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Climate change induced hybridization in flying squirrels

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Abstract

There is now unequivocal evidence for global climate change; however, its potential impacts on evolutionary processes remain unclear. Many species have responded to contemporary climate change through shifts in their geographic range. This could lead to increased sympatry between recently diverged species; likely increasing the potential for hybridization. Recently, following a series of warm winters, southern flying squirrels (*Glaucomys volans*) in Ontario, Canada rapidly expanded their northern range limit resulting in increased sympatry with the closely related northern flying squirrel (*Glaucomys sabrinus*). This provided the opportunity to test the prediction that contemporary climate change can act as a catalyst creating conditions for the formation of hybrid zones. Following extensive sampling and molecular analyses (nuclear and mitochondrial DNA), we identified the occurrence of hybridization between sympatric *G. sabrinus* and *G. volans*. There was evidence of backcrossing but not of extensive introgression, consistent with the hypothesis of recent rather than historic hybridization. To our knowledge, this is the first report of hybrid zone formation following a range expansion induced by contemporary climate change. This is also the first report of hybridization between North American flying squirrel species.

Keywords: climate warming, distribution, *Glaucomys sabrinus*, *Glaucomys volans*, global change, range expansion

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Introduction

Numerous anthropogenic impacts on biodiversity are now well documented; however, the effects of human activities on the evolutionary processes that contribute to biodiversity remain much less clear (Myers & Knoll, 2001). Interspecific hybridization is a particularly important consequence of anthropogenic disturbance because the process can occur rapidly and has important evolutionary consequences (Schluter, 2000; Coyne & Orr, 2004; Seehausen *et al*., 2008). A relatively large proportion of extant biodiversity is of recent evolutionary origin, <5 million years old, and these evolutionarily young species may be particularly vulnerable to hybridization (Seehausen *et al*., 2008). Although such a time period is sufficient for ecological speciation to occur (Schluter, 2000; Coyne & Orr, 2004), postzygotic barriers to reproduction are likely to be incomplete or nonexistent (Coyne & Orr, 2004).

There is now unequivocal evidence for contemporary global climate change (IPCC, 2007). It is thought that most species will respond to this climate change through shifts in their geographic range, rather than

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in situ adaptation, owing to constraints on rapid evolution (Parmesan, 2006). For many species, sufficient resources to support populations exist beyond their currently occupied range; however, they are limited by other factors, including climate (Parmesan *et al*., 1999; Walther *et al*., 2002). In these instances, global climate change can make available new areas by shifting climate regimes, leading to species range shifts. A consequence of these range shifts may be increased sympatry between recently diverged species (Parmesan, 2006). Similarly, the climatic fluctuations that characterized the Pleistocene led to species range expansions and contractions. These range fluctuations sometimes resulted in secondary contact between evolutionarily young species that subsequently hybridized (e.g., Ruedi *et al*., 1997; Melo-Ferreira *et al*., 2007). Thus, one might expect contemporary global climate change to promote instances of hybridization as a result of increased sympatry between formerly parapatric or allopatric, closely related species.

Recently (1995–2003), the southern flying squirrel (*Glaucomys volans*) rapidly expanded its northern range limit by approximately 200 km (Bowman *et al*., 2005). This species specializes in the eastern temperate deciduous forests of North America. The range expansion appears to have resulted from a series of warm winters, bringing *G. volans* into increased sympatry with its boreal forest congener, the northern flying squirrel (*Glaucomys sabrinus*) (Bowman *et al*., 2005). Subsequently, the northern range limit of *G. volans* abruptly contracted by 240 km in 1 year (2004), apparently due to cold winter conditions in combination with a low food supply (Bowman *et al*., 2005). The range boundary dynamics of the two flying squirrel species during this time provided a scenario whereby a climate change induced population expansion rapidly increased local sympatry between these two, normally parapatric species.

