

Directional gene flow patterns in disjunct populations of the black ratsnake (*Pantheropsis obsoletus*) and the Blanding's turtle (*Emydoidea blandingii*)

Briar J. Howes · Joseph W. Brown · H. Lisle Gibbs ·
Tom B. Herman · Stephen W. Mockford ·
Kent A. Prior · Patrick J. Weatherhead

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Abstract The estimation and maintenance of connectivity among local populations is an important conservation goal for many species at risk. We used Bayesian statistics and coalescent theory to estimate short- and long-term directional gene flow among subpopulations for two reptiles that occur in Canada as peripheral populations that are geographically disjunct from the core of their respective species' ranges: the black ratsnake and the Blanding's turtle. Estimates of directional gene flow were used to examine population connectivity and potential genetic source-sink dynamics. For both species, our estimates of directional short- and long-term gene flow were consistently lower than estimates inferred previously from F_{ST} measures. Short-

and long-term gene flow estimates were discordant in both species, suggesting that population dynamics have varied temporally in both species. These estimates of directional gene flow were used to identify specific subpopulations in both species that may be of high conservation value because they are net exporters of individuals to other subpopulations. Overall, our results show that the use of more sophisticated methods to evaluate population genetic data can provide valuable information for the conservation of species at risk, including bidirectional estimates of subpopulation connectivity that rely on fewer assumptions than more traditional analyses. Such information can be used by conservation practitioners to better understand the geographic scope required to maintain a functional metapopulation, determine which habitat corridors within a working landscape may be most important to maintain connectivity among subpopulations, and to prioritize subpopulations with respect to their potential to act as genetic sources within the metapopulation.

B. J. Howes (✉) · K. A. Prior
Parks Canada, Ecological Integrity Branch, 4th floor,
25 rue Eddy, Gatineau, QC, Canada K1A 0M5
e-mail: briar.howes@pc.gc.ca

J. W. Brown
Museum of Zoology, Bird Division, University of Michigan,
3015 Ruthven Museums Building, 1109 Geddes Avenue,
Ann Arbor, MI 48109-1079, USA

H. L. Gibbs
Department of Evolution, Ecology and Organismal Biology,
Ohio State University, 370 Aronoff Laboratory, 318 W. 12th
Avenue, Columbus, OH 43210, USA

T. B. Herman · S. W. Mockford
Department of Biology, Acadia University, Wolfville,
NS, Canada B4P 2R6

P. J. Weatherhead
Program in Ecology, Evolution and Conservation Biology,
University of Illinois, 606 E. Healey Street, Champaign,
IL 61820, USA

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Introduction

Understanding connectivity among local populations is a fundamental aspect of population ecology and is essential for managing populations of conservation concern (Hanski 1999; Clobert and Danchin 2001). Movement of individuals and their genes influences a number of ecological and evolutionary processes (Hanski and Gilpin 1997), including population persistence and adaptive response to environmental change (Frankham et al. 2002). Because the persistence of protected populations may be influenced by

the persistence of unprotected neighboring populations (Hanski 1999), it is crucial that managers of these species be aware of connections between populations. When natural dispersal rates among populations are reduced by anthropogenic habitat fragmentation, the probability of some local populations becoming extinct increases (Hanski 1999). As such, the identification and maintenance of connectivity patterns among local populations is an important conservation priority (e.g., Epps et al. 2007).

In this light, we re-analyzed data for peripheral populations of black ratsnakes (*Pantheropsis obsoletus*) and Blanding's turtles (*Emydoidea blandingii*). Our goal was to estimate short- and long-term gene flow among subpopulations, determine if subpopulations show genetic source-sink relationships within a metapopulation, and to contrast temporal patterns of gene flow estimates between the two species. Although the two species are ecologically very different, both metapopulations we consider occur in Canada as peripheral populations that are geographically disjunct from their respective species' core ranges and are of conservation concern: the eastern Ontario population of black ratsnakes is listed as Threatened (COSEWIC 2007), and the Nova Scotia population of Blanding's turtles is listed as Endangered (COSEWIC 2005).

Data for both species were previously analyzed using traditional F -statistics to investigate local population genetic structure. Loughheed et al. (1999) used six microsatellite loci to investigate regional genetic structure for the black ratsnake in eastern Ontario and determined that while there was substantial gene flow (based on Nm estimates from F_{ST} estimates) among communal hibernacula within a population, there was significant genetic differentiation over relatively short distances. Mockford et al. (2005) used five microsatellite loci to examine regional genetic structure for the Blanding's turtle in Nova Scotia and found that all pairs of populations were significantly genetically differentiated and that geographic distance rather than watershed seemed to be the principal influence on genetic structure. Indirect estimates of population connectivity and patterns of gene flow, such as the estimates described above, have traditionally been determined using F -statistics (Wright 1931). However, this approach has been criticized because it relies on unrealistic assumptions (Whitlock and McCaughley 1999), and poorly reflects contemporary gene flow (Paetkau et al. 2004). Recently developed genetic analyses incorporating coalescent theory (Kingman 1982) and Bayesian statistics (e.g. Beerli 2006) make few of the assumptions required by the F -statistic approach. Additionally, these analyses extract more information from the data relative to F -statistics and may allow more straightforward interpretation of genetic results (Slatkin and Barton 1989, Shoemaker et al. 1999, Pearse and Crandall 2004).

