ORIGINS OF GENETIC VARIATION AND POPULATION STRUCTURE OF FOXSNAKES ACROSS SPATIAL AND TEMPORAL SCALES

By

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Abstract

Understanding the events and processes responsible for patterns of within species diversity, provides insight into major evolutionary themes like adaptation, species distributions, and ultimately speciation itself. Here, I combine ecological, genetic and spatial perspectives to evaluate the roles that both historical and contemporary factors have played in shaping the population structure and genetic variation of foxsnakes (*Pantherophis gloydi*).

First, I determine the likely impact of habitat loss on population distribution, through radio-telemetry (32 individuals) at two locations varying in habitat patch size. As predicted, individuals had similar habitat use patterns, but restricted movements to patches of suitable habitat at the more disturbed site. Also, occurrence records spread across a fragmented region were non-randomly distributed and located close to patches of usable habitat, suggesting habitat distribution limits population distribution.

Next, I combined habitat suitability modeling with population genetics (589 individuals, 12 microsatellite loci) to infer how foxsnakes disperse through a mosaic of natural and altered landscape features. Boundary regions between genetic clusters were comprised of low suitability habitat (e.g. agricultural fields). Island populations were grouped into a single genetic cluster suggesting open water presents less of a barrier than non-suitable terrestrial habitat. Isolation by distance models had a stronger correlation with genetic data when including resistance values derived from habitat suitability maps, suggesting habitat degradation limits dispersal for foxsnakes.

At larger temporal and spatial scales I quantified patterns of genetic diversity and population structure using mitochondrial (101 cytochrome *b* sequences) and

microsatellite (816 individuals, 12 loci) DNA and used Approximate Bayesian computation to test competing models of demographic history. Supporting my predictions, I found models with populations which have undergone population size drops and splitting events continually had more support than models with small founding populations expanding to stable populations. Based on timing, the most likely cause was the cooling of temperatures and infilling of deciduous forest since the Hypisthermal. On a smaller scale, evidence suggested anthropogenic habitat loss has caused further decline and fragmentation. Mitochondrial DNA structure did not correspond to fragmented populations and the majority of foxsnakes had an identical haplotype, suggesting a past bottleneck or selective sweep.

Co-Authorship

This thesis was formatted in the manuscript format as outlined in the guidelines provided by The Department of Biology. All chapters are co-authored by Stephen Lougheed, who contributed financially and intellectually to the design and development and editing of the thesis. The first and second chapters were also co-authored by Gabriel Blouin-Demers, who contributed intellectually and logistically for both of those chapters.

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Chapter 1. General Introduction

Background

At its core, evolutionary biology seeks to understand the origins of diversity across hierarchical scales of organization, from individuals to species. Understanding the patterns and processes responsible for diversity provides insights into major evolutionary themes like adaptation, species distributions, and ultimately speciation itself. Similarly, insight into how human alterations on the landscape have modified and are modifying the organization, diversity and connectedness of populations is a central theme in conservation biology (e.g. Clark *et al*. 2010; Flight 2010; Zhu *et al*. 2010). Over the past 20 years our ability to quantify the patterns of genetic diversity within individuals, populations and species has greatly advanced our understanding of how geographic and demographic factors influence the microevolutionary processes (e.g. drift, gene flow, selection) that shape patterns of genetic variation (Wright 1978; Slatkin 1987). The spatial and temporal distribution of individuals, populations and usable habitat can therefore have marked impacts on the genetic diversity and population structure of species. Only in the last ten years, however, have landscape characteristics been routinely and explicitly combined with population genetic models producing the emerging field of landscape genetics (reviewed in: Manel *et al*. 2003). In brief, landscape genetics attempts to understand how topography, hydrology and habitat modulate the impact of microevolutionary processes on fine scale genetic population structure (Manel *et al*. 2003; Storfer *et al*. 2007; Holderegger & Wagner 2008).

Improvements in molecular genetics (Sunnucks 2000) and statistical tools (e.g. Manel *et al*. 2003; Guillot *et al*. 2009) and in the resolution and availability of digital imagery from Geographic Information Systems (GIS) have improved our ability to

quantify both population structure and landscape features, and to incorporate spatial data directly into spatial genetic analyses. New genetic assignment tests, many based on Bayesian perspectives (reviewed in: Manel *et al*. 2005), allow us to determine the number and extent of populations based on the distribution of genotypes and not solely on arbitrary geographic delineations of populations as was previously the common practice. Combined with geographic information, assignment tests can identify or confirm barriers on the landscape that function as impediments to gene flow (e.g. Zalewski *et al*. 2009; Pierson *et al*. 2010). Isolation by distance (IBD) models using populations (Wright 1943) or individuals (Rousset 2000) have also been a common way to examine genetic population structure in more continuously distributed populations. Incorporating landscape information in the form of least-cost paths (LCP) (Adriaensen *et al*. 2003) or more recently isolation by resistance (IBR) (McRae 2006) can similarly identify landscape features that promote or impede dispersal and gene flow in continuous populations (e.g. Lee-Yaw *et al*. 2009; Schwartz *et al*. 2009).

Despite these advances, few studies have simultaneously combined spatial, ecological and genetic analyses to take full advantage of these new techniques. For example, there are many habitat suitability modeling procedures available (reviewed in: Hirzel & Le Lay 2008) that have been used to establish habitat use preferences and develop habitat suitability maps (e.g. Livingston *et al*. 1990; Clark *et al*. 1993; Peeters & Gardeniers 1998; Hirzel *et al*. 2002). Despite the availability of these methods very few landscape genetic studies incorporate suitability modeling (but see: Wang *et al*. 2008). Rather many authors test a series of models (e.g. Cushman *et al*. 2006; Stevens *et al*. 2006; Pérez-Espona *et al*. 2008), which may not have a strong basis in the biology of the

focal species. Combining habitat suitability modeling in landscape genetics allows for testing how the amount and quality of habitat impacts genetic connectivity instead of simply identifying landscape features *post hoc*.

Given the importance of the spatial distribution of populations on genetic population structure it is not surprising that large-scale geographic and climatic events can have strong and lasting effects on the patterns of diversity within a species. For example, climatic oscillations during the glacial periods of the Pleistocene are considered a major cause in the divergence patterns within and between a number of temperate species across Europe and North America (Hewitt 1996; Hewitt 2000). Mountain (e.g. Nielson *et al*. 2001; McCormack *et al*. 2008) and island formation or isolation (e.g. Jordan & Snell 2008) have also been major contributors to within and between species diversity. These large-scale events, however, have not acted alone. Indeed smaller scale, more recent factors, such as natural or human-induced habitat loss and fragmentation (Costello *et al*. 2003; Zellmer & Knowles 2009) and current effective population sizes (Johansson *et al*. 2006) are also key determinants of contemporary population structure and often erase or at least dilute the signature of more historical effects (Zellmer & Knowles 2009).

Understanding the patterns of geographic variation within a species and the causal factors and processes, is fundamental for our understanding of evolution (Gould & Johnston 1972). The importance of making the link between microevolution and intraspecific variation with speciation was recognized by Avise *et al*. (1987) when they proposed the new discipline of phylogeography – merging phylogenetic methodology and interpretations with population genetics and considerations of geographical distributions.

From its inception, the field of phylogeography has typically examined within species gene genealogies in a geographic context, attempting to identify the events (e.g. glaciations, mountain formation) and/or demographic processes (e.g. population and range expansion, population bottlenecks) responsible (reviewed in: Hickerson *et al*. 2010). Until recently, however, phylogeography was not embedded within a rigorous hypothesis-testing framework. Rather traditional phylogeographic approaches typically inferred past population processes *post hoc* by testing for an association between deduced genetic patterns and geography to derive conclusions regarding myriad possible causative factors (e.g. nested clade analysis, Templeton 1998). Such *post hoc* forms of analysis lead to a high probability of false positives (Panchal & Beaumont 2007); i.e. spuriously attributing causation to some historical factor. The emergence of statistical phylogeography shows great promise in solving this issue, as it relies on testing competing models that are proposed *a priori* and can incorporate formal tests of uncertainty (Knowles & Maddison 2002). Although statistical phylogeographic methods and programs are increasingly available (e.g. Cornuet & Luikart 1996; Wegmann *et al*. 2010) these have not been widely used in the literature.

The study species

Foxsnakes (Fig. 1.1A) are relatively large (~1.5m), oviparous snakes native to the Great Lakes Basin (Ontario, Ohio, Michigan) and the north-central United States (Fig. 1.1B). The northern distribution of eastern (*Pantherophis gloydi*) and western foxsnakes (*P. vulpinus*), is quite unusual among temperate terrestrial squamates, which generally have at least a portion of their range extend south into regions that would not have been covered in ice sheets during the Pleistocene glacial maxima. Ectotherms must maintain

their body temperature through heat obtained from their environment, which is particularly difficult in temperate climates (Blouin-Demers & Weatherhead 2002; Row & Blouin-Demers 2006b) and for large (Bulté & Blouin-Demers 2010), oviparous (Gregory 2009) reptiles. Likely due, at least in part, to their thermoregulatory requirements, foxsnakes are marsh and prairie specialists (Ernst & Barbour 1989; Row *et al*. 2010). Open habitats like prairie and marshes often have higher temperatures than more closed forested habitats and thus, higher thermal quality in temperate climates (Blouin-Demers & Weatherhead 2002; Row & Blouin-Demers 2006a).

Within the current range of foxsnakes there are a number of significant geographic disjunctions. Particularly prominent is the large gap between eastern and western foxsnakes for which there has been speculation as to its cause and significance. For example, many authorities consider eastern and western foxnakes to be separate species, mainly based on this geographic divide (Collins 1991). The current range of foxsnakes would have been almost completely covered by ice sheets during the maximum glacial extent of the Pleistocene (~70 000 years before present) and there has been suggestion that eastern foxsnakes colonized their current range following an eastward extension of the prairie peninsula (post-glacial steppe) that existed approximately 2000-7000 years ago (Schmidt 1938; Webb 1981). This prairie habitat was subsequently replaced by deciduous forest, possibly leading to the split between eastern and western foxsnakes. Even within the present-day range of eastern foxsnakes, there are many disjunctions among isolated populations according to occurrence records dating back to the 1900's. Such disjunctions then possibly pre-date major European settlement and may have been caused by the aforementioned incursion of deciduous forest into southwestern Ontario following glacial

retreat. Like most northern temperate species, however, eastern foxsnakes have experienced more recent habitat fragmentation and loss due to human activities. This is particularly true for eastern foxsnakes, where extensive urban and agricultural development has occurred across their distribution within the Great Lakes basin. For example, in extreme southwestern Ontario, over 90 % of the marshes have been drained (Whitaker 1938). Thus, contemporary gaps in the distribution of eastern foxsnakes may have been caused by postglacial colonization coupled with changing environments, or by recent isolation of previously more connected populations because of land clearing and wetland drainage (last 100-200 years). Of course, these are not mutually exclusive explanations.

Due to the complex demographic and evolutionary history of most species, it is often difficult to define and disentangle the relative contribution of historical and contemporary processes that have shaped patterns of variation within species. Eckert et al. (2008) suggested defining historical processes as those that have had an effect in shaping current patterns of diversity, but are no longer in effect, whereas contemporary processes are those that continue to operate. The general goals of my thesis are to combine ecological, genetic and spatial perspectives to evaluate the roles that both historical and contemporary factors have played in shaping the genetic variation and population structure across the range of eastern and western foxsnakes. Collectively these studies bridge a number of conceptual and empirical gaps that persist in the ecological, population genetic and phylogeographic literature. Specific objects for each chapter are outlined below.