The evolutionary history of these species has been well characterized (Arbogast, 1999, 2007; Bidlack & Cook, 2001; Arbogast *et al*., 2005; Petersen & Stewart, 2006). Mitochondrial DNA (mtDNA) phylogenetic analyses suggest that there are at least three distinct *Glaucomys* lineages, and that they diverged contemporaneously in three forest refugia between 0.7 and 1.3 mya (Arbogast, 1999). One lineage is *G. volans* and the others are Pacific and continental lineages of *G. sabrinus* (Arbogast, 1999). Interestingly, Arbogast (1999) found evidence that the *G. sabrinus* lineages are not sister to each other; rather, the continental lineage is most likely sister to the *G. volans* lineage. Despite their well differentiated mtDNA genomes there is evidence of historical and contemporary gene flow between the two *G. sabrinus* lineages based upon nuclear markers (Arbogast *et al*., 2005). However, the *G. volans* lineage is clearly differentiated from both *G. sabrinus* lineages based upon morphology, as well as nuclear and mtDNA markers (Thorington *et al*., 1996; Arbogast *et al*., 2005; Arbogast, 2007).

There is genetic, fossil, and pollen evidence that suggests that the *G. volans* lineage was confined to, and expanded north from, a southeastern North American deciduous forest refuge during the most recent glacial maxima and that the continental *G. sabrinus* clade, which now occupies most of North America, expanded from a primarily coniferous south-central North American refuge at the same time (Arbogast, 1999, 2007; Arbogast *et al*., 2005). Remnant populations of *G. sabrinus* in the southern Appalachian Mountains are likely late-Pleistocene relict populations that became isolated in coniferous forest patches as the boreal forest shifted north (Arbogast, 1999; Arbogast *et al*., 2005). Today these two flying squirrel species are parapatric; the distribution of *G. sabrinus* remains closely associated with northern boreal forests and the *G. volans* distribution follows the hardwood forests of eastern North America. There are a few areas of unstable local sympatry where these forest types overlap in eastern North America (Bowman *et al*., 2005).

Together with parapatry, competition within the narrow contact zone (Weigl, 1978; Price *et al*., 1988; Wetzel & Weigl, 1994) may also contribute to reproductive isolation between *Glaucomys* species. Despite this, we became interested in the potential for hybridization between flying squirrel species when, during a study of range boundary dynamics (Bowman *et al*., 2005), a subset of the flying squirrels captured had intermediate morphological characteristics (body size measurements and pelage colour). Given the recent range expansion of *G. volans* observed in Ontario, we were interested in testing the prediction that contemporary anthropogenic climate change can act as a catalyst creating the formation of hybrid zones between evolutionarily young species. To do this we used two types of molecular markers, the maternally inherited mtDNA cytochrome *b* gene and biparentally inherited microsatellite loci, to test for the occurrence of hybridization between flying squirrel species in areas subjected to recent range perturbation.

Methods

Flying squirrel sampling

Live-trapping of animals took place in Ontario, Canada between 2002 and 2004 at 26 sites covering a latitudinal gradient from the north shore of Lake Erie (42.52°N) to the southern edge of the boreal forest (47.29°N) (42 971 trap nights; Fig. 1). These sites encompassed the

previously delineated northern range limit of *G. volans* (Stabb, 1988). In 2002 and 2003, many *G. volans* individuals were captured at distances of up to 200 km farther north than the range limit identified in 1988 (24 139 trap nights north of 1988 limit; 788 captures of 506 *G. volans*) (Fig. 1). In 2004 however, the species' northern range limit contracted by about 240 km to near the 1988 limit. Five sites north of this 1988 range limit were sampled in both 2003 and 2004. In 2003, there were 485 captures of 309 *G. volans* in 13 397 trap nights, whereas in 2004 no *G. volans* were captured in 12 945 trap nights. During the 2002–2004 surveys, we captured 232 *G. sabrinus* 376 times. This extensive trapping effort as well as another long-term data set from Algonquin Park suggests that *G. volans* expanded its range from the south beginning in the mid-1990s with a series of warm winters (Bowman *et al*., 2005). This range expansion resulted in increased sympatry with *G. sabrinus*, which inhabits mixed and coniferous forests in the region. We also conducted intensive live trapping and nest box surveys (540 nest boxes) aimed at determining the status of *G. sabrinus*, at 19 sites in Pennsylvania, USA. These surveys have confirmed a reduction in this species range compared with historic records (Mahan *et al*., 1999). The decline of *G. sabrinus* most recently led to the listing of this species as endangered in the state. The surveys in Pennsylvania documented that all remaining *G. sabrinus* populations in the state were sympatric with *G. volans*.

