

Impact of urban fragmentation on the genetic structure of the eastern red-backed salamander

Sarah Noël · Martin Ouellet · Patrick Galois · François-Joseph Lapointe

Received: 17 January 2006 / Accepted: 9 August 2006 / Published online: 28 September 2006
© Springer Science+Business Media B.V. 2006

Abstract Urban development is a major cause of habitat loss and fragmentation. Few studies, however, have dealt with fragmentation in an urban landscape. In this paper, we examine the genetic structure of isolated populations of the eastern red-backed salamander (*Plethodon cinereus*) in a metropolitan area. We sampled four populations located on a mountain in the heart of Montréal (Québec, Canada), which presents a mosaic of forested patches isolated by roads, graveyards and buildings. We assessed the genetic structure of these populations using microsatellite loci and compared it to the genetic structure of four populations located in a continuous habitat in southern Québec. Our results indicate that allelic richness and heterozygosity are lower in the urban populations. Exact differentiation tests and pairwise F_{ST} also show that the populations found in the fragmented habitat are genetically differentiated, whereas populations located in the continuous habitat are genetically homogeneous. These results raise conservation concerns for these populations as well as for rare or threatened species inhabiting urban landscapes.

Keywords Conservation genetics · Habitat fragmentation · Microsatellites · *Plethodon cinereus* · Urbanization

S. Noël (✉) · F.-J. Lapointe
Département de sciences biologiques, Université de Montréal, P.O. Box 6128, Succursale Centre-Ville, Montréal, Québec, Canada H3C 3J7
e-mail: sarah.noel-boissonneault@umontreal.ca

M. Ouellet · P. Galois
Amphibia-Nature, 4254 rue Garnier, Montréal, Québec, Canada H2J 3R5

Introduction

In the past few years, a decline of amphibian populations has been observed worldwide (Houlahan et al. 2000). Although the causes of this decline often remain unknown, numerous factors have been proposed, such as climate changes (Pounds et al. 1999), pollutants (Kucken et al. 1994), ultraviolet radiations (Blaustein et al. 1998), diseases (Berger et al. 1998, but see Ouellet et al. 2005b), acid rain (Beebee et al. 1990), and invasive species (Knapp and Matthews 2000). However, it is habitat modifications including habitat loss, degradation and fragmentation that represent the major factors affecting amphibian diversity (Green 1997; Dodd and Smith 2003).

Many studies have dealt with fragmentation caused by agricultural or logging activities (e.g., Kolozsvary and Swihart 1999; Vos et al. 2001). However, urban development has also been a major factor of landscape evolution and habitat fragmentation (Miller and Hobbs 2002). During the second half of the 20th century, the proportion of the human population living in urban areas has increased steadily, rising from 29% in 1950 to 47% in 2000 (United Nations Population Division 2004). In Canada, more than 78% of the population now live in urban centers (Statistics Canada 2000). As a consequence, urban agglomerations are sprawling at the expense of natural areas within and outside the cities.

Urbanization has a strong negative impact on biodiversity. For example, it has been shown to change species composition and increase the number of invasive species (Tait et al. 2005). Habitat loss due to urbanization is the principal cause of species endangerment in the mainland United States (Czech et al.

2000). To limit the effects of urbanization, parks are often created within metropolitan areas to retain a part of the original biodiversity of the region, and they are thus a very important component of landscapes. However, little is known about animal populations living in urban natural areas, and to what extent they are affected by urban challenges such as human disturbance, pet predation, proliferation of pest species and most importantly, habitat fragmentation.

Habitat fragmentation can be defined as the subdivision of a large area of habitat into smaller isolated patches (Wilcove et al. 1986). It usually implies a diminution in the total amount of habitat available and a change in the spatial configuration of the remaining habitat (Fahrig 2003). Fragmentation is thought to have severe effects on the genetic structure of populations. After fragmentation, the remaining populations are smaller and more susceptible to greater temporal variation in population size, which increase their extinction probabilities (Reed and Hobbs 2004). Following bottlenecks, populations may experience loss of rare alleles and a diminution of heterozygosity through genetic drift and inbreeding. These processes contribute to the erosion of genetic diversity and can lead to fitness reduction (Reed and Frankham 2003). Also, as genetic diversity represents the adaptive potential of a species, reduced genetic variability can inhibit the ability of a population to respond to rapid environmental changes (Young et al. 1996).