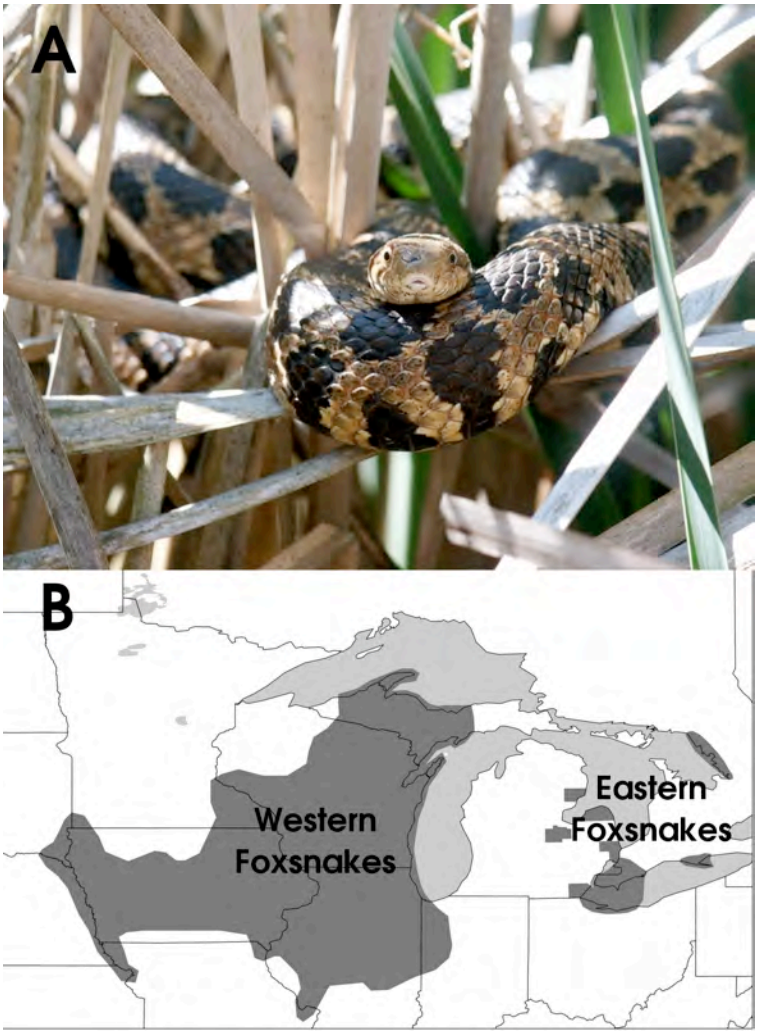

Figure 1.1. Eastern foxsnake basking at Point Pelee National Park, and B) the range of eastern and western foxsnakes derived from Conant & Collins (1991) and historical occurrence records from Ontario, Ohio and Michigan.

Chapter Objectives

1) Movement and habitat use of the Eastern Foxsnake (Pantherophis gloydi) in a fragmented landscape:

The decline in the size (i.e. habitat loss) and the degree of isolation (i.e. habitat fragmentation) of habitat patches have been suggested as leading causes of species extinction (Tilman *et al*. 1994; Fahrig 2002). Individual species, however, can be impacted differently with some species being limited to the remaining patches of suitable habitat (e.g. Greenwald *et al*. 2009) while others may modify habitat preferences to use or move through undesirable habitat (Githiru *et al*. 2007; Marchesan & Carthew 2008). To devise effective management strategies (e.g. habitat corridors) and predict how species respond to habitat changes we need detailed studies of habitat use and behaviour for species in fragmented landscapes. For the second chapter, I used radio-telemetry to quantify habitat use patterns at two locations varying in their degree of habitat fragmentation. I predicted that individuals at the more fragmented site would maintain their habitat use preferences and restrict their movements to within patches of suitable habitat. At the landscape scale I used occurrence records spread across a fragmented region and predicted that they would be non-randomly distributed and located close to patches of usable habitat.

2) Habitat distribution influences dispersal and fine-scale genetic population structure of eastern foxsnakes (Pantherophis gloydi) across a fragmented landscape:

Both theory (e.g. Wright 1948; Slatkin 1987) and empirical data (e.g. Postma & van Noordwijk 2005) show that dispersal has large impacts on the distribution of genetic variation. Studying factors that promote or impede dispersal has therefore been a central theme in evolutionary ecology (Greenwood & Harvey 1982) and conservation biology (Frankham *et al*. 2002). Across southwestern Ontario there are varying degrees of agricultural and urban development that have reduced and fragmented marsh and prairie habitat. Despite these changes, foxsnake occurrence records suggest foxsnakes occupy the extent of much of their former range and persist in areas where a number of other snake species have disappeared. It is likely, however, that this development has resulted in barriers to dispersal for foxsnakes.

In chapter 3, I determine the impact that both natural (lakes) and anthropogenic (e.g. roads, agricultural fields) barriers have had on dispersal patterns and resulting fine-scale genetic population structure of eastern foxsnakes. I first determine habitat use patterns at the landscape scale and develop a habitat suitability map across southwestern Ontario using Ecological Niche Factor analysis (ENFA) (Hirzel et al. 2002). Second, I quantify the genetic population structure using high-resolution DNA microsatellite markers and determine whether 1) the number and extent of genetic populations identified using assignment tests correlate with habitat distribution and landscape features, and 2) individual isolation by distance models and spatial autocorrelation analysis significantly improve when incorporating landscape derived resistance values.

3) Impacts of historical and contemporary processes on population structure:

Geographic variation within a species both reflects past evolution and shapes future evolutionary trajectories (Gould & Johnston 1972). Quantifying intraspecific genetic variation is essential to our understanding of evolution, including as a central goal, disentangling the relative contributions of historical demographic changes and contemporary processes. Recently, Approximate Bayesian computation (ABC) coupled with coalescent modeling has been employed in a statistical phylogenetic approach to explicitly test multiple hypotheses of causation of present day patterns (Beaumont *et al*. 2002). As with all Bayesian analysis, prior information can be incorporated in the form of prior distributions and the fit of competing models can be evaluated by comparing the marginal densities and computing a Bayes factor (Leuenberger & Wegmann 2010), making it an ideal approach statistical phylogeography (Knowles & Maddison 2002).

The glacial periods of the Pleistocene (Hewitt 1996; Hewitt 2000) have significantly impacted genetic variation for numerous North American species of herpetofauna (Austin *et al*. 2002; Zamudio & Savage 2003; Howes *et al*. 2006; Placyk Jr *et al*. 2007). I predict that this will also be the case for eastern foxsnakes. More recent natural and anthropogenic changes on the landscape have modified the distribution of available habitat, which has likely resulted in alterations to the size, extent and connectivity of foxsnake populations across their current range and impinged on genetic structure. In Chapter 4, I use both microsatellite and mitochondrial DNA markers to first establish the range wide genetic population structure and genetic diversity patterns. I subsequently use ABC analysis to compare competing population demographic models that are consistent with two hypotheses: 1) large populations, which have undergone

drops in population size and splitting events, and 2) small founding populations that have split from large populations and expanded to be stable. Following the choice of the most appropriate models, I estimate population parameters (e.g. effective population sizes, divergence times of populations) and make comparisons between eastern and western foxsnakes with respect to their respective colonization patterns.

Literature Cited

Adriaensen F, Chardon JP, Deblust G (2003) The application of 'least-cost'modelling as a functional landscape model. *Landscape and Urban Planning,* **64,** 233–247.

Austin JD, Lougheed SC, Neidrauer L, Chek AA, Boag PT (2002) Cryptic lineages in a small frog: the post-glacial history of the spring peeper, *Pseudacris crucifer* (Anura: Hylidae). *Molecular Phylogenetics and Evolution,* **25,** 316–329.

Avise J, Arnold J, Ball R, Bermingham E, Lamb T, Neigel J, Reeb C, Saunders N (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics,* **18,** 489–522.

Beaumont M, Zhang W, Balding D (2002) Approximate Bayesian computation in population genetics. *Genetics,* **162,** 2025-2035.

Blouin-Demers G, Weatherhead PJ (2002) Habitat-specific behavioural thermoregulation by black rat snakes (*Elaphe obsoleta obsoleta*). *Oikos,* **97,** 59–68.

Bulté G, Blouin-Demers G (2010) Implications of extreme sexual size dimorphism for thermoregulation in a freshwater turtle. *Oecologia,* **162,** 313–322.

Clark J, Dunn J, Smith K (1993) A multivariate model of female black bear habitat use for a geographic information system. *The Journal of Wildlife Management,* **57,** 519–526.

Clark R, Brown W, Stechert R, Zamudio K (2010) Roads, interrupted dispersal, and genetic diversity in timber rattlesnakes. *Conservation Biology,* **24,** 1–11.

Collins JT (1991) Viewpoint: a new taxonomic arrangement for some North American amphibians and reptiles. *Herpetological Review,* **22,** 42–43.

Conant R, Collins JT (1991) A field guide to amphibians and reptiles of eastern and central North America. Houghton Mifflin Company, Boston, Massachusetts.

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics,* **144,** 2001–2014.

Costello A, Down T, Pollard S, Pacas C, Taylor E (2003) The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species: an examination of microsatellite DNA variation in bull trout, *Salvelinus confluentus* (Pisces: Salmonidae). *Evolution,* **57,** 328–344.

Cushman SA, Mckelvey KS, Hayden J, Schwartz MK (2006) Gene flow in complex landscapes: Testing multiple hypotheses with causal modeling. *The American Naturalist,* **168,** 486–499.

Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. *Molecular Ecology,* **17,** 1170–1188.

Ernst CH, Barbour RW (1989) Snakes of Eastern North America. George Mason University Press, Fairfax, Virginia

Fahrig L (2002) Effect of habitat fragmentation on the extinction threshold: A synthesis. *Ecological Applications,* **12,** 346–353.

Flight P (2010) Phylogenetic comparative methods strengthen evidence for reduced genetic diversity among endangered tetrapods. *Conservation Biology,* **24,** 1307–1315.

Frankham R, Ballou JD, Briscoe DA (2002) Population fragmentation. *Introduction to Conservation Genetics*, Cambridge University Press, Cambridge, UK, pp. 309-334.

Githiru M, Lens L, Bennun L (2007) Ranging behaviour and habitat use by an Afrotropical songbird in a fragmented landscape. *African Journal of Ecology,* **45,** 581–589.

Gould S, Johnston R (1972) Geographic variation. *Annual Review of Ecology and Systematics,* **3,** 457–498.

Greenwald KR, Purrenhage JL, Savage WK (2009) Landcover predicts isolation in *Ambystoma* salamanders across region and speices. *Biological Conservation,* **142,** 2493–2500.

Greenwood PJ, Harvey PH (1982) The natal and breeding dispersal of birds. *Annual Review of Ecology and Systematics,* **13,** 1–21.

Gregory P (2009) Northern lights and seasonal sex: the reproductive ecology of cool-climate snakes. *Herpetologica,* **65,** 1–13.

Guillot G, Leblois R, Coulon A, Frantz A (2009) Statistical methods in spatial genetics. *Molecular Ecology,* **18,** 4734–4756.