DNA extraction and genotyping

DNA was extracted from approximately 20–30 hairs per squirrel, using a Qiagen DNeasy tissue kit protocol (Qiagen Inc., Valencia, CA, USA). The hair was suspended in 500 μL of 1 × lysis buffer (4 M urea, 0.2 M NaCl, 0.5% *n*-lauroyl sarcosine, 10 mM CDTA (1,2 cyclohexanediamine), 0.1 M Tris-HCL (pH 8.0) (Applied Biosystems Inc., FosterCity, CA, USA), treated with 15 units of Proteinase K (Qiagen Inc.) and incubated at 37 °C for 12 h. Samples were then extracted following the DNeasy tissue kit (Qiagen Inc.) protocol until the final step when the samples were eluted with 70 °C 0.1 TE (1.0 M Tris-HCl, 0.5 M EDTA).

Nine microsatellite loci, GS2, GS4, GS8, GS10, GS13 (Zittlau *et al*., 2000), SFS03, SFS14 (Fokidis *et al*., 2003), FLS1, and FLS6 (Winterrowd *et al*., 2005) were amplified by the polymerase chain reaction (PCR) using fluorescently labelled (NED, 6 FAM, and HEX) primers. The nine loci were amplified in multiplex reactions containing two to three loci per individual. Amplifications were performed in 10 μL volumes containing 3 μL of stock DNA, 0.2 mM of each dNTP, 1 × Qiagen PCR buffer, 0.3 – 0.5 μM of forward and reverse primer, and 0.5 U of Taq polymerase (Qiagen Inc.). All samples were amplified under the following conditions: 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 60 °C for 45 s, 74 °C for 30 s, and 65 °C for 45 min. Multiplex reactions were

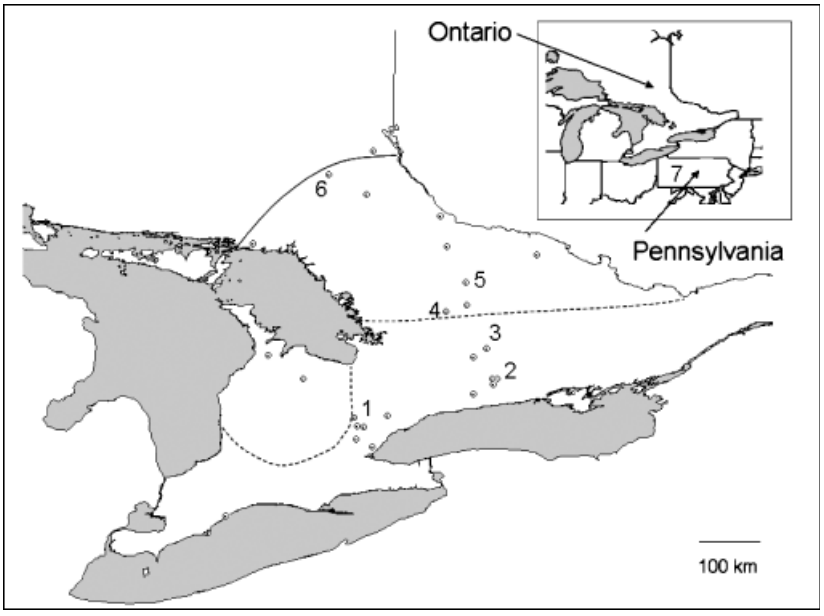

Fig. 1 Distribution of *Glaucomys sabrinus* and *Glaucomys volans* trapping sites in Ontario and Pennsylvania during 2002–2004. Putative hybrids were identified at numbered sites: (1) Aurora, (2) Peterborough, (3) Kawartha Highlands, (4) Dorset, (5) Algonquin Park, (6) Temagami, and (7) Pennsylvania. The solid line represents the northern limit of *G. volans* in 2003 following range expansion. Dashed lines represent the estimated northern range boundary of *G. volans* before the range expansion and following a range contraction during winter 2004 (Bowman *et al*., 2005).