Here we use Bayesian analytical methods to estimate patterns of population connectivity for both the black ratsnake and the Blanding's turtle. A particular advantage of these analyses is that they can provide estimates of effective population size and directional estimates of gene flow across different timescales (e.g. Beerli and Felsenstein 1999; Wilson and Rannala 2003). Examination of directional gene flow among populations can reveal genetic source-sink dynamics of a metapopulation, which can have important conservation implications. For example, populations that are net exporters of individuals (genetic sources) may be most valuable to protect from a conservation perspective, whereas those that are net importers (genetic sinks) may be at the highest risk of extinction. Comparison of directional gene flow patterns across different timescales can also offer insight into how population connectivity patterns may have changed over time. If discordance between estimates is detected, it could indicate that connectivity between populations has changed as a result of recent anthropogenic habitat fragmentation. Anthropogenic habitat fragmentation has occurred in both of our study sites, and in particular, road densities in both sites have recently increased. Road development may lead to decreased population connectivity in both species, especially as individuals of black ratsnakes and Blanding's turtles can travel large distances overland (black ratsnake—Blouin-Demers and Weatherhead 2002; Blanding's turtle—Power 1989, McMaster and Herman 2000), and are thus very susceptible to road mortality (Gibbs and Shriver 2002; Row et al. 2007).

We estimate short- (the past one to three generations) and long-term gene flow (averaged over the past n generations, where n = the number of generations the populations have been at equilibrium) to examine population connectivity patterns and genetic source-sink dynamics (based on migration among populations as detected by genetic methods) at two evolutionary scales. We identify subpopulations that are net exporters of individuals and therefore may be of disproportionate conservation value to the metapopulation. Finally, we determine whether short- and long-term estimates of gene flow are concordant for each species. We predicted that ratsnakes would show greater discordance between short- and long-term estimates than the Blanding's turtle because of their shorter generation time. The generation time for the Blanding's turtle is estimated to be approximately 37 years (Congdon et al. 1993), roughly double that of the black ratsnake (approximately 14 years; Blouin-Demers et al. 2002). A shorter generation time should facilitate detection of discordance between historical and contemporary connectivity patterns that may be altered by relatively recent anthropogenic changes to the landscape.

Methods

Sampling

Collection of microsatellite data for ratsnakes used in this study is described in Lougheed et al. (1999). We refer to a local sampling unit as a subpopulation, and the collective group of sampling units as a metapopulation. Sampled subpopulations within the metapopulation of eastern Ontario were Charleston Lake Provincial Park (CLPP), LaRue Mills (LRM), Murphy’s Point Provincial Park (MPPP), St. Lawrence Islands National Park (SLINP), and Queen’s University Biological Station (QUBS) (Fig. 1, Table 1).

Collection of microsatellite data for Blanding’s turtles used in this study is described in Mockford et al. (2005). Sampled subpopulations within the metapopulation of Nova Scotia were Kejimikujik National Park (KNP), McGowan Lake (ML), and Pleasant River (PR) (Fig. 2, Table 1).

Genetic structure

For both species, we tested for linkage disequilibrium between all pairs of loci and for departures from Hardy–Weinberg expectations using a Markov chain approximation of an exact test as implemented in GENEPOP web version 3.4 (Raymond and Rousset 1995).

We first examined genetic structure in both metapopulations using the program BAPS Version 3.2 (Corander et al. 2003) and the approach in which groups of individuals are clustered. We also used the program GENECLASS2, Version 2.0 (Piry et al. 2004), to examine genetic structure in both metapopulations based on assignment tests. Assignment probabilities of individuals were calculated

using a Bayesian procedure (Rannala and Mountain 1997) and Monte Carlo re-sampling techniques using 100,000 simulated individuals and a threshold of 0.01.

Short-term and long-term estimates of local gene flow

To estimate short-term gene flow (m = proportion of migrants) between subpopulation pairs we used the program BAYESASS, Version 1.3 (Wilson and Rannala 2003). BAYESASS estimates migration rates over the last several generations using Markov chain Monte Carlo techniques and does not assume that populations are in migration-drift or Hardy–Weinberg equilibrium. We ran the program for 2×10^7 iterations, following a burn-in of 10^7 iterations.

Samples were collected every 2,000 iterations to infer posterior probability distributions of migration rates. The first run used default settings for delta values for allele frequency, migration rate, and inbreeding, but subsequent runs incorporated different delta values to ensure that proposed changes between chains at the end of the run were between 40% and 60% of the total chain length (Wilson and Rannala 2003). Once a run achieved the acceptable proposed changes for allele frequency, migration rate, and inbreeding, two subsequent runs were performed using different random seed numbers to ensure consistency among runs. To examine the strength of the information in each dataset, 95% confidence intervals were determined for migration rates and compared to a scenario where there is no information in the data.