Hewitt G (2000) The genetic legacy of the Quaternary ice ages. *Nature,* **405,** 907–913.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role, in divergence and speciation. *Biological Journal of the Linnean Society,* **58,** 247–276.

Hickerson M, Carstens B, Cavender-Bares J, Crandall K, Graham C, Johnson J, Rissler L, Victoriano P, Yoder A (2010) Phylogeography's past, present, and future: 10 years after Avise, 2000. *Molecular Phylogenetics and Evolution,* **54,** 291–301.

Hirzel AH, Hausser J, Chessel D, Perrin N (2002) Ecolocial-niche factor analysis: how to compute habitat-suitability maps without absence data? *Ecology,* **83,** 2027–2036.

Hirzel A, Le Lay G (2008) Habitat suitability modelling and niche theory. *Journal of Applied Ecology,* **45,** 1372–1381.

Holderegger R, Wagner H (2008) Landscape genetics. *BioScience,* **58,** 199–207.

Howes B, Lindsay B, Lougheed S (2006) Range-wide phylogeography of a temperate lizard, the five-lined skink (*Eumeces fasciatus*). *Molecular Phylogenetics and Evolution,* **40,** 183–194.

Johansson M, Primmer C, Merilae J (2006) History vs. current demography: explaining the genetic population structure of the common frog (*Rana temporaria*). *Molecular Ecology,* **15,** 975–983.

Jordan M, Snell H (2008) Historical fragmentation of islands and genetic drift in populations of Galapagos lava lizards (*Microlophus albemarlensis complex*). *Molecular Ecology,* **17,** 1224–1237.

Knowles LL, Maddison WP (2002) Statistical phylogeography. *Molecular Ecology,* **11,** 2623–2635.

Lee-Yaw J, Davidson A, Mcrae B, Green D (2009) Do landscape processes predict phylogeographic patterns in the wood frog? *Molecular Ecology,* **18,** 1863–1874.

Leuenberger C, Wegmann D (2010) Bayesian computation and model selection without likelihoods. *Genetics,* **184,** 243–252.

Livingston S, Todd C, Krohn W, Owen Jr RB (1990) Habitat models for nesting bald eagles in Maine. *The Journal of Wildlife Management,* **54,** 644–653.

Manel S, Gaggiotti OE, Waples RS (2005) Assignment methods: matching biological questions with appropriate techniques. *Trends in Ecology & Evolution,* **20,** 136–142.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution,* **18,** 189–197.

Marchesan D, Carthew SM (2008) Use of space by the yellow-footed antechinus, *Antechinus flavipes*, in a fragmented landscape in South Australia. *Landscape Ecology,* **23,** 741–752.

McCormack J, Peterson A, Bonaccorso E, Smith T (2008) Speciation in the highlands of Mexico: genetic and phenotypic divergence in the Mexican jay (*Aphelocoma ultramarina*). *Molecular Ecology,* **17,** 2505–2521.

Mcrae BH (2006) Isolation by resistance. *Evolution,* **60,** 1551–1561.

Nielson M, Lohman K, Sullivan J (2001) Phylogeography of the tailed frog (*Ascaphus truei*): implications for the biogeography of the Pacific Northwest. *Evolution,* **55,** 147–160.

Panchal M, Beaumont M (2007) The automation and evaluation of nested clade phylogeographic analysis. *Evolution,* **61,** 1466–1480.

Peeters E, Gardeniers J (1998) Logistic regression as a tool for defining habitat requirements of two common gammarids. *Freshwater Biology,* **39,** 605–615.

Pérez-Espona S, Pérez-Barbería F, Mcleod J, Jiggins C, Gordon I, Pemberton J (2008) Landscape features affect gene flow of Scottish Highland red deer (*Cervus elaphus*). *Molecular Ecology,* **17,** 981–996.

Pierson J, Allendorf F, Saab V, Drapeau P, Schwartz M (2010) Do male and female black-backed woodpeckers respond differently to gaps in habitat? *Evolutionary Applications,* **3,** 263–278.

Placyk Jr JS, Burghardt G, Small R, King R, Casper G, Robinson J (2007) Post-glacial recolonization of the Great Lakes region by the common gartersnake (*Thamnophis sirtalis*) inferred from mtDNA sequences. *Molecular Phylogenetics and Evolution,* **43,** 452–467.

Postma E, Van Noordwijk AJ (2005) Gene flow maintains a large genetic difference in clutch size at a small spatial scale. *Nature,* **433,** 65–68.

Rousset F (2000) Genetic differentiation between individuals. *Journal of Evolutionary Biology,* **13,** 58–62.

Row JR, Blouin-Demers G, Lougheed SC (2010) Habitat distribution influences dispersal and fine-scale genetic population structure of eastern foxsnakes (*Mintonius gloydi*) across a fragmented landscape. *Molecular Ecology, 19, 5157-5171.*

Row JR, Blouin-Demers G (2006a) Thermal quality influences habitat selection at multiple spatial scales in milksnakes. *Ecoscience,* **13,** 443–450.

Row JR, Blouin-Demers G (2006b) Thermal quality influences effectiveness of thermoregulation, habitat use, and behaviour in milk snakes. *Oecologia,* **148,** 1–11.

Schmidt K (1938) Herpetological evidence for the postglacial eastward extension of the steppe in North America. *Ecology,* **19,** 396–407.

Schwartz MK, Copeland JP, Anderson NJ, Squires JR, Inman RM, Mckelvey KS, Pilgrim KL, Waits LP, Cushman SA (2009) Wolverine gene flow across a narrow climatic niche. *Ecology,* **90,** 3222–3232.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science,* **236,** 787–792.

Stevens V, Verkenne C, Vandewoestijne S, Wesselingh R, Baguette M (2006) Gene flow and functional connectivity in the natterjack toad. *Molecular Ecology,* **15,** 2333–2344.

Storfer A, Murphy MA, Evans JS, Goldberg CS, Robinson S, Spear SF, Dezzani R, Delmelle E, Waits LP (2007) Putting the 'landscape' in landscape genetics. *Heredity,* **98,** 128–142.

Sunnucks P (2000) Efficient genetic markers for population biology. *Trends in Ecology & Evolution,* **15,** 199–203.

Templeton A (1998) Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology,* **7,** 381–397.

Tilman D, May RM, Lehman CL, Nowak MA (1994) Habitat destruction and the extinction debt. *Nature,* **371,** 65–66.

Wang Y, Yang K, Bridgman C, Lin L (2008) Habitat suitability modelling to correlate gene flow with landscape connectivity. *Landscape Ecology,* **23,** 989–1000.

Webb T (1981) The past 11,000 years of vegetational change in eastern north america. *Bioscience,* **31,** 501–506.

Wegmann D, Leuenberger C, Neuenschwander S, Excoffier L (2010) ABCtoolbox: a versatile toolkit for approximate Bayesian computations. *BMC Bioinformatics,* **11,** 116.

Whitaker JR (1938) Agricultural gradients in southern Ontario. *Economic Geography,* **14,** 109–120.

Wright S (1948) On the roles of directed and random changes in gene frequency in the genetics of populations. *Evolution,* **2,** 279–294.

Wright S (1943) Isolation by distance. *Genetics,* **28,** 114-138.

Wright S (1978) Evolution and the genetics of populations: variability within and among natural populations. University of Chicago Press, Chicago, IL

Zalewski A, Piertney SB, Zalewska H, Lambin X (2009) Landscape barriers reduce gene flow in an invasive carnivore: geographical and local genetic structure of American mink in Scotland. *Molecular Ecology,* **18,** 1601–1615.

Zamudio K, Savage W (2003) Historical isolation, range expansion, and secondary contact of two highly divergent mitochondrial lineages in spotted salamanders (*Ambystoma maculatum*). *Evolution,* **57,** 1631–1652.

Zellmer A, Knowles L (2009) Disentangling the effects of historic vs. contemporary landscape structure on population genetic divergence. *Molecular Ecology,* **18,** 3593–3602.

Zhu L, Zhan X, Wu H, Zhang S, Meng T, Bruford M, Wei F (2010) Conservation implications of drastic reductions in the smallest and most isolated populations of giant pandas. *Conservation Biology,* **24,** 1299–1306.

Chapter 2: Movement and habitat use of the Eastern Foxsnake (*Pantherophis gloydi*) in a fragmented landscape

Abstract

Determining how animals respond to habitat loss and fragmentation requires detailed studies of habitat use and behaviour in regions that vary in their degree of habitat patch size and fragmentation. As predators, snakes are an important component of ecosystems, yet little is known about how they respond behaviourally to habitat loss. Using radio-telemetry at two locations that differ in size, we examined habitat use patterns at two spatial scales and movement patterns for the endangered eastern foxsnake. Movement patterns were similar at the two locations, but individuals exhibited greater variation in home-range size, and males and gravid females dispersed further from hibernation sites within the larger natural habitat patch. Individuals from both locations preferred marsh at the home range scale, but open semi-natural habitat at the location scale. Within the smaller habitat patch, however, these preferences were accentuated with snakes avoiding agricultural fields. At the landscape scale, individual occurrence records were found closer to and in areas with a higher density of useable habitat, than randomly distributed locations. As predators, snakes are an important component of ecosystems, yet ours is one of the few studies to examine how they respond to habitat loss and fragmentation.

Introduction

Habitat loss and fragmentation significantly reduce species diversity and abundance (Ludwig et al., 2009; Vignoli et al., 2009) and these human impacts are generally deemed to be the leading cause of species extinction (Tilman et al., 1994; Fahrig, 2002). Species with divergent life histories, however, can be impacted differently by habitat loss and fragmentation (Fahrig, 2002; Fahrig, 2007). Some species may be strictly limited to certain habitat types resulting in isolated populations in fragmented landscapes (Greenwald et al., 2009). Other species may show a more plastic response and modify habitat use patterns (Githiru et al., 2007) or be better adapted to moving through a fragmented landscape (Marchesan and Carthew, 2008). To devise effective management practices, we need detailed information on how individuals, populations, and even entire guilds respond to fragmented landscapes (Marchesan and Carthew, 2008), although such information is typically lacking for most organisms and landscapes.

Snakes are often one of the top terrestrial predators in biological communities (Schwaner and Sarre, 1988; Tzika et al., 2008) and significant predators of birds, mammals, amphibians, fish, and reptiles (Luiselli et al., 1998). Recent studies show that habitat loss and fragmentation can negatively impact snake diversity and abundance (Cagle, 2008; Driscoll, 2008; Vignoli et al., 2009). This can have large implications as reduced predator abundance can have potentially profound consequences for ecosystems (Paine, 1969; Duffy, 2002). Despite their importance as predators, however, there is little information on how most snakes respond behaviouraly to habitat loss and fragmentation (but see: Corey and Doody, 2010). Indeed, with the importance of edge and open habitat for thermoregulation in temperate climates, some fragmentation may be beneficial for

snakes (Row and Blouin-Demers, 2006c) to the detriment of their prey (Weatherhead and Blouin-Demers, 2004). Without explicit information linking fragmentation and snakes' responses to it, it is difficult for managers to incorporate these predators into management plans for landscapes.