Here, we assess the impact of urban fragmentation on the population genetic structure of the eastern red-backed salamander (*Plethodon cinereus*), a small terrestrial amphibian species distributed throughout eastern North America. Two different color morphs are found in most natural populations. The striped morph has a red stripe on the back and the tail, whereas the lead-backed morph is uniformly dark and lacks this stripe. This coloration pattern is controlled by a single gene for which the allele coding for the striped morph is dominant (Highton 1959). Additionally, erythristic morphs, which are entirely red, and albinos are also occasionally encountered in some populations (Reed 1908; Rosen 1971; Dyrkacz 1981). Like all members of the genus *Plethodon*, the eastern red-backed salamander does not have an aquatic larval stage (Petranka 1998). Females lay their eggs under logs, rocks and other natural cover objects and the eggs develop into tiny salamanders resembling the adults. Eastern red-backed salamanders are associated with mature forests with moist soils (Grover 1998; Bonin et al. 1999; Hyde and Simons 2001) and rarely venture across dry and hot habitats (Larson et al. 1984). Dispersal distances of *Plethodon cinereus* are not clearly

known. Although the mean daily movements are as low as 0.4 m, it has been shown that some individuals can easily travel 30 m to return to their territory when displaced. However, the homing ability is greatly reduced when displacement distances increase up to 90 m (Kleeberger and Werner 1982). *P. cinereus* usually occurs at high densities (Jaeger 1979) and often represents the most abundant species of salamander in forests (Burton and Likens 1975; Marsh and Beckman 2004). Eastern red-backed salamanders can even be encountered in highly patchy habitats, and thus seem to be resilient to fragmentation (Gibbs 1998a). However, the population sizes of isolated populations are probably reduced because small habitats usually harbor smaller populations than larger patches (Fahrig 2003) and because the eastern red-backed salamander is sensitive to edge effect (deMaynadier and Hunter 1998). Consequently, dispersal patterns and genetic diversity may be altered in isolated populations, but it is not clear to what extent habitat fragmentation affects the genetic structure of the eastern red-backed salamander.

In this paper, the effect of habitat fragmentation on the eastern red-backed salamander was evaluated by comparing isolated urban populations with populations located in a continuous habitat. First, we predicted that populations from the fragmented habitat would exhibit lower genetic diversity. Secondly, because all urban salamander populations are isolated by dispersal barriers, we expected to detect a higher level of population differentiation among these populations.

Methods

Sampling

Sampling was conducted in the summer and fall of 2004 on two mountains. Urban salamander populations were sampled from the Mount Royal (45°30' N, 73°35' W), which is a highly fragmented mountain (233 m high) located in the heart of Montréal (Québec, Canada). It is part of the Monteregian Hills, a series of small mountains formed during the Cretaceous by the intrusion of igneous rock into older sedimentary rock (Adams 1903; Ouellet et al. 2005a). The Mount Royal represents an important reservoir of biodiversity in the urban landscape. Unfortunately, it has been gradually fragmented by human activities since the arrival of the first European settlers over 350 years ago. A road, created around 1700, still divides the Mount Royal in two parts, and was probably the first main anthropogenic feature to fragment the habitat. Between 1852 and 1855, two

graveyards were established and have considerably contributed to the deforestation of the mountain in the following years. In 1924, a tramway line was constructed along an east/west axis, and was later transformed into a paved road. Intensive residential and commercial development has also occurred on the mountain throughout the years. This strong urbanization has already led to the extinction of at least four species of amphibians and reptiles on the mountain (M. Ouellet et al. unpublished results). Nowadays, the Mount Royal presents a mosaic of forested habitats, some of them still inhabited by populations of eastern red-backed salamanders. The Mount Royal populations (MR-1 to MR-4) are isolated from each other by roads, graveyards or buildings, and are separated by distances ranging from 0.9 to 3.3 km (Fig. 1A).

Salamanders were also collected from the Mount Mégantic (45°27' N, 71°09' W), which lies 190 km east of Montréal, and is the highest (1105 m) and the most pristine of the Monteregian Hills. It is part of a National Park, and it is protected against any form of commercial exploitation or deforestation. The mountain is entirely forested and hence represents a continuous habitat where the distribution of eastern

red-backed salamanders is constrained only by altitude. Since we were not able to circumscribe isolated populations in this continuous habitat, we selected four sampling sites (MM-1 to MM-4) distributed in a pattern similar to that of the Mount Royal. Those sampling sites were separated by distances ranging from 0.8 to 4.1 km (Fig. 1B).