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, Valencia, CA, USA). Amplified product from multiplex reactions were added in equal proportions and 10 μL was ethanol precipitated and resuspended in 5 μL of ddH20 for loading on the ABI 3730. Genotypes were scored using GENEMARKER software (SoftGenetics, State College, PA, USA).

In order to characterize typical mtDNA sequences for *G. sabrinus* and *G. volans*, we randomly selected 27 *G. volans* and 32 *G. sabrinus* that were highly assigned to their species in the Bayesian assignment test (mean assignments of 0.97 and 0.98, respectively) and characterized their cytochrome *b* genes. To further assess incidences of introgressive hybridization, we also sequenced cytochrome *b* mtDNA sequences of putative hybrids, and of individuals that assigned to one of the two species with assignments between 0.80 and 0.90. This allowed us to determine concordance between the two differentially inherited marker types (biparental for microsatellite loci and maternal for mtDNA).

Data analysis

We tested for deviations from Hardy–Weinberg equilibrium (HWE) with Bonferroni-corrected significance thresholds for each sample site, locus, and species using the ADEGENET package (Jombart, 2008) for R (R Development Core Team, 2008) and linkage equilibrium (LE) in GENEPOP 4 (Rousset, 2008).

Upon capture, squirrels were classified to species based upon morphological characters such as body size, tail length, and ventral pelage and tail colour. Subsequently, species identification and ancestry of each individual's microsatellite genotype was estimated using an individual-based Bayesian assignment test implemented in STRUCTURE 2.2 (Pritchard *et al*., 2000; Falush *et al*., 2003). Departures from LE and HWE can be a result of contemporary migration patterns and hybridization of species or populations. We modelled the sample as two populations and probabilistically assigned individual ancestry to those two populations such that LE and HWE were maximized. We implemented the admixture model with no *a priori* species identification or geographic information, allowing α (degree of admixture) to vary independently for each population, with a burn-in of 500 000 Markov Chain Monte Carlo iterations (MCMC), and run of 1 000 000 MCMC iterations. Particularly important output from this analysis for this study is the *Q* heuristic which denotes the proportion of an individuals genome assigned to a particular population.

We assessed the ability of the Bayesian analysis to assign individuals to species and to detect hybrids given our microsatellite markers by repeating the same analysis as above on simulated parental and first generation (F1) hybrid populations of individuals of known ancestry. This allowed us to quantify the range of expected *Q* values expected for each species and F1 hybrids and to assign individuals from the real data based upon these values. To do this we first selected 40 highly assigned individuals of each species ($Q > 0.98$) from nine sample sites where we had no evidence of sympatry. Assuming LE, random mating, and neutrality, we simulated new parental and F1 hybrid populations ($n = 1000$ per population). We built parental populations by randomly selecting alleles from their frequency distributions in their species. We built the F1 hybrid population by randomly selecting one allele each from the frequency distributions of alleles in each parental species. We then randomly selected 150 individuals from each of the *G. sabrinus* and *G. volans* populations, and 15 individuals from the F1 population and reanalysed these data using the same Bayesian assignment test described above (this process was repeated five times). We chose 150 parental types and 15 hybrid squirrels to approximately represent sample sizes from the real data. From these simulations, we could assess our ability to detect hybrids given our data set and determine an appropriate probability of assignment (*Q* value) to assign individuals to species or putative hybrid classes.