Southwestern Ontario has the highest density of species at risk in Canada (Environment Canada, 2009). Agricultural and residential development has eliminated over 90% of the marshes (Whitaker, 1938) and most natural habitat for terrestrial species, including many snakes. In this study, we used radio-telemetry in Essex county, southwestern Ontario, to determine the movement patterns and habitat use preferences for the endangered eastern foxsnake (*Pantherophis gloydi*) at two locations differing in habitat availability and total patch size. We recognize the limitations imposed on our conclusions because we only had a single large site and a single small site, but our study is nevertheless an important first step towards understanding the potential effects of habitat patch size on movement and habitat use patterns in snakes

Despite the extreme fragmentation across Essex county, foxsnakes remain distributed across most of their historical range (based on post-1900 occurrence records), albeit patchily. Foxsnakes are regarded as marsh and prairie specialists (Ernst and Barbour, 1989) and show significant genetic population structure across this region, with genetic clusters spatially coincident with remaining patches of suitable marsh and grassland habitat (DiLeo et al., 2010; Row et al., 2010). Because of this apparent habitat specificity and indirect genetic evidence of dispersal impeded by areas of agricultural fields, we predicted that foxsnake movements would be more restricted at the smaller of our two locations. We also use occurrence records spread across southwestern Ontario

and a recently developed habitat suitability map (Row et al., 2010) to determine the distances of individual occurrences from suitable habitat at a landscape scale. We predicted that occurrences would be non-randomly distributed and be significantly closer to patches of suitable habitat, again implying that habitat configuration is a limiting factor in their distribution across this region.

Methods

Study area and study animals

Throughout the season when snakes are active (mid-April – late September) of 2007 and 2008, we opportunistically hand captured and selected 32 eastern foxsnakes (*Pantherophis gloydi*) at Point Pelee National Park (PPNP; ~1500 ha) and Hillman Marsh Conservation Area (HMCA; ~350 ha) (Fig. 2.1) and implanted them with radio-tramitters (SI-2 transmitters, 2 year battery life, Holohil Systems Ltd., Ottawa, Ontario). We attempted to select individuals spaced evenly throughout each location. PPNP is located along the north shore of Lake Erie in southwestern Ontario. The park is reasonably undisturbed and most of the habitat is in a relatively natural state. HMCA is located approximated 5 km north of PPNP, is a smaller habitat patch, and is almost completed surrounded by roads and extensive agricultural fields (Fig. 2.1). Foxsnakes were located approximately every 2-3 days and at each location, we recorded the UTM coordinates and the general habitat type (marsh, prairie, agricultural field, open semi-natural).

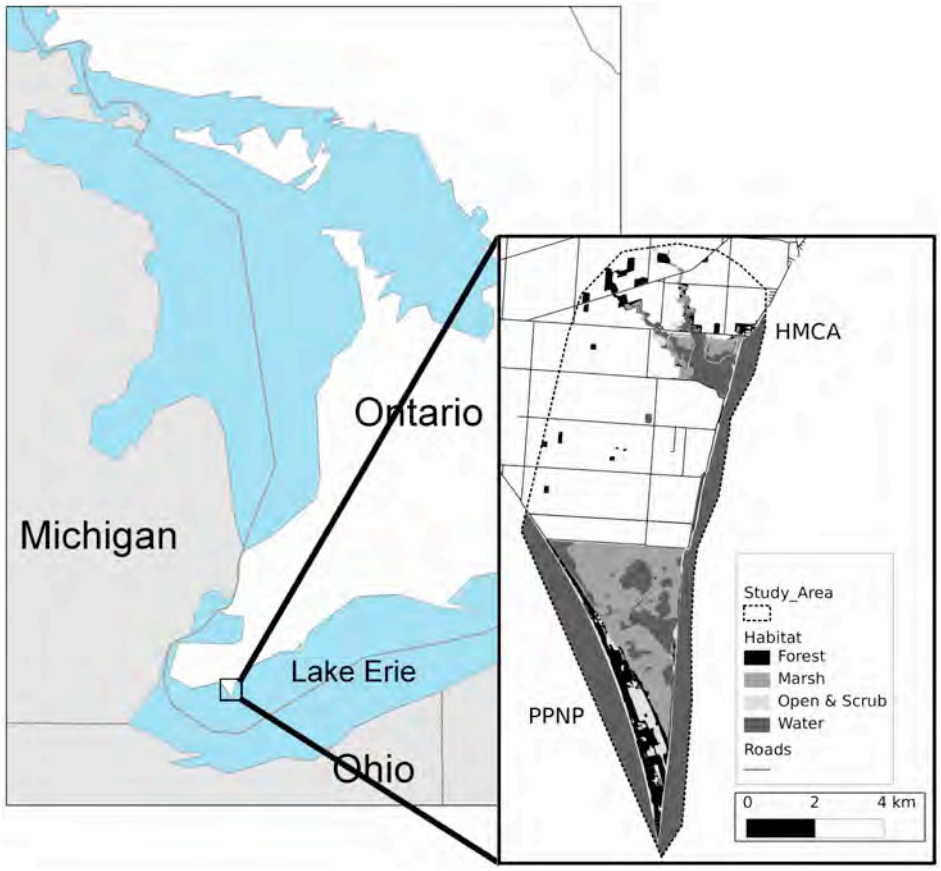

Figure 2.1. Map of study area showing the large (PPNP) and small (HMCA) habitat patches where foxsnakes were tracked using radio-telemetry. Undelineated habitat (white) primarily consists of agricultural fields.

Land cover maps

We used Ontario digital topographic maps (Ontario Base Map, Ontario Ministry of Natural Resources, scale of 1:10000) as base maps to delineate the major habitat types. These maps were generally out of date (collected from 1977-2000) and missing some important features (e.g., open semi-natural habitat). We therefore used 30 cm^2 resolution aerial photography taken in 2006 (SWOOP, Ontario Ministry of Natural Resources), to confirm existing habitat features and added new features resulting in a map with - open water, semi-natural open (prairie, dune, old unmaintained fields), marsh, forest, agriculture, and scrub (Fig. 2.1).

Movement Patterns

We used two movement summaries to determine if individuals were constrained within the smaller habitat patch. First, we estimated home-range size using minimum convex polygons (MCP). MCPs are simple and do not rely on the data having any underlying statistical distribution, which can bias home-range size results for herpetofauna (Row and Blouin-Demers, 2006b). Before calculating MCP home ranges, commutes (straight-line movements in areas not revisited throughout the active season) to and from hibernation sites were removed. Individuals that were not located at least 20 times within the core activity season were removed from the analysis. Second, we calculated maximum distance from hibernation site for each individual as a measure of dispersal distance. For both of our movement parameters, we tested for differences among reproductive classes (M = Male, NGF = Non-Gravid Female, GF = Gravid Female) and location using 2-way ANOVAs. For this and subsequent ANOVAs, interactions were

included in the model, but removed and not reported if non-significant. Because females shift reproductive classes between years, we considered individuals tracked in consecutive years to be independent for all analyses.

As a measure of movement rate, we also calculated distance moved per day for each reproductive class and location. Temperate zone snakes exhibit seasonal variation in movement patterns (Blouin-Demers and Weatherhead, 2002b; Row and Blouin-Demers, 2006c; Kapfer et al., 2008). We therefore split individuals into their respective reproductive class and divided the active season in three based on the biology of foxsnakes: Mating (May 21 – June 19), Gestation (June 20 – July 20), and Post-Gestation (July 21 – August 31). We subsequently calculated distance moved per day (sum of distance moved /number of days elapsed in season) for each reproductive class and location within each season and tested for differences using a 3-way ANOVA.

For all analyses the distribution of residuals was examined to determine if the assumptions of normality and homogeneity of variance were upheld, and we applied transformations or used equivalent non-parametric tests when violated. All statistical analyses were performed in JMP version 5.1 (SAS Institute Inc., Cary, NC). All means are reported ± standard error.

Habitat use

We first compared habitat use to availability using compositional analysis (Aebischer et al., 1993). At the location scale (selection of locations within the home-range), we compared the proportions of used habitat types to the proportions of habitat types available within the home range. At the home range scale (selection of the entire home-range within the study area), we compared the proportions of habitat types within

the home range of each individual to an availability circle centered on the hibernation site of that individual (or first location if hibernation site was unknown) with a radius equal to the maximum length of their home-range (Row and Blouin-Demers, 2006a). Habitat proportions were computed in ArcView 3.2 (ESRI, Redlands, CA) using the Animal Movement Extension (Hooge and Eichenlaub, 1997).

Compositional analysis does not examine inter-individual variation (Calenge and Dufour, 2006). We therefore examined variation between individuals at both scales using an eigen analysis of selection ratios, which maximizes the difference between use and availability onto one or two factor scores and assesses variation among individuals (Calenge and Dufour, 2006). Compositional and eigen analysis were done in R (R Core Development Team, Vienna, Austria) using the *adehabitat* package (Calenge, 2007).

Landscape scale

Row et al. (2010) developed a habitat suitability map for eastern foxsnakes across southwestern Ontario using 722 occurrence records and an Ecological Niche Factor Analysis (see Appendix 2). They grouped the habitat across southwestern Ontario into 4 suitability classes: unsuitable, marginal, suitable, and optimal. Using the habitat suitability map and occurrence records, we determined the propensity of individuals to travel and persist with low amounts of suitable habitat by calculating 1) the distance from occurrence records to usable habitat (marginal-optimal) and 2) the area of suitable habitat surrounding (1.5 km buffer) each occurrence record. We compared these values to an equal number of locations (722) randomly distributed across the study area using a one-way ANOVA.

Results

Movement patterns

We tracked 17 individuals at HMCA resulting in 20 (NGF = 7; GF = 7; M = 9) snake years (3 individuals were tracked in both years) and we tracked 15 individuals at PPNP resulting in 16 (NGF = 5; GF = 5; M = 6) snake years (one individual was tracked in both years). Mean MCP home range area was larger for individuals at PPNP (mean = 50 ± 10.5 ha) than at HMCA (mean = 31 ± 9.39 ha); however, a 2-way ANOVA revealed that there was no significant difference for mean MCP area between the reproductive classes ($R^2 = 0.02$, $F_{2,35} = 0.25$, $p = 0.78$) or location ($R^2 = 0.04$, $F_{1,35} = 1.20$, $p = 0.28$) possibly due to the large variation among individuals. Due to two outliers (see below), the assumption of normality was not met, but the lack of significance was confirmed using a non-parametric Kruskal–Wallis test. The range in MCP area was higher for individuals at PPNP (min = 4.8 ha, max = 163.9 ha, range 159.0 ha) than at HMCA (min = 8.4, ha, max = 75.5 ha, range 67.1 ha) mainly due to two outliers at PPNP (~150 ha home ranges).

Maximum distance to hibernation site did not significantly vary by reproductive class ($R^2 = 0.03$, $F_{2,39} = 0.67$, $p = 0.52$) or location ($R^2 = 0.04$, $F_{1,39} = 1.77$, $p = 0.19$) nor was the interaction significant ($R^2 = 0.07$, $F_{2,39} = 1.44$, $p = 0.24$). One female tracked for 2 years at PPNP (the only female not to become gravid over the 2 years) had much lower movement rates than all other individuals. When this female was removed, all reproductive classes at PPNP had a longer maximum distance to their hibernation sites and location became marginally significant ($R^2 = 0.11$, $F_{2,36} = 4.01$, $p = 0.05$; Fig. 2.2A).