To estimate long-term gene flow [$M = m/(m \times \mu)$, where m = migration rate and $(m \times \mu)$ = mutation rate] between population pairs and THETA ($\Theta = 4N_e\mu$; where N_e = effective population size, and μ = mutation rate) we used

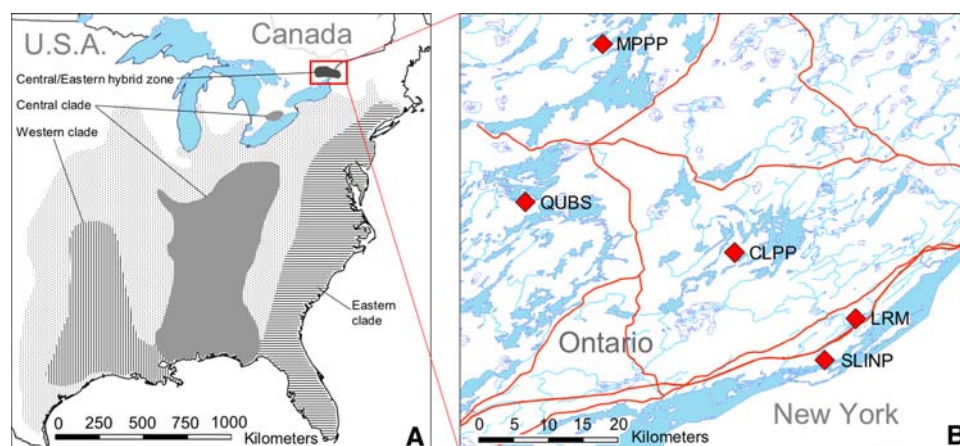


Fig. 1 Geographic distribution of the black ratsnake (shaded in grey), with mitochondrial clades indicated according to Burbrink et al. (2000) and Gibbs et al. (2006) (a). Local distribution of sampled subpopulations in eastern Ontario (b). Sample sites included in this study are indicated by red diamonds and their abbreviations are as

follows: CLPP (Charleston Lake Provincial Park), LRM (LaRue Mills), MPPP (Murphy’s Point Provincial Park), QUBS (Queen’s University Biological Station), and SLINP (St. Lawrence Islands National Park). Red lines represent primary roads

Table 1 Inferred values of Θ for sampled subpopulations of black ratsnake and Blanding's turtle as estimated by MIGRATE

Subpopulation	<i>n</i>	Mean Θ (95% CIs)
Black ratsnake		
Charleston Lake Provincial Park (CLPP)	23	0.144 (0.075–0.21)
LaRue Mills (LRM)	34	0.30 (0.21–0.38)
Murphy's Point Provincial Park (MPPP)	60	0.27 (0.21–0.32)
Queen's University Biological Station (QUBS)	132	0.72 (0.70–0.94)
St. Lawrence Islands National Park (SLINP)	37	0.10 (0.055–0.15)
Blanding's turtle		
Kejimikujik National Park (KNP)	43	1.19 (0.96–1.45)
McGowan Lake (ML)	40	1.53 (1.31–1.78)
Pleasant River (PR)	27	1.12 (0.96–1.28)

See Figs. 1 and 2 for geographic locations of subpopulations.

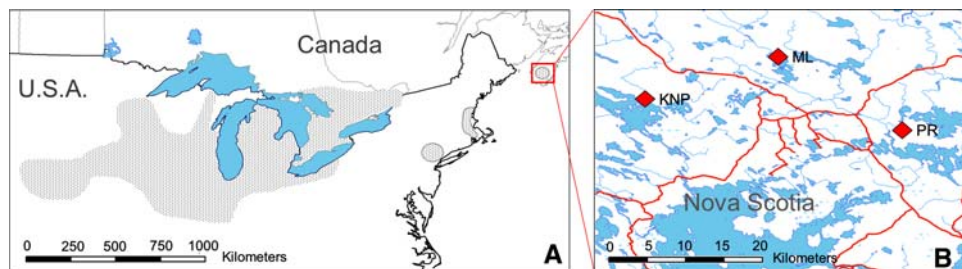


Fig. 2 Geographic distribution of the Blanding's turtle (shaded in grey) (a), and local distribution of sampled subpopulations in southern Nova Scotia (b). Sample sites included in this study are

indicated by red diamonds and their abbreviations are as follows: KNP (Kejimikujik National Park), ML (McGowan Lake), and PR (Pleasant River). Red lines represent primary roads

the program MIGRATE, Version 2.1.3 (Beerli and Felsenstein 1999, 2001). This program estimates historical migration rates and effective population sizes using coalescence theory and Markov chain Monte Carlo techniques. It assumes that populations are in migration-drift equilibrium, but does not require that populations are in Hardy–Weinberg equilibrium. Parameter distributions were estimated using the Bayesian implementation of MIGRATE (Beerli 2006). Similar analysis settings were applied to both species. Microsatellite mutation was modeled as a continuous Brownian process. Following a burn-in of 5×10^4 iterations, 2×10^5 genealogies were recorded at a sampling increment of 50 iterations. Exponential priors (minimum, mean, maximum) were placed on both Θ (0, 1, 5) and M (0, 10, 30). An adaptive heating scheme using 4 simultaneous Markov chains was implemented to increase the efficiency of searches. The program was run several times for each species using different random number seeds and results were generally stable, suggesting that Markov chains had converged on the stationary distribution. We converted long-term estimates of gene flow (M) to proportion of migrants (m) from population i to population j using the formula: $m_{i \rightarrow j} = (M_{i \rightarrow j})/\mu$ (where $\mu = 5.4 \times 10^{-4}$; Goldstein et al. 1995), so that these values would be more comparable to estimates of m produced by BAYESASS. Additionally, we converted long-term estimates of gene flow (M) to the average number of effective migrants

($N_e m$) from population i to population j using the formula: $N_e m_{i \rightarrow j} = (\Theta_i M_{i \rightarrow j})/4$.