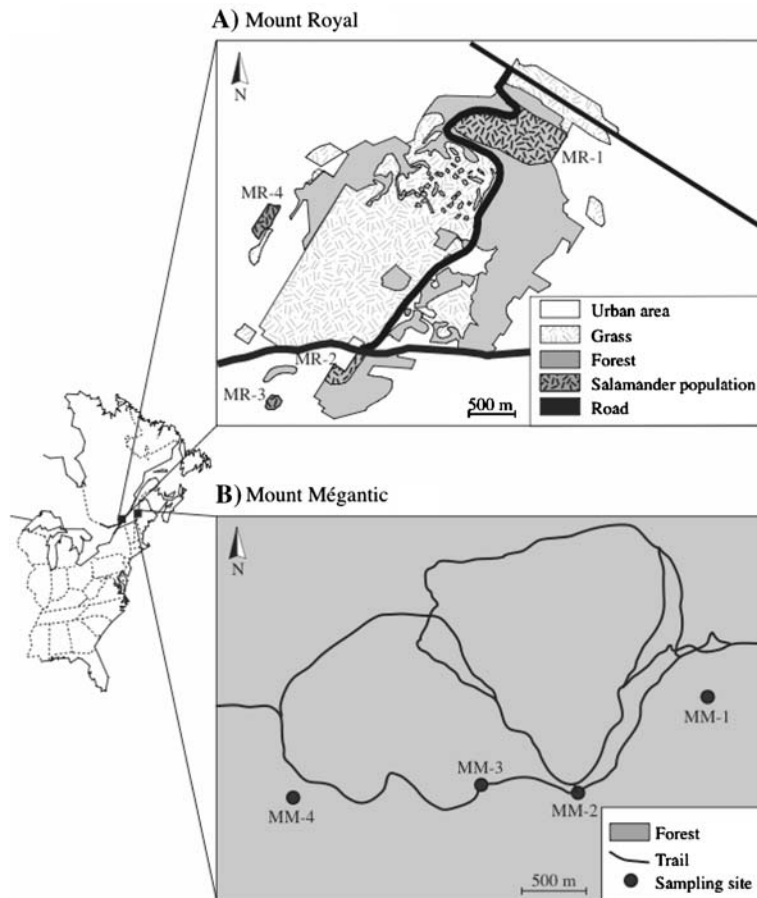
In each of the eight populations on the two mountains, we collected from 25 to 30 specimens by active searches under natural cover objects. Tail-tips were cut and stored in 95% ethanol at room temperature until further DNA extraction.

Genotyping

DNA was extracted using a standard phenol–chloroform protocol (Sambrook et al. 1989) with phase-lock gel (Murphy and Hellwig 1996). We used seven microsatellite loci (PcII14, PcJX06, PcJX24, PcLI16, PcLX16, PcLX23, and PcXF08) designed for *P. cinereus* (Connors and Cabe 2003). All forward primers were fluorescently labeled.

Amplifications were carried out in 10 µl volumes including 10 mM Tris–HCl, 0.1% Triton X-100, 50 mM

Fig. 1 Map of eastern North America with inserts showing the populations of the eastern red-backed salamander (*Plethodon cinereus*) sampled from the fragmented habitat of the Mount Royal (A) and the continuous habitat of the Mount Mégantic (B)



KCl, 0.3–0.7 mM MgCl₂, 0.25 mM of each dNTP, 0.2 μM of each primer, 0.5 U of *Taq* DNA polymerase, and 50–100 ng of template DNA. PCR conditions were as followed: after initial denaturation at 94°C for 2 min, cycling was performed for 30 cycles of 30 s at 94°C, 45 s at 61°C and 30 s at 72°C, with a final extension of 5 min at 72°C for all loci, except PcII14 for which annealing temperature was 57°C. PCR products were separated on an acrylamide gel using an ABI prism 3100 genetic analyzer (Applied Biosystems Inc.). We determined all genotypes using Genescan analysis 3.1.2 (Applied Biosystems Inc.).

Analyses

The frequency of red-backed and lead-backed morphs for each population was computed as a mean to evaluate population differentiation with morphological characters. Differences between mountains were assessed with a Fisher exact test. Linkage disequilibrium across all populations and deviation from Hardy–Weinberg equilibrium were tested with Genepop version 3.2 using a Markov Chain method to estimate the exact probability of each test (Raymond and Rousset 1995). We estimated genetic diversity using observed heterozygosity (H_o), expected heterozygosity (H_e) and allelic richness, a measure of the number of alleles per locus corrected for differences in population size with a resampling procedure (Petit et al. 1998). We also recorded the number of private alleles in each population. All parameters were computed using FSTAT version 2.9.3.2 (Goudet 2002). To test the significance of the differences in H_o , H_e , and allelic richness between Mount Royal and Mount Mégantic populations, we used the non-parametric test implemented in FSTAT with 10,000 permutations. The one-tailed permutation test was used because we expected the populations in the fragmented landscape to have smaller heterozygosity and allelic richness values.