A 3-way ANOVA determined that distance moved per day varied significantly with season ($R^2 = 0.11$, $F_{2,125} = 8.81$, $p < 0.001$) and season*reproductive class ($R^2 = 0.09$,

$F_{4,125} = 3.46$, $p < 0.01$), but not by reproductive class ($R^2 = 0.03$, $F_{2,125} = 2.65$, $p > 0.07$) or location ($R^2 < 0.001$, $F_{1,125} = 0.001$, $p = 0.97$). All other interactions were non-significant (all p-values > 0.52). Because of the interaction between reproductive class and season, we used separate one-way ANOVAs to compare reproductive classes within seasons grouping over locations. Within the gestation period, the effect of reproductive class was significant ($R^2 < 0.21$, $F_{1,43} = 5.62$, $p = 0.007$) and Tukey HSD tests revealed that gravid females moved more than the other two classes (Fig. 2.2B). Although males and gravid females appeared to have higher movement rates than non-gravid females in the mating season (Fig. 2.2B), this difference was not significant ($R^2 < 0.12$, $F_{1,39} = 2.05$, $p = 0.095$). In the post gestation period, there was some evidence that non-gravid females have higher movement rates than the other two groups (Fig. 2.2B), but this difference was not significant ($R^2 < 0.09$, $F_{1,43} = 1.19$, $p = 0.157$).

Habitat use

Compositional analysis at the location scale revealed that individuals at HMCA used habitat within their home-range non-randomly ($\lambda_{20,5} = 0.02$, $p > 001$; Fig. 2.3A) and individuals preferred open dry habitat to all others. For this and subsequent tests, significant differences in rank at alpha = 0.05 are represented by ">>" and non-significant by ">". Habitat ranks were open >> marsh > agriculture > shrub > forest. Individuals at PPNP were also found to use habitat non-randomly ($\lambda_{20,5} = 0.02$, $p > 001$; Fig. 2.3B) and snakes also preferred open to all other habitats types: open >> marsh > forest, with marsh and forest being > than dense shrub, but >> agriculture.

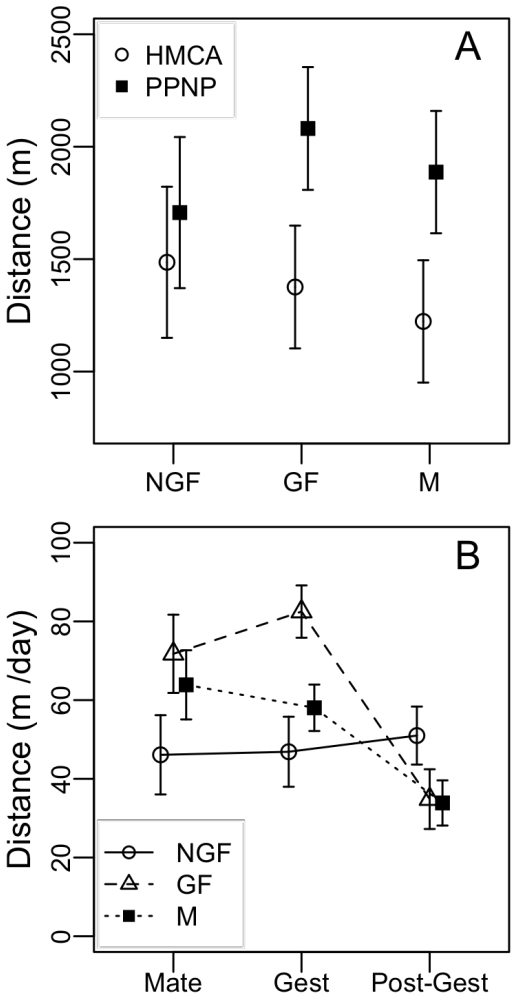

Figure 2.2. A) Mean (± standard error) maximum distance from hibernation sites for non-gravid females (NGF), gravid females (GF), and male (M) eastern foxsnakes from a large (PPNP) and small (HMCA) habitat patch in southwestern Ontario, and B) Mean distance (± standard error) moved per day varied differently across season for radio-tracked M, GF and NGF eastern foxsnakes combined over the two locations (PPNP & HMCA).

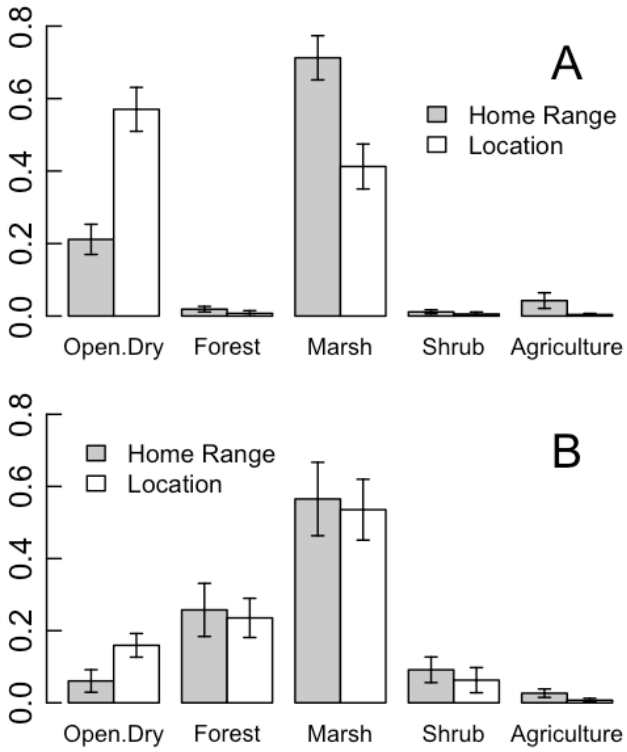

Figure 2.3. Mean proportion (± standard error) of radio-telemetry locations within five habitat types compared to habitat composition within minimum convex polygon home-ranges for radio-tracked eastern foxsnakes at A) a highly fragmented (HMCA) and, B) a site with little fragmentation (PPNP) in southwestern Ontario.

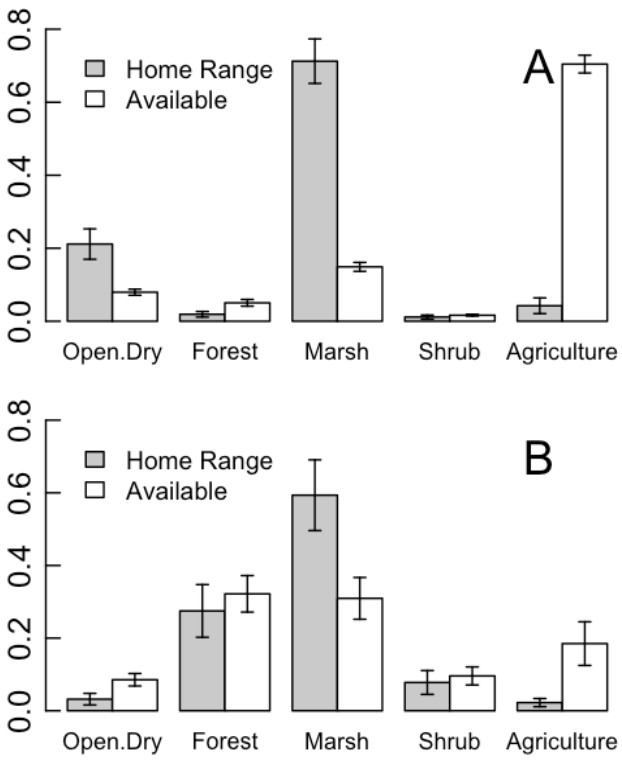

Figure 2.4. Mean habitat proportions (± standard error) within minimum convex polygon home-ranges compared to available habitat composition (circle centered on the hibernation site with a radius equal to the home-range length for each individual) for radio-tracked eastern foxsnakes at, A) a highly fragmented (HMCA) and B) a site with little fragmentation (PPNP) in southwestern Ontario.

Eigen analysis reduced most of the variation to the first axis (94%), with all individuals having varying degrees of preference for open habitat while avoiding the other habitats (Figure A1.1A Appendix 1). At PPNP, 87% of the variation was explained by the first two axes (axis 1 – 63%, axis 2 – 24%). As with HMCA, the majority of individuals preferred open dry habitat to the other habitats at this scale. There was much more variation among individuals, however, and many demonstrated little apparent preference for any habitat (values close to zero for both axes) at this scale (Figure A1.1B Appendix 1).

Using compositional analysis at the home range scale, we determined that habitat use was significantly different from random for snakes at HMCA ($\lambda_{20,5} = 0.14$, $p > 001$; Fig. 2.4A) and marsh was significantly preferred over all other habitat types, and all habitat types were preferred over agriculture (ranks: marsh >> open >> shrub > forest >> agriculture). For snakes at PPNP, habitat use was also significantly different from random ($\lambda_{20,5} = 0.34$, $p = 0.009$; Fig. 2.4B) and marsh was again preferred over all other habitat types: marsh >> forest > open > shrub > agriculture.

The first two axes of the eigen analysis explained most of the variation (99%) observed at HMCA. All individuals had positive values on the first axis, which explained most of the variation (≈89%), with all individuals demonstrating preference for marsh and open dry habitat and avoidance for the other habitat types (Figure A1.2A Appendix 1). There was some variation among individuals on the second axis, which explains less variation (9%), demonstrating some variation in preference for open dry habitat within the home range.

At PPNP there was more individual variation, but the first two axes of the eigen analysis still explained a large proportion of the total variation (86%). Most variation was explained by the first axes (axis 1 – 70%, axis 2 – 16%), and all except two individuals still had negative values on the first axis representing a preference for marsh habitat (Figure A1.2B Appendix 1). The second axis mainly separated individuals preferring open dry and shrubby habitats versus forest habitat with about half the individuals showing a weak preference for each.

Landscape Scale

The distance of foxnake occurrences from usable habitat (marginal-optimal) was significantly lower than for random locations ($F_{1,1443} = 287.22$, $p < 0.001$; Fig. 2.5A). Approximately 15 % (111 records) of occurrence records were outside usable habitat as we defined it. The greatest distance that any individual was found from usable habitat was 4.6 km, but only 11 (~1.5%) records were >1.5 km (average maximum distance from hibernation site for radio tracked snakes) from usable habitat. Random locations were much further from suitable habitat, with 588 records (81 %) placed outside usable habitat and 147 (20%) locations > 1.5 km from usable habitat (Fig. 2.5A). There was also significantly more usable habitat within a 1.5 km buffer surrounding foxsnake occurences (mean = 385 ± 175 ha) than random locations (mean = 126 ± 153 ha) ($F_{1,1443} = 891.77$, $p < 0.001$;Fig. 2.5B). Only 14 (~2%) of occurrence records were found in areas with < 1 ha of usable habitat, whereas, 126 (~17%) random records had <1 ha of surrounding usable habitat.