Concordance of short- and long-term gene flow estimates

To determine if short- and long-term estimates of gene flow for each species were correlated we performed two tests. First, we calculated Spearman's r coefficients (ρ) between bidirectional estimates of gene flow for each population pair (Fraser et al. 2007a). Second, we compared the asymmetric matrices of bidirectional short-term and long-term estimates of gene flow using a Mantel test as implemented in CADM (Congruence Among Distance Matrices; Legendre and Lapointe 2004). This test allowed us to test statistically the congruence of these temporally different estimates of population connectivity over 9,999 permutations.

Results

After sequential Bonferroni correction (Rice 1989), only two of 75 pairwise tests for linkage disequilibrium were significant in the black ratsnake dataset, and no pairwise tests for linkage disequilibrium were significant in the Blanding's

turtle dataset, suggesting that loci used for both species were unlinked. For ratsnakes, 2 of 30 tests for Hardy–Weinberg departures were significant after sequential Bonferroni correction: one for EM2 (QUBS) and one for EMS34 (SLINP) (see Lougheed et al. 1999 for primer details). For Blanding’s turtles, 2 of 14 tests for Hardy–Weinberg departures were significant after sequential Bonferroni correction: one for CC7 (PR) and one for Eb11 (ML) (see Mockford et al. 2005 for primer details).

Genetic structure and gene flow in the black ratsnake

Bayesian clustering of groups of individuals revealed four independent clusters that were generally concordant with our sampled subpopulations of black ratsnakes in eastern Ontario (1. CLPP/QUBS, 2. LRM, 3. MPPP, and 4. SLINP) (Fig. 3). Bayesian analyses revealed that the proportion of individuals assigned to their population of origin (the population in which they were sampled) ranged from 0.52 to 0.82, with a mean of 0.69. The proportion of individuals assigned to other populations ranged from 0 to 0.30, with a mean of 0.075 (Table 2). Estimates of Θ produced by MIGRATE for the 5 subpopulations ranged from 0.10 to 0.72, with a mean of 0.31 (Table 1).

Estimates of short-term gene flow were significantly asymmetric (i.e., non-overlapping 95% confidence intervals) for only one pair of black ratsnake subpopulations in eastern Ontario (Table 4): QUBS had greater gene flow into CLPP than vice versa. The largest estimate of short-term gene flow occurred from QUBS to CLPP (0.23), whereas the smallest estimate of short-term gene flow occurred from CLPP to QUBS (0.0024). No pairs of subpopulations showed

significant asymmetric long-term estimates of gene flow (Table 4). The largest estimate of long-term gene flow occurred from CLPP to QUBS (0.0040), whereas the smallest estimate of long-term gene flow occurred from LRM to SLINP (0.0010).

QUBS had the highest net emigration rate (sum of outgoing gene flow minus the sum of incoming gene flow) according to the short-term estimates of gene flow, and yet this subpopulation had the smallest net emigration rate according to the long-term estimates of gene flow. The subpopulation with the highest net emigration rate according to the long-term estimates of gene flow was LRM. Short-term estimates of net emigration rate were negative in CLPP (−0.29) and SLINP (−0.022), and long-term estimates of net emigration were negative in CLPP (−0.0020) and QUBS (−0.0022) (Table 5).

Short- and long-term bidirectional estimates of gene flow across subpopulation pairs were not significantly correlated (Spearman’s $r = + 0.041$, $P = 0.87$). Additionally, there was no concordance between matrices of short- and long-term pairwise estimates of gene flow ($r = + 0.52$, $P = 0.48$), suggesting that recent dispersal patterns are not consistent with long-term gene flow patterns among subpopulations.

Genetic structure and gene flow in the Blanding’s turtle

Bayesian clustering of groups of individuals revealed two independent clusters that were generally concordant with our sampled subpopulations of Blanding’s turtles in Nova Scotia (1. KNP, and 2. ML/PR) (Fig. 4). Bayesian analyses revealed that the proportion of individuals assigned to their