We sought to compare our model-based STRUCTURE analysis to an alternative analysis to assess concordance with a different analytical approach. Individual-based principal components analysis (PCA) of microsatellite genotypes is a good option for comparisons as it makes fewer assumptions regarding the underlying structure of the data than STRUCTURE (no assumption of HWE and LE; Jombart, 2008). We implemented PCA using the ADEGENET package (Jombart, 2008) as an interface for importing genetic data into the ADE4 package (Dray & Dufour, 2007) within the R statistical language (R Development Core Team, 2005). This method performs a PCA on a matrix of allele frequencies with each row in the matrix representing an individual and each column representing an allele. Alleles were coded as 0 if absent in an individual, 0.5 if heterozygous, and 1 if homozygous. The first and subsequent axes explained the most and then progressively less variation in the data such that the first axis is associated with the most differentiated individuals and so on. We plotted each species and the hybrids identified in the STRUCTURE analyses and 95% confidence ellipses around them. We tested for concordance between STRUCTURE and PCA scores with Kruskall–Wallis tests.

Finally, the correspondence, or lack thereof, between an individual's mtDNA and microsatellite ancestry was used to infer hybrid fertility. Individuals assigned to one species, but with mtDNA of the other species, should be a result either of mating between hybrids or between a hybrid and parental type. Using a similar criterion, we could also assess the relative timescale over which hybridization between these species has taken place (i.e., contemporary or historical). For example, an individual that was highly assigned (defined here as $Q > 0.95$) to one species based upon microsatellite DNA, but with mtDNA of the opposite species, would likely be the result of hybridization and multiple generations of backcrossing to a parental type.

Results

Microsatellites

We profiled 271 flying squirrels, of which 153 and 118 were identified in the field as *G. sabrinus* and *G. volans*, respectively. These totals include the few squirrels where ambiguous morphological characteristics rendered the field identifications provisional. Of the nine microsatellite loci screened in both *G. sabrinus* and *G. volans*; five loci amplified diagnosable microsatellite alleles in both species (SFS3 and SFS15 (Fokidis *et al*., 2003); GS4, GS8, and GS10 (Zittlau *et al*., 2000)). There were a total of 69 alleles from the five loci with a mean of 14 alleles per locus (range 11–19). Each locus contained between one and six species-specific alleles. There was no evidence of deviation from LE or HWE after Bonferroni corrections for multiple tests ($P < 0.05$) with species and sample sites considered separately.

Species identification and admixture

Bayesian assignment clearly differentiated the two species and supported field species identifications based on morphology (Fig. 2). Mean probability of assignment to species was 0.97 to *G. sabrinus* and 0.98 to *G. volans*. A subset of squirrels, however, appeared to be of mixed ancestry (Fig. 2). The same assignment test performed on simulated parental and F1 hybrid genotypes suggested that it was likely not possible, even with the large number of simulated individuals, to generate F1 hybrids with $Q > 0.80$ given the number of loci used and their variability and so we considered individuals that assigned to a species with $Q > 0.20$ and < 0.80 to be putative hybrids and those outside of this range as species (Fig. 3). By this criterion there were 11 putative hybrids (4% of sampled squirrels) in our sample. These hybrids occurred only in regions where species where known to be sympatric at some point during our study (Fig. 1).

Like the Bayesian assignment test, the PCA clearly distinguished the two species and all 11 putative hybrids were intermediate between the two species clusters in the biplot (Fig. 4). Two of the putative hybrids clustered outside of the hybrid 95% confidence ellipse closer to *G. sabrinus*. Nonparametric Kruskal–Wallis tests indicated that scores on the first and second axes were strongly correlated with species and hybrid status as determined from assignment tests ($P < 0.0001$), however the third was not ($P = 0.86$).

Of the 11 putative hybrids, eight carried *G. volans* mtDNA. For the squirrels assigned to a species, mtDNA and microsatellites were concordant for all except one squirrel. This squirrel had a *G. sabrinus* microsatellite genotype according to our STRUCTURE analysis ($Q = 0.89$) and PCA; however, it had a *G. volans* mtDNA haplotype. The STRUCTURE assignment for this squirrel was greater than our criterion for hybrids and thus it was most likely a product of backcrossing.