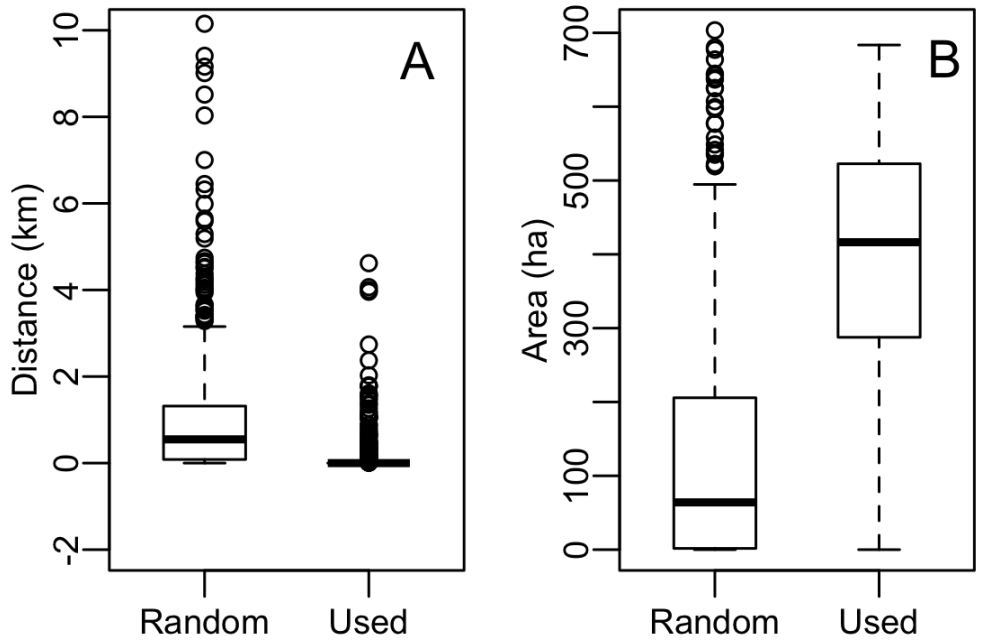

Figure 2.5. A) Distance to usable (marginal-optimal) habitat, and B) and amount of usable habitat within a 1.5 km buffer surrounding foxsnake occurrence records and randomly generated points across southwestern Ontario.

Discussion

Although habitat fragmentation has been shown to have a negative effect on snake diversity and abundance (Luiselli and Capizzi, 1997; Mac Nally and Brown, 2001; Vignoli et al., 2009), little is known about the response of individuals and populations to habitat patch size and fragmentation. There are limitations to our conclusions because we only had a single large site and a single small site; however, our study is an important first step towards understanding the potential effects of habitat fragmentation and patch size on movement and habitat use patterns in snakes. Thus, this study will be useful to land managers attempting to understand and minimize the impact of habitat fragmentation.

Using radio-telemetry, Corey and Doody (2010) found that individual carpet pythons (*Morelia spilota*) in disturbed habitats in Australia had lower movement rates than in a less disturbed habitat, but found no difference in space use (e.g. home-range size) between the sites. Here we found most movement patterns of foxsnakes from the two sites to be similar, but there were some differences that suggest movements are constrained in smaller habitat patches and this, in turn, implies that the significant genetic structure across this region (Row et al., 2010) in part arises because movements are hindered for snakes in smaller habitat patches. First, mean MCP home-range size did not differ significantly between locations, but the range in values was much greater for individuals at PPNP. This was mainly due to two individuals with extremely large home ranges (~150 ha), but does imply that patch size may limit home range size. Similarly, when one outlier non-gravid female was removed, all reproductive classes had significantly higher distances from their hibernation sites at PPNP compared to

individuals at HMCA, demonstrating their ability to travel further distances in larger expanses of natural habitat.

We found no difference between locations for distance moved per day, but detailed consideration of individual locations showed that reproductive males and gravid females both tended to have increased movement (distance/day) during the mating and gestation periods whereas an increase was not evident in non-gravid females, which had similar movement patterns in all three seasons. Many other studies on snakes have reported increased male movement rates during the mating season in comparison to the other reproductive classes, which is likely due to mate searching (Blouin-Demers and Weatherhead, 2002a; Carfagno and Weatherhead, 2008; Kapfer et al., 2008). Many females made long distance movements to and from nesting locations, which likely accounts for the increased movement of gravid females compared to non-gravid females during the mating and gestation seasons.

Our fine-scale radio-telemetry results indicate that foxsnakes are strict habitat specialists and are restricted mainly to marsh and prairie habitat as reported previously in the general literature (Ernst and Barbour, 1989). Overall, habitat use patterns at both locations showed little absolute difference. We did find a difference in patterns depending on scale (marsh at home range scale, open habitat at location scale) suggesting that individuals are using these habitats for different reasons, which has been reported for other reptiles (Compton et al., 2002). A possible reason for this disparity may be a compromise between suitable retreat and/or basking sites and foraging habitat. Individuals would often spend long periods of time basking and resting beside or under shelter such as rocks or snags, which appear to be more abundant in areas surrounding the

marsh. Further studies testing prey abundance and distribution of shelters would be required to test if perhaps prey abundance is higher in marshes leading to the differences between scales.

Despite the overall similarities between sites, individuals at HMCA had stronger habitat selection patterns with less variability among individuals. These differences are likely due to the amount and distribution of habitat within locales, with individuals at HMCA not having to travel through undesirable natural habitat such as forests and dense shrub habitat. It does demonstrate, however, the unwillingness of foxsnakes at HMCA to use, or even move through, agricultural fields despite the abundance of this habitat type at this location. No individual was ever located directly within an agricultural field, likely due to a lack of cover. Agricultural fields are bare throughout spring and lack dead vegetation and other shelter (e.g., rocks or logs) that would be present in more natural open habitat.

Our radio-telemetry analysis looked at fine scale patterns at only two locations and so it is impossible to eliminate other site-specific effects (e.g., distribution of hibernation sites, habitat quality) that could be affecting movement patterns independent of patch size or fragmentation. There are also much smaller patches of habitat across the range of foxsnakes that still appear to be inhabited. It would be interesting to confirm whether movement patterns of resident snakes are confined to these smaller patches, or whether these individuals are more inclined to move through the agricultural matrix at these locations. We did track three individuals in a small privately owned patch of open dry habitat (~8-10 ha, much smaller than HMCA) embedded within a dense agricultural mosaic. Although not included in our analyses due to small sample sizes, these three

individuals also did not use agricultural fields, but did traverse agricultural fields to use small patches of semi-natural open habitat in other areas (e.g., large hedge rows, drainage ditches, restored private ponds, and prairie habitat). Further detailed work in such habitat patches will increase our understanding of dispersal patterns across this region.

At the landscape scale, the vast majority of occurrence records were close to usable habitat, at distances that our radio-telemetry data indicate foxsnakes can easily traverse. The fact that some individuals were found outside of suitable habitat at all, however, suggests that individuals in more impoverished habitats are travelling through or utilizing smaller patches and/or different habitats than individuals observed at HMCA, which never travelled into agricultural fields.

Management Implications

Recent landscape genetics studies have suggested that habitat loss and fragmentation can impact snake population genetic structure (Jansen et al., 2008; Clark et al., 2010) and reduce abundance and diversity (Cagle, 2008; Vignoli et al., 2009). Given their importance as predators in many landscapes (Schwaner and Sarre, 1988; Tzika et al., 2008) and the scale of habitat fragmentation occurring globally, effective management strategies are required to maintain snake populations. The broad occupancy of foxsnakes across much of their former range (compared to historical records) in a heavily fragmented region, implies foxsnakes may have adapted well to the extensive habitat loss and fragmentation in this region or that there is a prolonged lag between habitat loss and ultimate demise of these small populations. Our results, combined with the results of DiLeo et al. (2010) and Row et al. (2010) suggest, however, that foxsnake populations are limited by the distribution of the small patches of suitable habitat remaining. These results

demonstrate the importance of maintaining relatively close (>1.5 km) habitat connections between populations, but imply that it is possible that connections may be maintained through the use of habitat islands and/or habitat corridors (Rosenberg et al., 1997).

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Literature Cited

Aebischer, N. J., P. A. Robertson, and R. E. Kenward. 1993. Compositional analysis of habitat use from animal radio-tracking data. Ecology 74:1313–1325.

Blouin-Demers, G., and P. J. Weatherhead. 2002a. Habitat-specific behavioural thermoregulation by black rat snakes (*Elaphe obsoleta obsoleta*). Oikos 97:59–68.

Blouin-Demers, G., and P. J. Weatherhead. 2002b. Implications of movement patterns for gene flow in black rat snakes (*Elaphe obsoleta*). Canadian Journal of Zoology 80:1162–1172.

Cagle, N. 2008. Snake species distributions and temperate grasslands: A case study from the American tallgrass prairie. Biological Conservation 141:744–755.

Calenge, C. 2007. Exploring habitat selection by wildlife with *adehabitat*. Journal of Statistical Software 22:1–19.

Calenge, C., and A. B. Dufour. 2006. Eigenanalysis of selection ratios from animal radio-tracking data. Ecology 87:2349–2355.

Carfagno, G., and P. J. Weatherhead. 2008. Energetics and space use: intraspecific and interspecific comparisons of movements and home ranges of two Colubrid snakes. Journal of Animal Ecology 77:416–424.

Corey, B., and J. Doody. 2010. Anthropogenic influences on the spatial ecology of a semi-arid python. Journal of Zoology 281:293–302.

Clark, R., W. Brown, R. Stechert, and K. Zamudio. 2010. Roads, interrupted dispersal, and genetic diversity in timber rattlesnakes. Conservation Biology 24:1–11.

Compton, B. W., J. M. Rhymer, and M. Mccollough. 2002. Habitat selection by wood turtles (*Clemmys insculpta*): an application of paired logistic regression. Ecology 83:833–843.

DiLeo, M., J. R. Row, and S. C. Lougheed. 2010. Discordant patterns of population structure for two co-distributed snake species across a fragmented Ontario landscape. Diversity and Distributions 16:571–581.

Driscoll, D. A. 2008. Extinction and outbreaks accompany fragmentation of a reptile community. Ecological Applications 14:220–240.

Duffy, J. E. 2002. Biodiversity and ecosystem function: the consumer connection. Oikos 99:210–219.

Ernst, C. H., and Barbour, R. W. 1989. Snakes of Eastern North America. George Mason University Press, Fairfax, Virginia

Environment Canada. 2009. Species at risk in Ontario. Environment Canada, Ottawa, Ontario. Available from http://www.on.ec.gc.ca/wildlife/sar/sar-e.html (accessed March 2010)

Fahrig, L. 2002. Effect of habitat fragmentation on the extinction threshold: A synthesis. Ecological Applications 12:346–353.

Fahrig, L. 2007. Non-optimal animal movement in human-altered landscapes. Functional Ecology 21:1003–1015.

Githiru, M., L. Lens, and L. Bennun. 2007. Ranging behaviour and habitat use by an Afrotropical songbird in a fragmented landscape. African Journal of Ecology 45:581–589.

Greenwald, K. R., J. L. Purrenhage, and W. K. Savage. 2009. Landcover predicts isolation in *Ambystoma* salamanders across region and speices. Biological Conservation 142:2493–2500.

Hooge, P. N., and B. Eichenlaub. 1997. Animal movement extension to ArcView. ver. 1.1. Alaska Science Center - Biological Science Office, U.S. Geological Survey, Anchorage, Alaska.

Jansen, K. P., H. R. Mushinsky, and S. A. Karl. 2008. Population genetics of the mangrove salt marsh snake, *Nerodia clarkii compressicauda*, in a linear, fragmented habitat. Conservation Genetics 9:401–410.

Kapfer, J., J. Coggins, and R. Hay. 2008. Spatial ecology and habitat selection of bullsnakes (*Pituophis catenifer sayi*) at the northern periphery of their geographic range. Copeia 2008:815–826.

Ludwig, T., I. Storch, and R. F. Graf. 2009. Historic landscape change and habitat loss: the case of black grouse in Lower Saxony, Germany. Landscape Ecology 24:533–546.

Luiselli, L., G. C. Akani, and D. Capizzi. 1998. Food resource partitioning of a community of snakes in a swamp rainforest of south-eastern Nigeria. Journal of Zoology 246:125–133.