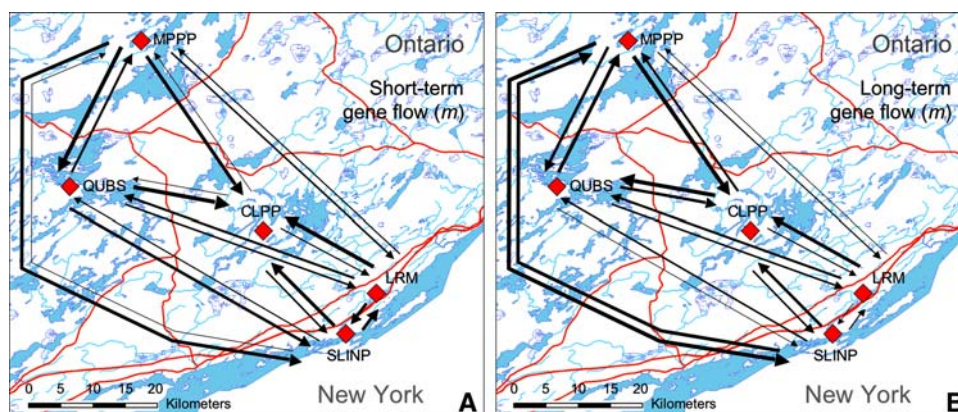


Fig. 3 Local gene flow (m = proportion of migrants) patterns among subpopulations of the black ratsnake in eastern Ontario based on short-term estimates of gene flow (a) and long-term estimates of gene flow (b). Arrows indicate direction of gene flow between each subpopulation pair, and the relative thickness of each arrow represents the relative amount of directional gene flow. Sample sites included in

this study are indicated by red diamonds and their abbreviations are as follows: CLPP (Charleston Lake Provincial Park), LRM (LaRue Mills), MPPP (Murphy’s Point Provincial Park), SLINP (St. Lawrence Islands National Park), and QUBS (Queen’s University Biological Station). Red lines represent primary roads

Table 2 Results of Bayesian population level assignment tests performed for five Ontario populations of the black ratsnake in GENECLASS2

Sampled subpopulation	Assigned subpopulation				
	CLPP	LRM	MPPP	QUBS	SLINP
CLPP	0.52	0.043	0.087	0.30	0.043
LRM	0.029	0.82	0	0.088	0.059
MPPP	0.13	0.017	0.57	0.27	0
QUBS	0.13	0.015	0.038	0.80	0.015
SLINP	0.054	0.027	0.054	0.11	0.76

Values represent the proportion of individuals assigned to each sampled subpopulation. Diagonal values are in bold and represent the proportion of individuals assigned to the subpopulation in which they were sampled

population of origin ranged from 0.37 to 0.73, with a mean of 0.59. The proportion of individuals assigned to other populations ranged from 0 to 0.59, with a mean of 0.20 (Table 3). Estimates of Θ produced by MIGRATE for the 3 populations ranged from 1.12 to 1.53, with a mean of 1.28 (Table 1).

No pairs of Blanding's turtle subpopulations showed asymmetric short-term estimates of gene flow (i.e. non-overlapping 95% confidence intervals) and there were no statistically significant estimates of short-term gene flow among any of the local populations (Table 4). The largest estimate of short-term gene flow occurred from PR to ML (0.16), whereas the smallest estimate of short-term gene flow occurred from KNP to PR (0.012). Only one subpopulation pair showed asymmetric long-term estimates of gene flow: ML had greater gene flow into PR than vice versa (Table 4). The largest estimate of long-term gene flow occurred from ML to PR (0.0054), whereas the smallest estimate of long-term gene flow occurred from PR to ML (0.0030).

Table 3 Results of Bayesian population level assignment tests performed for three Nova Scotia subpopulations of the Blanding's turtle in GENECLASS2

Sampled subpopulation	Assigned subpopulation		
	KNP	ML	PR
KNP	0.73	0.23	0
ML	0.25	0.68	0.075
PR	0.037	0.59	0.37

Values represent the proportion of individuals assigned to each sampled subpopulation. Diagonal values are in bold and represent the proportion of individuals assigned to the subpopulation in which they were sampled

KNP had the highest net emigration rate according to both the short- and long-term estimates of gene flow, suggesting that this is currently an important genetic source subpopulation for the metapopulation, and has been so historically. Short-term estimates of net emigration were negative in ML (-0.27), whereas long-term estimates of net emigration rate were negative in PR (-0.0029) (Table 5).

Similar to the black ratsnake, short- and long-term bidirectional estimates of gene flow across subpopulation pairs in the Blanding's turtle were not significantly correlated (Spearman's $r = -0.14$, $P = 0.79$), and there was a lack of concordance between matrices of short- and long-term pairwise estimates of gene flow (M) ($r = 0.54$, $P = 0.66$).

Discussion

Population dynamics in the black ratsnake

Bayesian clustering of groups of individuals of black ratsnake and population-level assignment tests were generally

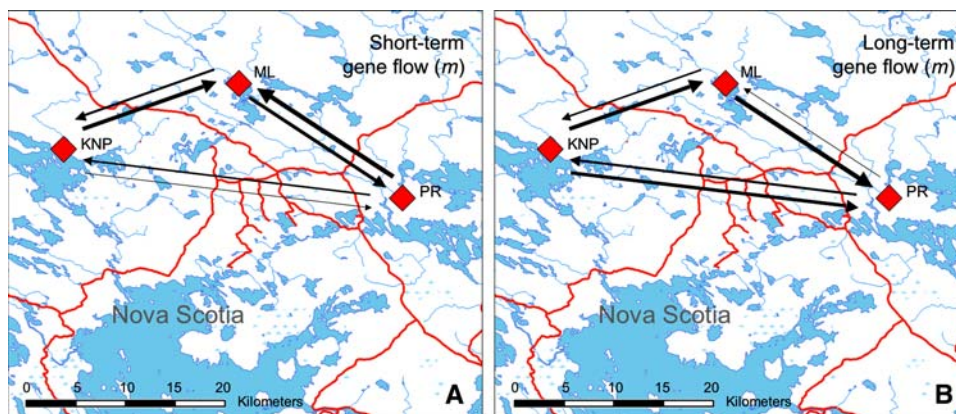


Fig. 4 Local gene flow (m = proportion of migrants) patterns among subpopulations of the Blanding's turtle in southern Nova Scotia based on short-term estimates of gene flow (a) and long-term estimates of gene flow (b). Arrows indicate direction of gene flow between each subpopulation pair, and the relative thickness of each arrow represents