Evidence of hybrid fertility

All *G. sabrinus* were monomorphic for a private allele at the SFS15 locus. Six of the 11 putative hybrids were heterozygous for this allele, one was homozygous

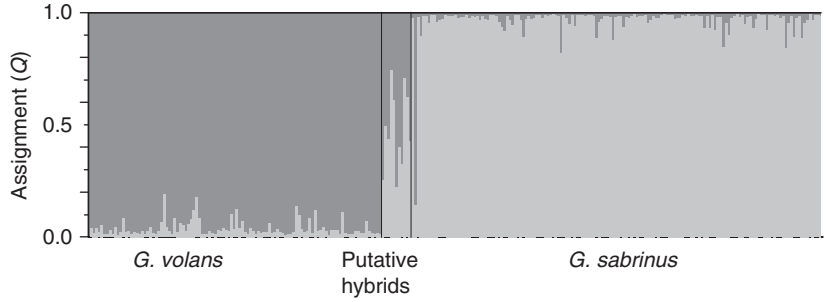

Fig. 2 A barplot of the proportion of ancestry (Q) assigned to field-identified northern (*Glaucomys sabrinus*; $n = 153$) and southern (*Glaucomys volans*; $n = 118$) flying squirrel species based upon Bayesian clustering of individual microsatellite genotypes. Eleven putative hybrids are highlighted in the centre of the plot.

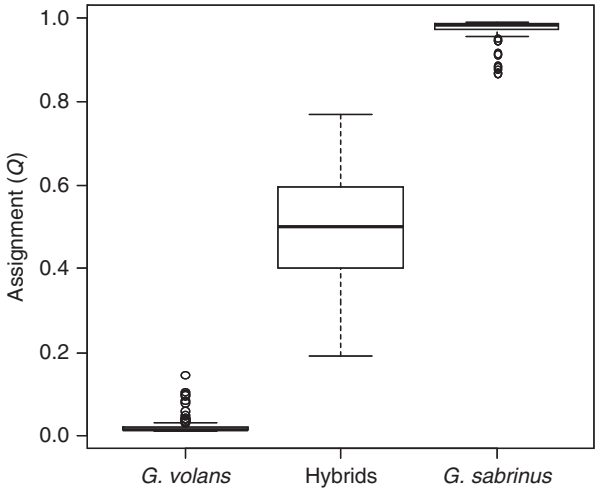

Fig. 3 Boxplots of the range of probability of assignment values (Q) based upon Bayesian clustering of simulated individual microsatellite genotypes for 150 southern flying squirrels (*Glaucomys volans*), 150 northern flying squirrels (*Glaucomys sabrinus*) and 15 F1 hybrids. There was no overlap in Q for any population and so we considered individuals assigned to a population with $0.20 < Q < 0.80$ as likely F1 hybrids.