Luiselli, L., and D. Capizzi. 1997. Influences of area, isolation and habitat features on distribution of snakes in Mediterranean fragmented woodlands. Biodiversity and Conservation 6:1339–1351.

Mac Nally, R., and G. Brown. 2001. Reptiles and habitat fragmentation in the box-ironbark forests of central Victoria, Australia: predictions, compositional change and faunal nestedness. Oecologia 128:116–125.

Marchesan, D., and S. M. Carthew. 2008. Use of space by the yellow-footed antechinus, *Antechinus flavipes*, in a fragmented landscape in South Australia. Landscape Ecology 23:741–752.

Paine, R. T. 1969. A note on trophic complexity and community stability. The American Naturalist 103:91–93.

Rosenberg, D. K., B. R. Noon, and E. C. Meslow. 1997. Biological corridors: form, function, and efficacy. BioScience 47:677–687.

Row, J. R., G. Blouin-Demers, and S. C. Lougheed (2010) Habitat distribution influences dispersal and fine-scale genetic population structure of eastern foxsnakes (*Mintonius gloydi*) across a fragmented landscape. Molecular Ecology*, 19, 5157-5171*

Row, J. R., and G. Blouin-Demers. 2006a. Thermal quality influences habitat selection at multiple spatial scales in milksnakes. Ecoscience 13:443–450.

Row, J. R., and G. Blouin-Demers. 2006b. Kernels are not accurate estimators of home-range size for herpetofauna. Copeia 2006:797–802.

Row, J. R., and G. Blouin-Demers. 2006c. Thermal quality influences effectiveness of thermoregulation, habitat use, and behaviour in milk snakes. Oecologia 148:1–11.

Schwaner, T. D., and S. D. Sarre. 1988. Body size of tiger snakes in southern Australia, with particular reference to *Notechis ater serventyi* (*Elapidae*) on Chappell Island. Journal of Herpetology 22:24–33.

Tilman, D., R. M. May, C. L. Lehman, and M. A. Nowak. 1994. Habitat destruction and the extinction debt. Nature 371:65–66.

Tzika, A. C., S. Koenig, R. Miller, G. Garcia, C. Remy, and M. C. Milinkovitch. 2008. Population structure of an endemic vulnerable species, the Jamaican boa (*Epicrates subflavus*). Molecular Ecology 17:533–544.

Vignoli, L., I. Mocaer, L. Luiselli, and M. Bologna. 2009. Can a large metropolis sustain complex herpetofauna communities? An analysis of the suitability of green space fragments in Rome. Animal Conservation 12:456–466.

Weatherhead, P. J., and G. Blouin-Demers. 2004. Understanding avian nest predation: why ornithologists should study snakes. Journal of Avian Biology 35:185–190.

Whitaker, J. R. 1938. Agricultural gradients in southern Ontario. Economic Geography 14:109–120.

Chapter 3: Habitat distribution influences dispersal and fine-scale genetic population structure of eastern foxsnakes (*Pantherophis gloydi*) across a fragmented landscape

Abstract

Dispersal is a fundamental attribute of species in nature, and shapes population dynamics, evolutionary trajectories and genetic variation across spatial and temporal scales. It is increasingly clear that landscape features have large impacts on dispersal patterns. Thus, understanding how individuals move through landscapes is essential for predicting impacts of landscape alterations. Information on dispersal patterns, however, is lacking for many taxa, particularly reptiles. Eastern foxsnakes (*Pantherophis gloydi*) are marsh and prairie specialists that avoid agricultural fields, but they have persisted across a fragmented region in southwestern Ontario and northern Ohio. Here, we combined habitat suitability modeling with population genetic analyses to infer how foxsnakes disperse through a habitat mosaic of natural and altered landscape features. Boundary regions between the eight genetic clusters, identified through assignment tests, were comprised of low suitability habitat (e.g. agricultural fields). Island populations were grouped into a single genetic cluster, and comparatively low F_{ST} values between island and mainland populations suggest open water presents less of a barrier than non-suitable terrestrial habitat. Isolation by resistance and least-cost path analysis produced similar results with matrices of pairwise individual genetic distance significantly more correlated to matrices of resistance values derived from habitat suitability than models with an undifferentiated landscape. Spatial autocorrelation results matched better with assignment results when incorporating resistance values rather than straight-line distances. All analyses used in our study produced similar results suggesting that habitat degradation limits dispersal for foxsnakes, which has had a strong effect on the genetic population structure across this region.

Introduction

Both evolutionary theory (e.g. Wright 1948; Slatkin 1987) and empirical data (e.g. Postma & van Noordwijk 2005) show that dispersal has large impacts on how genetic variation is distributed among populations. Indeed, estimating dispersal and gene flow is key to understanding local adaptation (Postma & van Noordwijk 2005), population genetic models of diversification (Slatkin 1987), and population connectivity and persistence for species of conservation concern (e.g. Cegelski *et al*. 2003). Thus, studying factors that promote or impede dispersal has long been a central theme in evolutionary ecology (Greenwood & Harvey 1982) and conservation biology (Frankham *et al*. 2002).

Recent studies show that species' habitat preferences coupled with landscape features modulate dispersal patterns influencing genetic population structure (e.g. Piertney *et al*. 1998; Castric *et al*. 2001). Thus, understanding how individuals disperse through complex landscapes is essential for predicting the impact that landscape alterations (e.g. habitat fragmentation) have on populations and for devising effective schemes to mitigate their effects (e.g. habitat corridors) (Fahrig 2007). Information on dispersal patterns, however, is lacking for many taxa, and this is particularly true for terrestrial reptiles (Bowne & Bowers 2004), despite their importance as top predators in many ecosystems (Schwaner & Sarre 1988; Tzika *et al*. 2008).

Methods for spatially quantifying genetic population structure and landscape effects have been developing rapidly (Manel *et al*. 2003; Balkenhol *et al*. 2009; Guillot *et al*. 2009). A popular technique for quantifying landscape effects is the assignment test (reviewed in Manel *et al*. 2005), which allows researchers to identify boundaries between genetic clusters and to move away from arbitrary delineations of populations based on

geographic location alone (Manel *et al*. 2005; Zalewski *et al*. 2009). Combining assignment tests with surface interpolation of posterior probabilities (Guillot *et al*. 2005; Murphy *et al*. 2008; Pierson *et al*. 2010) and admixture coefficients (Durand *et al*. 2009) can diagnose population boundaries and regions of admixture on the landscape, but is under-utilized in the literature (but see: Murphy *et al*. 2008), particularly with three or more clusters.

When populations are continuously distributed, spatial genetic structure has traditionally been quantified using isolation by distance (IBD) models (individuals or populations) (Wright 1943; Rousset 2000; Frantz *et al*. 2009). Landscape effects can be incorporated into IBD models by using Mantel's non-parametric permutation tests (Mantel 1967; Slatkin 1993) to compare the fit of the relationship between matrices of genetic distinctiveness and straight-line geographic distance or matrices of resistance values based on landscape features. Traditionally, resistance values have been calculated using a 'least cost' path (LCP) model (Adriaensen *et al*. 2003) based on the estimated propensity for organisms to travel through different habitat types. A related and potentially more powerful method, isolation by resistance (IBR) (McRae 2006), uses circuit theory to quantify the amount of potential connectivity between populations and accommodates larger and/or more habitat corridors between populations. IBR approaches thus far appear to produce better results than least cost paths (McRae & Beier 2007), but have not been thoroughly evaluated with multiple empirical datasets, especially those at fine scales.

Spatial autocorrelation analysis (Slatkin & Arter 1991) is another potentially powerful approach in the landscape genetics tool kit that compares the relatedness of

individuals within spatial categories of increasing magnitude to the relatedness of randomly distributed pairs of individuals. Researchers often equate the scale of spatial genetic structure in continuous populations as the geographic distance of positive spatial autocorrelation (Epperson & Li 1997). Populations, species or sexes that show positive spatial autocorrelation across greater spatial extents are viewed as having greater dispersal ability (Beck *et al*. 2008; Hardy *et al*. 2008). As with IBD models, incorporating pairwise least-cost paths or resistance values into spatial autocorrelation analysis instead of straight-line distances would seem more biologically realistic. Although this is easily accomplished using most popular spatial autocorrelation software, this is rarely tested in natural populations.

Eastern foxsnakes (*Pantherophis gloydi*) are marsh and prairie specialists, but have persisted across southwestern Ontario and northern Ohio where most of these habitat types have been converted to agricultural land (Whitaker 1938). Here, we evaluate the effects that habitat conversion, loss and fragmentation have had on this marshland-prairie specialist, and infer how foxsnakes disperse through a complex habitat mosaic of natural and altered landscape features. Specifically, we combine the results of habitat suitability modeling and genetic patterns inferred using assignment tests with spatial interpolation, IBD (with IBR and LCP models) and spatial autocorrelation analysis to address the following questions:

1) Does the number and extent of genetic populations identified using Bayesian assignment methods correlate with current habitat distribution patterns and landscape features (e.g. road and urban barriers; lake barriers)?

2) Does the predictive ability of isolation models and spatial autocorrelation analysis significantly improve when using landscape resistance values derived from habitat suitability modeling?

Although studies increasingly deploy these methods to incorporate landscape structure into population genetic analysis, few fully combine ecological, genetic and spatial analysis. For example, to our knowledge ours is one of the first studies to combine the results of habitat suitability modeling with genetic analysis (but see: Wang *et al*. 2008). Thus, as a secondary goal, we compare results among the three methods and also determine if IBR outperforms LCP analysis for a relatively fine-scale individual dataset.

Methods

Genetic sampling and microsatellite screening

Over the season when snakes are active of 2006 – 2009, we hand captured foxsnakes from across southwestern Ontario, took a small blood sample (~200 ml stored in 95% ethanol) from the caudal vein and visually determined the sex. We also took tissues from individuals killed on roads and acquired samples from researchers working in other regions (Ohio and Michigan) leading to a total of 585 samples (Fig. 3.1). This sampling range represents the majority of the distribution of two geographically disjunct regions (large distribution gap between Norfolk county and other populations) of eastern foxsnakes, and comprises close to 60% of the current range of eastern foxsnakes (Fig. A2.1 Appendix 2).

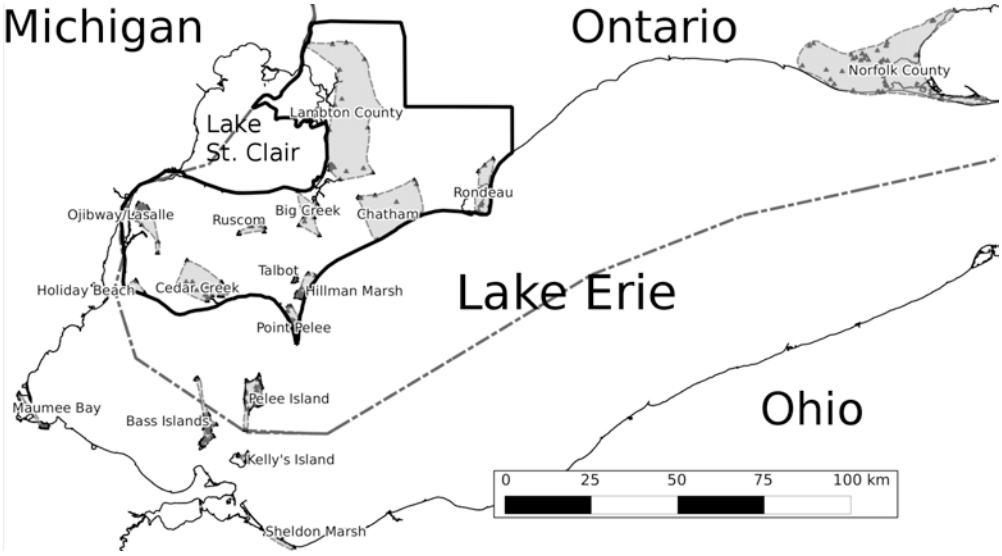

Figure 3.1. Map of study area with black triangles representing sample locations of eastern foxsnakes and grey polygon outlining populations for display purposes (see Fig. 3.2). Dark black line outlines region where detailed habitat modeling was completed.