To examine the population structure within and between mountains, we performed Fisher exact tests of population differentiation across all populations and for all pairwise comparisons in Genepop version 3.2 (Raymond and Rousset 1995). We also assessed population structure by an analysis of molecular variance (AMOVA, Excoffier et al. 1992) and all pairwise F_{ST} were tested for significance in Arlequin 2.0 (Schneider et al. 2000). To measure dispersal among populations on each mountain, we performed assignment tests (Wasser and Strobeck 1998; Berry et al. 2004) using the Bayesian approach of Rannala and Mountain (1997) implemented in GeneClass2 (Piry et al. 2004).

Sequential Bonferroni corrections were applied whenever necessary to correct for multiple comparisons (Rice 1989).

Results

A total of 221 *P. cinereus* were sampled from the two mountains. Striped individuals largely predominated in all populations, with a single lead-backed individual found on Mount Mégantic (99.0% striped) and four on Mount Royal (96.7% striped). The morphotype frequencies were not statistically different on the two mountains ($P = 0.243$).

All loci were polymorphic except PcJX24 for which a single allele was observed. This locus was thus excluded from further analyses. The number of alleles per locus ranged from 5 (PcLI16) to 18 (PcII14), with a mean of 12 alleles per locus and a total of 72 different alleles. No significant linkage disequilibrium was observed for any pair of loci after correcting for multiple comparisons. All populations were in Hardy–Weinberg equilibrium except MR-1 and MM-1 for the PcLX16 locus, but all loci were used for further analyses. Number of alleles, number of private alleles, allelic richness, observed heterozygosity (H_o) and expected heterozygosity (H_e) for each population are given in Table 1. One-tailed permutation tests revealed that allelic richness ($P = 0.008$), H_o ($P = 0.0117$) and H_e ($P = 0.0117$) are significantly higher for Mount Mégantic.

Fisher exact tests of population differentiation revealed a highly significant difference between allelic frequencies of the Mount Royal and Mount Mégantic populations, when all individuals from the same mountain were pooled together ($P < 0.0001$). Within Mount Royal, differentiation was significant for all populations ($P < 0.0001$). Moreover, all pairs of population were clearly differentiated on the basis of allelic frequencies (Table 2). On the other hand, the populations from Mount Mégantic had similar allelic frequencies ($P = 0.5201$) and no pairwise comparison was significant (p ranging from 0.1570–0.8308). F_{ST} values were consistent with these results and were only significant for pairwise comparisons among Mount Royal populations (Table 2). Probabilities associated with F_{ST} in Mount Mégantic pairwise comparisons ranged from 0.3145 to 0.9365. A F_{ST} of 0.1882 was obtained between the two mountains when all individuals from the same mountain were pooled together. The AMOVA revealed that 79.5% of the molecular variance is explained by within-population variation. The partitioning of genetic variation between mountains ($P = 0.0293$) and among populations within

Table 1 Sample sizes (*n*), number of private alleles and estimates of genetic diversity averaged over all microsatellite loci for the populations of eastern red-backed salamanders (*Plethodon cinereus*) sampled from a fragmented habitat (Mount Royal) and a continuous habitat (Mount Megantic)

Populations	<i>n</i>	No. alleles	No. private alleles	Allelic richness	<i>H_o</i> ^a	<i>H_e</i> ^b
Mount Royal	120	44	16	4.18	0.42	0.44
MR-1	30	31	8	4.82	0.42	0.46
MR-2	30	30	5	4.67	0.38	0.40
MR-3	30	25	1	3.97	0.49	0.66
MR-4	30	20	2	3.24	0.41	0.44
Mount Megantic	101	62	20	6.85	0.64	0.74
MM-1	25	42	4	6.86	0.61	0.74
MM-2	26	38	4	6.90	0.64	0.75
MM-3	25	42	6	7.29	0.69	0.75
MM-4	25	44	6	6.37	0.62	0.76

^a *H_o*: Observed heterozygosity

^b *H_e*: Expected heterozygosity

Table 2 Pairwise *F_{ST}* values (upper triangular matrix) and probabilities of exact population differentiation tests (lower triangular matrix) for the four eastern red-backed salamander (*Plethodon cinereus*) populations of the Mount Royal

	MR-1	MR-2	MR-3	MR-4
MR-1	–	0.0439*	0.0604*	0.0326*
MR-2	0.0009*	–	0.1133*	0.1081*
MR-3	<0.0001*	<0.0001*	–	0.0270*
MR-4	0.0002*	<0.0001*	<0.0001*	–

All tests were performed with 10,000 permutations. Significant tests at the 0.05 level are indicated with an asterisk

mountains (*P* < 0.0001) were also significant (Table 3). The success rate of assignment tests was twice as good for the Mount Royal (53%) than for Mount Megantic (23%), indicating a higher dispersal rate among the continuous habitat of the Mount Megantic.