the relative amount of directional gene flow. Sample sites included in this study are indicated by red diamonds and their abbreviations are as follows: KNP (Kejimikujik National Park), ML (McGowan Lake), and PR (Pleasant River). Red lines represent primary roads

Table 4 Directional gene flow estimates for each subpopulation pair in a metapopulation of black ratsnake in eastern Ontario and Blanding’s turtle in southern Nova Scotia

Subpopulation pair	Nm based on F_{ST} results ^a	Short-term gene flow (m)	Long-term gene flow (m)
Black ratsnake			
CLPP to LRM	0.06	0.0039 (0–0.021)	0.0016 (0.00090–0.0025)
CLPP to MPPP	7.15	0.0030 (0–0.017)	0.0030 (0.0020–0.0046)
CLPP to QUBS	80.40	0.0023 (0–0.011)	0.0040 (0.0025–0.0054)
CLPP to SLINP	9.18	0.0045 (0–0.022)	0.0027 (0.0017–0.0038)
LRM to CLPP	0.06	0.033 (0.00069–0.099)	0.0033 (0.0024–0.0042)
LRM to MPPP	2.06	0.0038 (0–0.020)	0.0015 (0.0007–0.0026)
LRM to QUBS	2.67	0.0070 (0–0.028)	0.0032 (0.0023–0.0046)
LRM to SLINP	1.63	0.0087 (0–0.041)	0.0010 (0.00030–0.0018)
MPPP to CLPP	7.15	0.031 (0.0003–0.11)	0.0036 (0.0021–0.0049)
MPPP to LRM	2.06	0.0044 (0–0.023)	0.0019 (0.00030–0.0039)
MPPP to QUBS	7.17	0.088 (0–0.048)	0.0033 (0.0022–0.0043)
MPPP to SLINP	3.72	0.017 (0.00048–0.053)	0.0039 (0.0028–0.0050)
QUBS to CLPP	80.40	0.23 (0.13–0.31)	0.0032 (0.0017–0.0052)
QUBS to LRM	2.67	0.0058 (0–0.031)	0.0022 (0.0012–0.0030)
QUBS to MPPP	7.17	0.0070 (0–0.042)	0.0034 (0.0018–0.0049)
QUBS to SLINP	8.81	0.012 (0–0.047)	0.0015 (0.00060–0.0026)
SLINP to CLPP	9.18	0.022 (0.00027–0.07)	0.0032 (0.0013–0.0054)
SLINP to LRM	1.63	0.012 (0–0.043)	0.0017 (0.00090–0.0031)
SLINP to MPPP	3.72	0.0025 (0–0.014)	0.0034 (0.0021–0.0049)
SLINP to QUBS	8.82	0.0035 (0–0.018)	0.0020 (0.0013–0.0028)
Blanding’s turtle			
KNP to ML	5.80	0.15 (0.028–0.26)	0.0049 (0.0035–0.0065)
KNP to PR	1.76	0.012 (0.00013–0.052)	0.0042 (0.0033–0.0050)
ML to KNP	5.80	0.017 (0.00015–0.060)	0.0034 (0.0026–0.0042)
ML to PR	5.70	0.027 (0.00015–0.12)	0.0054 (0.0044–0.0065)
PR to KNP	1.76	0.013 (0.00031–0.046)	0.0037 (0.0029–0.0044)
PR to ML	5.70	0.16 (0.054–0.30)	0.0030 (0.0021–0.0040)

Short-term estimates of gene flow and long-term estimates of gene flow represent the mean migration rate and 95% confidence interval (in brackets) for a pair of subpopulations

^a F_{ST} values used for Nm calculations are from Loughheed et al. (1999; black ratsnakes) and Mockford et al. (2005; Blanding’s turtles)

Table 5 Summary of net immigration and emigration rates among subpopulations in metapopulations of black ratsnake in eastern Ontario and Blanding’s turtle in southern Nova Scotia

Subpopulation	Total emigration (m)		Total immigration (m)		Net emigration (m)	
	Short-term	Long-term	Short-term	Long-term	Short-term	Long-term
Black ratsnake						
CLPP	0.014	0.011	0.30	0.013	–0.29	–0.0020
LRM	0.052	0.0090	0.026	0.0074	+0.026	+0.0016
MPPP	0.060	0.013	0.016	0.011	+0.044	+0.0013
QUBS	0.26	0.010	0.020	0.012	+0.24	–0.0022
SLINP	0.020	0.010	0.042	0.0091	–0.022	+0.0012
Blanding’s turtle						
KNP	0.16	0.0091	0.030	0.0071	+0.13	+0.0020
ML	0.043	0.0088	0.31	0.0079	–0.27	+0.00092
PR	0.17	0.0067	0.039	0.0096	+0.13	–0.0029

