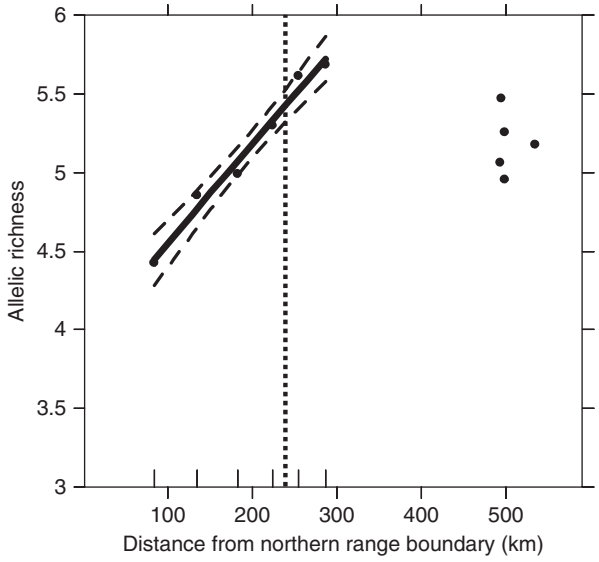

Fig. 5 A plot of *G. volans* allelic richness corrected for sample size vs. the distance from the northern range limit during the range expansion. The dashed line represents the point from which the expansion began.

population demography. We found a genetic signature of rapid *G. volans* range expansion in Ontario, Canada consistent with demographic data suggesting that they recently expanded their range in the region (Bowman *et al*., 2005) and supported our predicted genetic patterns of the early stages of rapid range expansions. There was spatial autocorrelation of allele frequencies in a latitudinal cline along the axis of expansion, consistent with waves of founders with high levels of gene flow. We also found a decline in allelic richness beginning from the point of expansion north to the 2003 *G. volans* range boundary. Despite the decline in allelic richness in the region of expansion we found no evidence for genetic bottlenecks in *G. volans*. We found no evidence for spatial autocorrelation of allele frequencies or spatial trends in allelic richness for the stationary species *G. sabrinus*. There was no evidence of genetically distinguishable clusters of individuals or isolation-by-distance in either *G. volans* or *G. sabrinus* suggesting populations in Ontario approximate panmixia. There was however, greater differentiation among *G. sabrinus* sample sites than *G. volans*. Together, trapping data (Bowman *et al*., 2005), a spatial cline in allele frequencies, a lack of isolation-by-distance, and no evidence for significant genetic structure or bottlenecks in *G. volans* suggest that *G. volans* expanded north from their contiguous range in a continuous wave of expansion with high levels of gene flow. Despite high gene flow, the decline in allelic richness suggested that the spatial movement of alleles was heterogenous, possibly a result of a tendency for dispersing individuals to originate from the range frontier (i.e., a subsampling effect).

Range expansions are common features of many species histories. Reduced genetic diversity is a well established consequence of historic range expansions (Excoffier *et al*., 2009; Sexton *et al*., 2009) and has been described across diverse taxa (e.g., insects, Cooper *et al*., 1995; Leotard *et al*., 2009; plants, Hewitt, 1999; mammals, Santucci *et al*., 1998; reptiles, Howes and Lougheed, 2008). The decline in genetic diversity observed for *G. volans* in the region of range expansion was consistent with the signature of historic range expansions (Excoffier *et al*., 2009) and our results suggest that this loss can occur over short time frames and with rapid continuous expansion. The evolutionary consequences of reduced diversity at range margins are not well known but there does not seem to be a general trend for reduced fitness in peripheral populations over relatively short time scales (e.g., Samis & Eckert, 2009). Low genetic variability may decrease evolutionary potential however, and increase the risk of extinction (Hoffmann & Blows, 1994). The pace of range shifts associated with contemporary climate change is fast and with a net directional trend as populations expand into newly suitable habitat and contract from newly unsuitable regions (Parmesan, 2006). Despite a large expansion front of individuals with high levels of gene flow, there was an immediate signature of reduced genetic diversity following this rapid range expansion. This suggests that recently shifted populations could have reduced genetic diversity and possibly reduced evolutionary potential; in extreme cases this could lead to inbreeding depression.

Another feature of historic range expansions is genetic differentiation between peripheral and more central individuals (Hallatschek *et al*., 2007; Hallatschek & Nelson, 2009). We found no evidence however, for isolation-by-distance. Differentiation of peripheral populations is likely a result of rare allele or mutation surfing and genetic drift at the expanding front of a population (Hallatschek *et al*., 2007; Excoffier & Ray, 2008; Hallatschek & Nelson, 2009). Microcosm experiments suggest that clines will develop before isolation-by-distance and suggest that there was likely insufficient time for new mutations to occur and accumulate or for a detectable signature of genetic drift although isolation-by-distance remains a prediction for the future (Hallatschek *et al*., 2007; Hallatschek & Nelson, 2009).

The overall lack of genetic structure in both species is consistent with previous research suggesting little differentiation across their range (Arbogast, 1999, 2007; Bidlack & Cook, 2001; Arbogast *et al*., 2005; Petersen & Stewart, 2006). The lack of structure is likely a consequence of population bottlenecks occurring in glacial

refugia and a subsequent range expansion following glacial retreat, an expansion process that has likely continued until the present day. Further, during the range expansion in Ontario, Bowman *et al*. (2005) estimated a rate of expansion for *G. volans* of 22 km per year and a diffusion coefficient greater than an order of magnitude larger than many other similarly sized animals. Given this potential for rapid long range movements it is perhaps not surprising that we have found largely panmictic population structure.

There is strong evidence for a broad trend of population range shifts following contemporary climate change and these are predicted to continue (Walther *et al*., 2002; Parmesan & Yohe, 2003). Impacts of range fluctuations on genetic and ecological diversity as a result of Pleistocene climate oscillations are well documented for many species (Hewitt, 2000). Past, natural climate oscillations tended to be evolutionarily limiting as gene pools that began to diversify and adapt in the relatively short periods between oscillations had their selective regimes altered, hybridized after re-established contact between diverging populations, or went extinct (Jansson & Dynesius, 2002). Our data suggest that the genetic consequences of historic climate change will likely be similar and perhaps amplified for contemporary anthropogenic climate change given the pace of change over the last 100 years and the likely steady increase in carbon emissions in the near future (IPCC, 2007; Excoffier *et al*., 2009). Indeed, an accumulating number of the predicted consequences of climate-change related range shifts have been identified in this study system in a very short period of expansion. These evolutionarily young species have recently been shown to hybridize in areas of increased and recent sympatry following the range expansion (Garroway *et al*., 2010). Now, we have shown that the range expansion of *G. volans* has reduced the genetic diversity in the area of range movement. These findings underscore the potential for rapid range shifts to affect biodiversity.

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