Discussion

Population differentiation

The comparison of populations sampled from fragmented and continuous landscapes allowed us to clearly assess the impact of habitat fragmentation on the genetic structure of the eastern red-backed salamander. Microsatellite analysis indicated a strong population differentiation on the Mount Royal, similar

to what has been observed for numerous amphibian populations inhabiting fragmented habitats (e.g., Hitchings and Beebee 1997; Gibbs 1998b; Pabijan et al. 2005). This differentiation challenges the conception that all populations from formerly glaciated areas are genetically uniform (Highton and Webster 1976). Eastern red-backed salamanders are sedentary animals with home range limited to tens of square meters, which may explain the population differentiation observed (Kleeberger and Werner 1982). However, they have been shown to travel across open fields up to 55 m (Marsh et al. 2004). In this study, populations were separated by non-hospitable habitat by 900 m or more, a distance sufficient to prevent important migration among populations. Moreover, some populations were separated by roads, which are known to have a strong negative effect on animal populations

Table 3 Analysis of molecular variance (AMOVA) assessing the genetic structure of eastern red-backed salamander (*Plethodon cinereus*) populations from Mount Royal and Mount Megantic

	df	Sum of squares	Variance components	% variation	<i>P</i>
Between mountains	1	89.13	0.39	18.27	0.0293
Among populations within mountains	6	25.82	0.05	2.24	<0.0001
Within populations	434	730.32	1.68	79.49	<0.0001
Total	441	845.27	2.12		

The probabilities were assessed with 10,000 permutations

through roadkills (Ehman and Cogger 1985; Kuhn 1987; Fahrig et al. 1995; Forman and Alexander 1998). In addition, roads disturb their surrounding habitat through noise and pollutants, which may reduce the densities of the eastern red-backed salamander (Marsh and Beckman 2004).

Unlike microsatellite markers, the analysis of morphotype frequency did not reveal any differences among populations of the Mount Royal. Greer (1973) suggested that the distribution of morph frequency in *P. cinereus* was correlated to climate, with unstriped morphs generally being more prevalent where the climate is warmer (Lotter and Scott 1977). Because all populations were sampled in the northern part of the species distribution, it may explain why striped individuals were largely predominant. However, differences in morph frequencies are known to occur over less than 10 km (Highton 1977), such that other factors should be involved to explain the variation in morph frequency. Although the mechanism is not yet fully understood, several hypotheses have been proposed, such as ecological isolation and differential fitness (e.g., Thurow 1961; Brown 1965; Pflingsten and Walker 1978).

Genetic diversity

All molecular estimates of genetic diversity were lower for the fragmented Mount Royal populations. Lower genetic diversity for populations in fragmented habitat has been observed for a variety of amphibian species (e.g., Hitchings and Beebee 1997, 1998; Andersen et al. 2004). However, in a similar study involving eastern red-backed salamander populations isolated for a comparable timescale (200–300 years), Gibbs (1998b) did not find significant differences in the genetic diversity of populations in fragmented and continuous habitat. In this particular study, genetic diversity was measured using three Random Amplified Polymorphic DNA markers (RAPDs, Welsh and McClelland 1990; Williams et al. 1990). Although these markers have been widely used in population genetics (e.g., Kimberling et al. 1996; Zeisset and Beebee 2003), they present serious drawbacks, such as uncertain homology and reproducibility concerns (Muralidharan and Wakeland 1993; Schierwater and Ender 1993; Rieseberg 1996). But most importantly, RAPDs are dominant markers. Thus it takes more markers to obtain a similar level of resolution with respect to co-dominant markers. This may explain, in part, the similarity in genetic diversity observed by Gibbs (1998b) for salamander populations living in fragmented and unfragmented habitat.

The diminution of genetic diversity may be related to the decrease of population size induced by fragmentation and subsequent genetic drift (Frankham 1996). Although the actual sizes of the Mount Royal populations are unknown, we have many reasons to believe that they are rather small. First, the remaining *P. cinereus* are confined to small patches, which harbor smaller populations than larger patches (Fahrig 2003). Secondly, the Mount Royal is frequented by 3 millions visitors every year for recreational activities, including mountain biking, and these visitors often wander outside established paths, thus perturbing even more the suitable habitat for salamanders (M. Ouellet et al. unpublished results). Finally, eastern red-backed salamanders are sensitive to habitat modifications induced by the proximity of forest edges (Marsh and Beckman 2004).