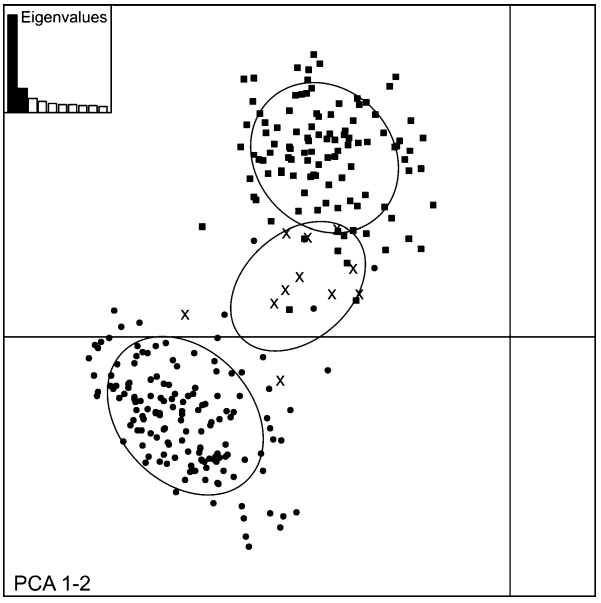

Fig. 4 PCA plot of eigenvector 1 vs. 2 showing clustering of *Glaucomys sabrinus* (solid circles; $n = 150$), *Glaucomys volans* (solid squares; $n = 110$) and F1 hybrid (X's $n = 11$) microsatellite genotypes in concordance with STRUCTURE results. Hybrids were identified as individuals with Q values of $<80\%$ (see text for details). 95% confidence ellipses also are shown. PCA, principal components analysis.

(hybrid 11), and two did not carry it (hybrids 5 and 6) (Table 1). This allele was also heterozygous in four individuals assigned to *G. volans* ($Q = 0.81$–0.88) and clustered with the hybrids in the PCA (Fig. 4). Together, this collection of 15 individuals and the above mentioned individual with discordant nuclear and mtDNA provided strong evidence of hybrid fertility and successful backcrosses to both species. Squirrels that were assigned to one species, but that contained markers (private allele or mtDNA) specific to the other species must have been the progeny of hybrids; either backcrosses to one parental species or, perhaps less likely, mating between hybrids. There was no evidence of extensive introgression of mtDNA or the private, fixed *G. sabrinus* allele however, despite evidence of some level of fertility and successful backcrosses. This suggests that that the hybridization is of recent origin likely coinciding with recent sympatry.

Discussion

Our data are consistent with the hypothesis that the expansion of *G. volans* north into the *G. sabrinus* range in Ontario has resulted in the formation of a new hybrid zone. Nuclear and mtDNA, and field based morphological identifications differentiated the majority of individuals into species and identified a small subset as hybrids. Despite the fact that we found evidence of successful backcrosses to both parental types we found no evidence of thorough introgression of mtDNA or the private, fixed *G. sabrinus* allele. The absence of introgression in a region strongly implies that interbreeding has not taken place (Currat *et al*., 2008). Therefore, we suggest that the hybridization was recent, coinciding with the recent increase in sympatry. Darwin's finches (*Geospiza* spp.) provide an example of introgression when hybridization occurs at low levels over longer time periods (Freeland & Boag, 1999). Despite an annual frequency of hybridization of $<5\%$, there was extensive sharing of mtDNA and nuclear DNA among species (Freeland & Boag, 1999).

In addition to climate change effects, habitat loss may have contributed to the recent increase in sympatry between flying squirrel species. This is particularly true in the Pennsylvania study area, where pressures associated with urban development have resulted in the loss of substantial amounts of coniferous forest, the preferred habitat of *G. sabrinus* (Mahan *et al*., 1999). Habitat loss may result in species packing into remaining fragments, leading to increased opportunities for hybridization (e.g., Bowman *et al*., 2002; Buttel *et al*., 2002).

An alternative interpretation of our data is that hybridization between these species is not new, but instead results from the movement of a previously undetected hybrid zone and perhaps a climate change mediated increase in the instances of hybridization.

Table 1 Details of individuals that classified as hybrids ($n = 11$) based upon assignment of microsatellite ancestry (Q) to either northern or southern flying squirrel species (*Glaucomys sabrinus* or *Glaucomys volans*) between of 0.20 and 0.80 probability as calculated from Bayesian assignment tests using the software STRUCTURE