The last column represents the sum of all emigration rates minus the sum of all immigration rates

consistent with each other and with previous genetic differentiation analyses based on F_{ST} (Lougheed et al. 1999). Results showed that each sampled subpopulation clustered independently from all other sampled subpopulations, except for QUBS and CLPP, which clustered together. Results from previous research indicated that this subpopulation pair had the lowest F_{ST} value of any eastern Ontario subpopulation pair (Lougheed et al. 1999). This is not just a function of geographic distance. Indeed, Lougheed et al. (1999) noted that although geographic distance is a good predictor of population differentiation, the relation is imperfect. Thus, our results confirm that population connectivity involves factors in addition to geographic distance.

When we converted our long-term gene flow estimates to estimates of average effective migrants, we found that $N_e m$ values among subpopulations ranged from 0.085 to 1.06, suggesting low to moderate gene flow among local populations. These $N_e m$ values were lower than Nm values predicted by F_{ST} measures, which ranged from 1.6 to 80.1 (Lougheed et al. 1999; Table 4). Our results generally agree with long-term ecological data collected in the QUBS subpopulation. Telemetry data showed that particularly mobile individuals from hibernacula up to 8 km apart could mate together, but that individuals from hibernacula separated by 15 km did not come together to mate (Blouin-Demers and Weatherhead 2002).

We do not have the data to compare observed metapopulation structure with other metapopulations of ratsnakes, but $N_e m$ has been estimated using MIGRATE for other snakes at comparable spatial scales. Manier and Arnold (2005) examined metapopulation dynamics in the terrestrial garter snake (*Thamnophis elegans*) and the common garter snake (*T. sirtalis*) in California and found that the mean pairwise $N_e m$ among subpopulations was 0.32 for the terrestrial garter snake and 0.54 for the common garter snake. Bittner and King (2003) examined metapopulation dynamics in the Lake Erie populations of the garter snake, and found that the mean pairwise $N_e m$ among subpopulations was 0.34. These values are comparable to those we found for ratsnakes (mean pairwise $N_e m = 0.38$), which from this limited data set suggests gene flow may be similar among snake species.

Finally, the mean Θ of our sampled subpopulations (0.31; range: 0.10–0.72) falls within the values found in other temperate snakes. Manier and Arnold (2005) found a mean Θ of 0.13 for subpopulations of both the terrestrial and common garter snake in California, whereas Bittner and King (2003) estimated a mean Θ of 1.31 for subpopulations of Lake Erie garter snakes. To generate estimates of effective population size, we assumed an average microsatellite mutation rate of 5.4×10^{-4} mutations per locus per generation (Goldstein et al. 1995). Based on this

mutation rate, effective population sizes of our sampled subpopulations ranged from 48 in the smallest subpopulation (SLINP) to 331 in the largest subpopulation (QUBS). At least one of these estimates of effective population size is concordant with a population size estimate based on long-term demographic data: Blouin-Demers and Weatherhead (2002) predicted a population size of 338 mature individuals for QUBS, based on their knowledge of the number of hibernacula and number of mature individuals at each hibernaculum in the subpopulation. Further investigation into the observed patterns of discordance between short- and long-term estimates of gene flow in the ratsnake is warranted. For instance, it is unclear how such patterns of discordance relate to changes in landscape or anthropogenic development. The apparent reversal of historic and contemporary patterns of gene flow between CLPP and QUBS is particularly intriguing. It is possible that historic patterns of forest clearing may have shifted subpopulations from genetic sources to sinks. To the extent that such patterns could be reconstructed it would be informative for future conservation. Relating changes in landscape use to changes in net migration patterns could also help resolve current population trends. Long-term demographic data (Weatherhead et al. 2002) indicate that both QUBS and SLINP subpopulations are declining, even though our analyses found that QUBS is currently a net exporter, whereas SLINP is currently a net importer. Reconciling these discrepancies will also be important for future conservation efforts.

Population dynamics in the Blanding's turtle

Bayesian clustering of groups of individuals and population-level assignment tests were generally consistent with each other and with previous genetic differentiation analyses based on F_{ST} (Mockford et al. 2005). Results showed that KNP clustered independently, whereas ML and PR clustered together.

When we converted long-term gene flow estimates to values of $N_e m$, we found that the mean pairwise $N_e m$ value among subpopulations was 2.45 (ranging from 1.54 to 3.84), suggesting moderate to high gene flow among subpopulations. These $N_e m$ values were lower than Nm values predicted by F_{ST} measures, which ranged from 1.76 to 5.80 (Mockford et al. 2005). Although field studies have not detected movement between subpopulations, individuals have been observed moving more than 15 km over terrestrial and aquatic habitat (Power 1989), so the high gene flow estimates are not entirely unexpected.