Based on simulations, the reduction of genetic variability due mainly to genetic drift should be detectable after 10 generations in small populations (Lacy 1987). While the generation time of eastern red-backed salamanders is not clearly known, it is generally assumed to be from 5 to 10 years (Gibbs 1998b). The first instance of fragmentation on the Mount Royal was the construction of a road separating populations MR-2 and MR-3 from the others in 1700. Although it was at first only a small country road, it was probably enough to somewhat reduce gene flow. Nowadays, this road is a major avenue that acts as a strong barrier to salamander migration. Using these figures, it appears that the eastern red-backed salamander populations may have been fragmented for at least 30 generations, a sufficient amount of time to observe the effects of genetic drift.

The observed loss of genetic diversity is worrying for the urban populations of the eastern red-backed salamander. Because genetic diversity is highly correlated with fitness, and because low genetic diversity reduces the ability of a species to adapt to changing environments, the long term survival of these populations may be at risk (Young et al. 1996; Reed and Frankham 2003). Moreover, the populations are facing other important threats such as habitat loss caused by residential and commercial development projects and habitat degradation caused by recreational activities.

The eastern red-backed salamander is a widely distributed species encountered almost in every mature forest, and which could reach impressive densities (Jaeger 1979). Yet, our results show that this common species can be severely affected by habitat fragmentation, and this raises concern for rare or threatened species inhabiting such urban landscapes.

Acknowledgements We would like to thank D. Fournier, P. Graillon, S. Marquis, M.-J. Morin, R. Pétel, É. Richard and N. Tessier for their contribution to this study and all members of the Laboratoire d'Écologie Moléculaire et Évolution for their comments on an earlier draft of this manuscript. This study was supported by the Direction des sports, des parcs et des espaces verts de la Ville de Montréal, the Parc national du Mont-Mégantic, Amphibia-Nature, a NSERC scholarship to S. Noël and NSERC grant no. 0155251 to F.-J. Lapointe. Our sampling protocol was approved by the Comité de déontologie de l'expérimentation des animaux of the Université de Montréal.