Squirrel	Sample site	Field ID	Sex	Mass (g)	mtDNA	*G. sabrinus* microsat. ancestry (Q)	*G. volans* microsat. ancestry (Q)	*G. sabrinus* private fixed allele
Hybrid 1	Algonquin Park	*G. volans*	f	66	*G. volans*	0.222	0.778	Heterozygous
Hybrid 2	Kawaratha Highlands	*G. sabrinus*	m	76	*G. volans*	0.266	0.734	Heterozygous
Hybrid 3	Algonquin Park	*G. volans*	m	68	*G. volans*	0.321	0.679	Heterozygous
Hybrid 4	Dorset	*G. sabrinus*	m	67	*G. volans*	0.401	0.599	Heterozygous
Hybrid 5	Temagami	*G. sabrinus*	–	–	*G. sabrinus*	0.409	0.591	Not present
Hybrid 6	Kawaratha Highlands	*G. volans*	m	65	*G. volans*	0.435	0.565	Not present
Hybrid 7	Algonquin Park	*G. volans*	f	77	*G. volans*	0.487	0.513	Heterozygous
Hybrid 8	Algonquin Park	*G. volans*	m	73	*G. volans*	0.606	0.394	Heterozygous
Hybrid 9	Pennsylvania	*G. volans*	f	71	*G. volans*	0.628	0.372	Heterozygous
Hybrid 10	Aurora	*G. sabrinus*	m	85	*G. sabrinus*	0.697	0.303	Heterozygous
Hybrid 11	Peterborough	*G. sabrinus*	f	122	*G. sabrinus*	0.747	0.253	Homozygous

mtDNA, mitochondrial DNA.

Hybrid zone movement is not uncommon (Buggs, 2007); contemporary climate change has been suggested as a possible mechanism for the movement of a cricket (*Allonemobius fasciatus* and *A. socius*) hybrid zone in the eastern United States (Britch *et al*., 2001). However, we do not consider this to be the most parsimonious interpretation of our data. For this to be a case of hybrid zone movement, given the apparent lack of introgression, we would have to assume strong selection against hybrids. An assumption of strong selection against hybrids such that we would detect no thorough introgression seems unlikely to hold given our evidence for hybrid fertility and backcrossing.

The natural history of *Glaucomys* species suggests some nonexclusive hypotheses for mechanisms by which range expansion could have increased the likelihood of hybridization. Low densities of *G. volans* on the expanding edge of the species range may have reduced mate choice options (likely for *G. volans* females) leading to a relaxation of assortative mating. Second, these species remain active throughout the winter and social thermoregulation is important for winter survival during the cold months (Muul, 1968; Stapp *et al*., 1991). This could lead to tolerance of the other species within nests for social thermoregulation whereas typically *G. volans* are expected to aggressively evict *G. sabrinus* (Weigl, 1978). Mating behaviour begins in late winter when squirrels are still nesting in large winter aggregations and so this scenario could lead to mating opportunities (Muul, 1968; Stapp *et al*., 1991). Finally, each species can carry the parasite *Strongyloides robustus*; however, it appears to only be deleterious to *G. sabrinus* (Weigl, 1968). The distribution of the nematode is likely limited by cold temperatures (Wetzel & Weigl, 1994; Pauli *et al*., 2004) and so may not have been able to expand its range along with *G. volans* populations, leading to a removal of what may be a reproductive barrier between the flying squirrel species.

The conservation implications of hybridization are not straightforward (Allendorf *et al*., 2001). Anthropogenically induced hybridization is generally considered undesirable (Allendorf *et al*., 2001), particularly when one of the parental species is vulnerable, such as *G. sabrinus*, which is endangered in Pennsylvania. Species effectively become extinct if all the parental genotypes are hybridized (Rhymer & Simberloff, 1996). However, hybridization might sometimes play an important role in adaptation and speciation (Seehausen, 2004; Mallet, 2007). The role of hybridization in sustaining and contributing to biodiversity is context dependent (Seehausen *et al*., 2008) and can be positive if it increases genetic variability and creates new gene combinations that increase the potential to adapt. This could be particularly important for these species in Ontario if it increases their potential to quickly adapt to the changing climate. Regardless of any potential adaptive advantage that hybrids may have, species concepts are the *sine qua non* of endangered species legislation. Further, the breakdown of reproductive barriers between young ecological species will result in a net loss of biodiversity with likely impacts on ecosystem function. This can quickly alter the evolutionary trajectory of species. Given that we are presently experiencing rapid global change, we suspect that there are many more examples of recent hybridization among young species that remain to be documented.

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