To our knowledge, this is the first study that has inferred bidirectional gene flow estimates in a freshwater turtle. However, mean Nm based on F_{ST} measures among subpopulations of the neotropical freshwater turtle (*Hydromedusa*

maximiliana) was estimated to be 0.59 (Souza et al. 2002), much lower than the N_m estimates for Blanding's turtle reported in our study, despite the fact that subpopulations of the Blanding's turtle were separated by greater distances.

The mean Θ of the three sampled subpopulations was 1.28 (ranging from 1.12 to 1.53). Again, assuming a microsatellite mutation rate of 5.4×10^{-4} mutations per locus per generation (see above), effective population sizes of our sampled subpopulations ranged from 516 in the smallest population (PR) to 707 in the largest population (ML). To our knowledge, estimates of effective population sizes have not yet been published for other freshwater turtle populations. These estimates of effective population size are greater than estimates of population size based on long-term demographic data that have been reported for two of the three subpopulations in the metapopulation (KNP = 132 and ML = 79, see Mockford et al. 2005 and references therein).

As with ratsnakes, our results raise several questions about the factors responsible for gene flow patterns. What long-term changes in landscape have contributed to the apparent decrease in relative gene flow between KNP and PR and the apparent increase in relative gene flow from PR to ML? Similarly, why has ML switched from being a net exporter to a net importer while PR has made the opposite change? To address these questions would likely require long-term demographic data combined with long-term environmental and habitat use data.

Differences between previous F_{ST} results and our results

Gene flow estimates inferred from F_{ST} measures were consistently higher than those inferred from statistical analyses based on Bayesian theory, coalescent theory, and Markov chain Monte Carlo techniques. It has been suggested that F_{ST} measures cannot be translated into accurate estimates of gene flow (Whitlock and McCaughley 1999). Moreover, our estimates of pairwise population gene flow are bidirectional, offering new insight into population connectivity patterns. For instance, while previous F_{ST} measures identified high connectivity between QUBS and CLPP, our contemporary gene flow estimates revealed that this connectivity is maintained almost exclusively via gene flow from QUBS to CLPP, suggesting that the persistence of CLPP depends disproportionately on QUBS. Finally, estimating directionality of gene flow provides information with respect to genetic source-sink dynamics of a metapopulation that may be relevant to conservation efforts. Given that genetically identified source subpopulations should be prioritized for conservation (Manier and Arnold 2005), our results provide input into which subpopulations might be prioritized for conservation. For the black

ratsnake, short-term estimates of gene flow indicate that QUBS is an extremely important genetic source subpopulation to the metapopulation, and thus may have increased conservation value relative to other subpopulations. In addition, LRM and MPPP were the only two subpopulations that were identified as genetic sources based on both short-term and long-term estimates of gene flow. Thus, these two subpopulations may also have increased conservation value relative to other subpopulations in the metapopulation. For the Blanding's turtle, short-term estimates of gene flow indicate that both KNP and PR are genetic source subpopulations to the metapopulation, and thus may have increased conservation value relative to ML. KNP was the only subpopulation that was identified as a genetic source based on both short-term and long-term gene flow estimates, which further supports its high conservation value.

Discordances between temporal methods of estimating gene flow

Discordance between temporal methods was detected in both of our focal species, and has been detected elsewhere (Fraser et al. 2004, 2007a; Hendry and Taylor 2004; but see Hänfling and Weetman 2006). Contrary to our prediction that a shorter generation time should result in greater discordance between short- and long-term estimates for ratsnakes than for Blanding's turtles, the two species showed similar levels of discordance. Discordance between estimates of short- and long-term gene flow could reflect differential reproductive fitness between migrants and non-migrants (e.g. individual dispersal does not result in gene flow if migrants do not reproduce in their new population) or true temporal variation in population dynamics, perhaps due to a change in landscape use (Austin et al. 2004). Certainly, there has been substantial landscape alteration in both study systems, as a result of anthropogenic influence. For instance, road density in the greater park ecosystem of SLINP and KNP was recently estimated to be 0.69 km/km² and 0.38 km/km², respectively (Rivard et al. 2000; Donald Rivard, pers. comm.), clearly much higher than was the case historically.

Discordance could also result from different assumptions of the two methods; contrary to long-term methods used in MIGRATE, short-term methods used in BAYESASS do not assume migration-drift equilibrium and constant migration rates. Additionally, MIGRATE assumes that the microsatellite data fit the stepwise mutation model, whereas BAYESASS does not make this assumption. While data for both species did not qualitatively appear to grossly violate this assumption (given that allele sizes show relatively continuous distributions at each locus), we cannot discount the possibility that results are influenced by the differing

assumptions of the methods we employed and how the data fit these assumptions. Ultimately, genetic samples collected over multiple temporal scales can discount methodological differences between approaches and reveal true biological patterns over time (see Fraser et al. 2007a, b).

We currently lack the information to determine whether the cause of the discordance between gene flow estimates reflects differing assumptions of the two methods or true temporal variation in population dynamics. However, our net emigration approach allows us to directly compare population export patterns rather than gene flow estimates, and to a large extent this removes the effect of different methodologies, thereby supporting the notion that population dynamics vary temporally in both systems. In the face of limited resources, short-term estimates of gene flow are valuable in prioritizing populations for conservation and in targeting specific populations for further demographic study (Manier and Arnold 2005).

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