References

- Adams FD (1903) The Monteregian Hills – a Canadian petrographical province. *J Geol* 2:239–282
- Andersen LW, Fog K, Damgaard C (2004) Habitat fragmentation causes bottlenecks and inbreeding in the European tree frog (*Hyla arborea*). *Proc Biol Sci* 271:1293–1302
- Beebee TJC, Flower RJ, Stevenson AC, Patrick ST, Appleby PG, Fletcher C, Marsh C, Natkanski J, Rippey B, Battarbee RW (1990) Decline of the natterjack toad *Bufo calamita* in Britain: paleoecological, documentary and experimental evidence for breeding site acidification. *Biol Conserv* 53:1–20
- Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocumbe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips KR, Marantelli G, Parkes H (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proc Natl Acad Sci USA* 95:9031–9036
- Berry O, Tocher MD, Sarre SD (2004) Can assignment tests measure dispersal? *Mol Ecol* 13:551–561
- Blaustein AR, Kiesecker JM, Chivers DP, Hokit DG, Marco A, Belden LK, Hatch A (1998) Effects of ultraviolet radiation on amphibians: field experiments. *Am Zool* 38:799–812
- Bonin J, Desroches J-F, Ouellet M, Leduc A (1999) Les forêts anciennes: refuges pour les salamandres. *Nat Can* 123(1):13–18
- Brown JL (1965) Stability of color phase ratio in populations of *Plethodon cinereus*. *Copeia* 1965:95–98
- Burton TM, Likens GE (1975) Salamander populations and biomass in the Hubbard Brook Experimental Forest, New Hampshire. *Copeia* 1975:541–546
- Connors LM, Cabe PR (2003) Isolation of dinucleotide microsatellite loci from red-backed salamander (*Plethodon cinereus*). *Mol Ecol Notes* 3:131–133
- Czech B, Krausman PR, Devers PK (2000) Economic associations among causes of species endangerment in the United States. *BioScience* 50:593–601
- deMaynadier PG, Hunter Jr ML (1998) Effects of silvicultural edges on the distribution and abundance of amphibians in Maine. *Conserv Biol* 12:340–352
- Dodd Jr CK, Smith LL (2003) Habitat destruction and alteration: historical trends and future prospects for amphibians. In: Semlitsch RD (ed) *Amphibian conservation*. Smithsonian Institution, Washington, DC, pp 94–112
- Dyrkacz S (1981) Recent instances of albinism in North American amphibians and reptiles. *Society for the Study of Amphibians and Reptiles Herpetological Circular* No. 11
- Ehman H, Cogger H (1985) Australia's endangered herpetofauna: a review of criteria and policies. In: Grigg GC, Shine R, Ehmann HFW (eds) *Biology of Australasian frogs and reptiles*. Royal Zoological Society of New South Wales, Sydney, pp 435–447
- Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479–491
- Fahrig L (2003) Effects of habitat fragmentation on biodiversity. *Ann Rev Ecol Syst* 34:487–515
- Fahrig L, Pedlar JH, Pope SE, Taylor PD, Wegner JF (1995) Effect of road traffic on amphibian density. *Biol Conserv* 73:177–182
- Forman RTT, Alexander LE (1998) Roads and their major ecological effects. *Ann Rev Ecol Syst* 29:207–231
- Frankham R (1996) Relationship of genetic variation to population size in wildlife. *Conserv Biol* 10:1500–1508
- Gibbs JP (1998a) Distribution of woodland amphibians along a forest fragmentation gradient. *Landsc Ecol* 13:263–268
- Gibbs JP (1998b) Genetic structure of redback salamander *Plethodon cinereus* populations in continuous and fragmented forests. *Biol Conserv* 86:77–81
- Goudet J (2002) FSTAT, a software to estimate and test gene diversities and differentiation statistics from codominant markers (version 2.9.3.2). Available from <http://www2.unil.ch/popgen/softwares/fstat.htm>
- Green DM (1997) Perspectives on amphibian population declines: defining the problem and searching for answers. *Herpetol Conserv* 1:291–308
- Greer AEJ (1973) Adaptive significance of the color phases of the red-backed salamander. *Yearb Am Philos Soc* 1973:308–309
- Grover MC (1998) Influence of cover and moisture on abundances of the terrestrial salamanders *Plethodon cinereus* and *Plethodon glutinosus*. *J Herpetol* 32:489–497
- Highton R (1959) The inheritance of the color phases of *Plethodon cinereus*. *Copeia* 1959:33–37
- Highton R (1977) Comparison of microgeographic variation in morphological and electrophoretic traits. In: Hecht M, Steer W, Wallace B (eds) *Evolutionary biology*. Plenum Publishing, New York, pp 397–436
- Highton R, Webster TP (1976) Geographic protein variation and divergence in populations of the salamander *Plethodon cinereus*. *Evolution* 30:33–45
- Hitchings SP, Beebee TJC (1997) Genetic substructuring as a result of barriers to gene flow in urban *Rana temporaria* (common frog) populations: implications for biodiversity conservation. *Heredity* 79:117–127
- Hitchings SP, Beebee TJC (1998) Loss of genetic diversity and fitness in common toad (*Bufo bufo*) populations isolated by inimical habitat. *J Evol Biol* 11:269–283
- Houlahan JE, Findlay CS, Schmidt BR, Meyer AH, Kuzmin SL (2000) Quantitative evidence for global amphibian population declines. *Nature* 404:752–755
- Hyde EJ, Simons TR (2001) Sampling plethodontid salamanders: sources of variability. *J Wildl Manage* 65:624–632
- Jaeger RG (1979) Seasonal spatial distributions of the terrestrial salamander *Plethodon cinereus*. *Herpetologica* 35:90–93
- Kimberling DN, Ferreira AR, Shuster SM, Keim P (1996) RAPD marker estimation of genetic structure among isolated northern leopard frog populations in the south-western USA. *Mol Ecol* 5:521–529
- Kleeberger SR, Werner JK (1982) Home range and homing behavior of *Plethodon cinereus* in northern Michigan. *Copeia* 1982:409–415
- Knapp RA, Matthews KR (2000) Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. *Conserv Biol* 14:428–438
- Kolozsvary MB, Swihart RK (1999) Habitat fragmentation and the distribution of amphibians: patch and landscape correlates in farmland. *Can J Zool* 77:1288–1299

- Kucken DJ, Davis JS, Petranka JW, Smith CK (1994) Anakeesta stream acidification and metal contamination-effects on a salamander community. *J Environ Qual* 23:1311–1317
- Kuhn J (1987) Strabentod der Erdkröte (*Bufo bufo* L.) Verlustquoten und Verkehrsaufkommen, Verhalten auf der Straße. Beiheft Veröffentlichungen Naturschutz Landschaftspflege Baden-Württemberg 41:175–176
- Lacy R (1987) Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. *Conserv Biol* 1:143–158
- Larson A, Wake DB, Yanev KP (1984) Measuring gene flow among populations having high-levels of genetic fragmentation. *Genetics* 106:293–308
- Lotter F, Scott Jr NJ (1977) Correlation between climate and distribution of the color morphs of the salamander *Plethodon cinereus*. *Copeia* 1977:681–690
- Marsh DM, Beckman NG (2004) Effects of forest roads on the abundance and activity of terrestrial salamanders. *Ecol Appl* 14:1882–1891
- Marsh DM, Thakur KA, Bulka KC, Clarke LB (2004) Dispersal and colonization through open fields by a terrestrial, woodland salamander. *Ecology* 85:3396–3405
- Miller JR, Hobbs RJ (2002) Conservation where people live and work. *Conserv Biol* 16:330–337
- Muralidharan K, Wakeland EK (1993) Concentration of primer and template qualitatively affects products in random-amplified polymorphic DNA PCR. *Biotechniques* 14:362–364
- Murphy NR, Hellwig RJ (1996) Improved nucleic acid organic extraction through use of a unique gel barrier material. *Biotechniques* 21:934–936
- Ouellet M, Galois P, Pétel R, Fortin C (2005a) Les amphibiens et les reptiles des collines montérégiennes: enjeux et conservation. *Nat Can* 129(1):42–49
- Ouellet M, Mikaelian I, Pauli BD, Rodrigue J, Green DM (2005b) Historical evidence of widespread chytrid infection in North American amphibian populations. *Conserv Biol* 19:1431–1440
- Pabijan M, Babik W, Rafinski J (2005) Conservation units in north-eastern populations of the Alpine newt (*Triturus alpestris*). *Conserv Genet* 6:307–312
- Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conserv Biol* 12:844–855
- Petranka J (1998) Salamanders of the United States and Canada. Smithsonian Institution, Washington, DC
- Pfingsten RA, Walker CF (1978) Some nearly all black populations of *Plethodon cinereus* (Amphibia, Urodela, Plethodontidae) in northern Ohio. *J Herpetol* 12:163–167
- Piry S, Alapetite A, Cornuet J-M., Paetkau D, Baudouin L, Estoup A (2004) GeneClass2: a software for genetic assignment and first generation migrants detection. *J Hered* 95:536–539
- Pounds JA, Fogden MPL, Campbell JH (1999) Biological response to climate change on a tropical mountain. *Nature* 398:611–615
- Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. *Proc Nat Acad Sci USA* 94:9197–9201
- Raymond M, Rousset F (1995) Genepop (Version 1.2): population genetics software for exact tests and ecumenicism. *J Hered* 86:248–249
- Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. *Conserv Biol* 17:230–237
- Reed DH, Hobbs GR (2004) The relationship between population size and temporal variability in population size. *Anim Conserv* 7:1–8
- Reed HD (1908) A note on the coloration of *Plethodon cinereus*. *Am Nat* 42:460–465
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution* 43:223–225
- Rieseberg LH (1996) Homology among RAPD fragments in interspecific comparisons. *Mol Ecol* 5:99–105
- Rosen M (1971) An erythristic *Plethodon cinereus cinereus* from Ste Foy, Portneuf county, Québec. *Can Field-Nat* 85:326–327
- Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
- Schierwater B, Ender A (1993) Different thermostable DNA-polymerases may amplify different RAPD products. *Nucleic Acids Res* 21:4647–4648
- Schneider S, Roessli D, Excoffier L (2000) Arlequin: a software for population genetics data analysis. Department of Anthropology, University of Geneva, Geneva
- Statistics Canada (2000) Human activity and the environment. Report 11-509-F. Statistics Canada, Ottawa
- Tait CJ, Daniels CB, Hill RS (2005) Changes in species assemblages within the Adelaide Metropolitan Area, Australia, 1836–2002. *Ecol Appl* 15:346–359
- Thurrow G (1961) A salamander color variant associated with glacial boundaries. *Evolution* 15:281–287
- United Nations Population Division (2004) World urbanization prospects: the 2003 revision. United Nations Department of Economic and Social Affairs, New York
- Vos CC, Antonisse-De Jong AG, Goedhart PW, Smulders MJM (2001) Genetic similarity as a measure for connectivity between fragmented populations of the moor frog (*Rana arvalis*). *Heredity* 86:598–608
- Wasser PM, Strobeck C (1998) Genetic signatures of interpopulation dispersal. *Trends Ecol Evol* 13:43–44
- Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res* 18:7213–7218
- Wilcove DS, McLellan CH, Dobson AP (1986) Habitat fragmentation in the temperate zone. In: Soulé ME (ed) Conservation biology. Sinauer, Sunderland, pp 237–256
- Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res* 18:6531–6535
- Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. *Trends Ecol Evol* 11:413–418
- Zeisset I, Beebe TJC (2003) Population genetics of a successful invader: the marsh frog *Rana ridibunda* in Britain. *Mol Ecol* 12:639–646