We extracted DNA from blood and tissue using QIAGEN (Venlo, Netherlands) DNeasy blood and tissue kit following the manufacturer's protocols. All samples were genotyped for 11 microsatellite loci (FS24, FS50, FS33, FS52, FS67, FS82, FS77, FS63, FS09B, FS42B, FSV16B, accession # EU294198 – EU294208) developed specifically for this species (Row *et al*. 2008) and one additional locus (EOB10, accession # AF544655) developed for eastern ratsnakes (*Pantherophis obsoleta*) (Blouin-Demers & Gibbs 2003). PCR reaction mixes were made up of 10 ng of genomic DNA, 1X Taq buffer with $(NH_4)_2SO_4$ (Fermentas), 0.2 μM forward and reverse primer, 0.1 mM of each nucleotide, 0.03 μM of Well RED fluorescent-labeled M13 primer (Boutin-Ganache *et al*. 2001), 0.25 U of DNA Taq polymerase (Fermentas) and concentrations of $MgCl_2$ specific to the microsatellite (Row *et al*. 2008). PCRs were done in a GeneAmp 9700 or 2700 (Applied Biosystems) using the cycling profile: 5 min denaturation at 95°C; 35 cycles of 30 s at 95°C, 30 s at 55°C and 30 s at 72°C; and a final extension of 72°C for 5 min. PCR products were run on a Beckman Coulter CEQ 8000 capillary automated sequencer and microsatellite sizes were scored using CEQ 8000 Genetic Analysis System.

Previous studies using these same loci found neither deviations from Hardy-Weinberg Equilibrium (HWE) nor linkage equilibrium (Row *et al*. 2008), nor were null alleles prevalent (DiLeo *et al*. 2010). Because we use additional populations and loci (EOB 10), we again tested for departures from HWE (100 batches, 1000 iterations per batch) and linkage equilibrium (100 batches, 1000 iterations) using Fisher's exact tests as implemented in Genepop 4.0.1 (Raymond & Rousset 1995) and used MICRO-

CHECKER 2.2.3 (van Oosterhout *et al*. 2004) to test for scoring errors and null alleles. We split our samples into our 16 geographically defined "populations" (Fig. 3.1; Appendix 3); excluding the Chatham population) where we had samples with >10 individuals. In the MICRO-CHECKER analysis only samples from the 8 populations identified by clustering analysis were used.

Landscape quantification and habitat suitability

Across southwestern Ontario (Fig. 3.1) we used Ontario digital topographic maps (Ontario Base Map, Ontario Ministry of Natural Resources, scale of 1:10000) as base maps for the major habitat types. These maps were generally out of date (collected from 1977-2000) and missing some important features (e.g. open semi-natural habitat). We therefore overlaid a grid (~5 km^2) and, using 30 cm^2 resolution aerial photography taken in 2006 (SWOOP, Ontario Ministry of Natural Resources), we confirmed existing habitat features and added new features (> 15 m^2 in size) resulting in a map with: open water, semi-natural open habitat, marsh, forest, residential/urban, agriculture, roads, and small creeks/drains. Using these maps and 722 occurrence records spread across this region (Fig. A2.1 Appendix 2) we used Ecological Niche Factor Analysis (ENFA) (Hirzel *et al*. 2002) to determine landscape scale habitat preference patterns and develop two (40 m X 40 m resolution) habitat suitability maps: 1) a ranked habitat suitability map with suitability scores between 0 and 100, and 2) a grouped habitat suitability map with 4 habitat suitability classes: unsuitable, marginal, suitable and optimal, based on a plot of the predicted frequency of evaluation points in each habitat class to the expected frequency based on a random model (Fig. A2.2a; Appendix 2) (Hirzel *et al*. 2006). Both of these ecologically derived habitat suitability maps were used to develop landscape

conductance and resistance scores (See: *Isolation by resistance and least-cost path analysis*). Ninety-nine percent of individuals were found in habitat with a suitability ranking > 2 out of 100 suggesting that they rarely travel in low quality habitat. We therefore added a fifth 'barrier' habitat class (habitat suitability = ranking between 0-2) for some of the fine-scale genetic analyses. A detailed account of the methods and results of the ENFA analysis can be found in Appendix 2.

Assignment tests

Because of their superiority at detecting fine scale population structure when genetic clusters are spatially distinct (Chen *et al*. 2007) (which is expected for a relatively low vagility taxon such as snakes), we chose clustering programs that explicitly incorporate spatial information. There are now multiple techniques for individual clustering with spatial options, each of which make different assumptions about the data (Guillot *et al*. 2009). We therefore used two approaches to test whether our results were consistent. We used the program Bayesian Analysis of Population Structure (BAPS 5.1) (Corander *et al*. 2008) because of the low subjectivity involved in the methods for choosing the number of clusters (k). BAPS outputs a probability for the number of clusters, which may or may not match the maximum number of clusters (max k). Because the probability for the number of clusters can vary with max k, we ran BAPS using individual spatial clustering with 20 replicates for each of max k set to 10, 15, 20 and 25. We conducted admixture analysis using the number of clusters chosen in the non-admixture analysis with 200 iterations, 200 reference individuals and 20 iterations for each reference individual (Corander & Marttinen 2006).

We also used TESS 1.3 (Chen *et al*. 2007), which has a spatial option and allows for a detailed admixture analysis (Durand *et al*. 2009). Using TESS we ran 70,000 (20,000 burn-in) MCMC iterations 10 times from k = 3 to k = 12 using non-admixture analysis. The ideal cluster number was chosen based on when the Deviance Information Criterion (DIC) values reached a plateau and/or the Q-matrix of individual posterior probabilities stabilized (no additional clusters became apparent). Following the choice of the number of clusters, we ran an additional 60 replicates for that number of clusters and averaged the top models (based on DIC) in CLUMPP 1.2 (Jakobsson & Rosenberg 2007) and displayed clusters using DISTRUCT 1.1 (Rosenberg 2004). We chose the number of models to average based on the distribution of DIC values. We estimated admixture proportions, using the number of clusters established with the non-admixture analysis, with a conditional auto-regressive (CAR) Gaussian model with a trend degree of two (Besag 1975; Durand *et al*. 2009). We again conducted 60 runs with 70,000 (20,000 burnin) MCMC iterations, and averaged the top runs in CLUMPP 1.2 and displayed the results using DISTRUCT 1.1.

For the non-admixture analysis, we considered an individual as a migrant if its genotype implied that it originated from a population other than where it was captured ($p > 80\%$ of non-membership) and an individual that did not assign to any population with $p > 80\%$ as having unknown ancestry. In the admixture analysis, BAPS tests for individuals showing significant levels of admixture ($\alpha = 0.05$) (Corander & Marttinen 2006) and comparing DIC values in TESS can establish if using admixture provides a better fit for the data (Durand *et al*. 2009). For comparative purposes we also ran a non-spatial, admixture analysis using Structure 2.3.3 (Pritchard *et al*. 2000) (see Appendix 4).

We subsequently determined the extent of differentiation between and patterns of genetic diversity within identified genetic clusters by calculating pairwise F_{ST} (Weir & Cockerham 1984) and Joust's D (Jost 2008) between all clusters and expected heterozygosity (H_e - corrected for sample size; (Nei 1978)), mean number of alleles, standardized allelic richness (Hurlbert 1971) and mean F_{IS} within clusters using Microsatellite analyzer 4.05 (Dieringer & Schlotterer 2003) and SMOGD 1.2.5 (Crawford 2010).

Spatial kriging

Using the R (R Development Core Team 2009) package *spatial 7.2* and *gstat 0.9,* we mapped the extent of genetic clusters and identified barriers between clusters using ordinary kriging surface interpolation (Ripley 1981) of admixture proportions. For all clusters, we fit a zero polynomial (constant) trend surface regression with an exponential covariance function to the admixture proportions (psill = 1, nugget = 0) for each cluster, with a range parameter of 100 000 (100 km), and extrapolated the trend over the study area at a resolution of 500 m. This resulted in 8 maps, equal to the number of genetic clusters, with each map extrapolating the admixture proportions (proportion of genotype belonging to that particular cluster) across the study area. We identified common barriers by combining kriging maps of all the clusters and taking the maximum values. Therefore, we considered areas with low admixture values in the combined map to be boundary regions between genetic clusters. The surface interpolation extrapolates trends beyond areas with samples, so patterns in zones with large sampling gaps and in non-usable habitat (e.g. lakes) must be interpreted with caution.

If habitat quality was impacting genetic population structure you would expect areas with low habitat quality to correlate with genetic boundaries (i.e. low admixture proportions). We determined whether genetic boundaries were spatially related to regions of low habitat suitability by overlaying the habitat suitability map on top of the kriging surface maps across southwestern Ontario where detailed habitat suitability maps were developed (Fig. 3.1). Subsequently, we tested whether mean admixture proportions were lower (i.e. boundary regions between genetic clusters) within lower habitat suitability classes using a one-way ANOVA.

Isolation by resistance and least-cost path analysis

Across southwestern Ontario, where detailed habitat maps were available (Fig. 3.1), we conducted IBR and LCP analysis. Resistance values for the analysis are often not derived from ecological data and most studies test a series of models with a variety of costs assigned to landscape features (e.g. Schweiger *et al*. 2004; Cushman *et al*. 2006; Quéméré *et al*. 2010). A rarely used alternative approach is to use the results of habitat suitability modeling (Wang *et al*. 2008). We used a method similar to Wang *et al*. (2008) and derived landscape costs using habitat suitability scores derived from the ENFA analysis (see: Appendix 2). In the IBR analysis we used the conductance settings, with higher values (i.e. higher suitability) having a greater conductance (i.e. lower landscape resistance). Using the habitat suitability scores we derived 6 models based on: 1) the habitat suitability values produced from the ENFA analysis, 2) the 5 grouped habitat suitability classes (barrier, unsuitable, marginal, suitable and optimal -see *Landscape quantification and habitat suitability*), and 3) suspected barriers on the landscape (e.g. major highways and urban centers) (Table 3.1). Values for grouped models (Cond2,

Cond3, Cond5, Cond6) were the average value of the Predicted/Expected score of the ENFA analysis (Fig A2.2a; Appendix 2) within that habitat suitability class. These models were compared to a model with all landscape values equal to a conductance of 1, which is analogous to a straight-line distance model, but bounded by the study area and therefore a more direct comparison between models (Lee-Yaw *et al*. 2009). Pairwise resistance scores between individuals were calculated using CIRCUITSCAPE 3.5 (McRae 2006). CIRCUITSCAPE uses electrical theory to measure electrical resistance (measured in ohms) between sampling locations based on the assigned resistance or conductance values (in our case conductance) provided for the landscape (McRae et al. 2008).

We used a similar method to develop landscape resistance values for the LCP analysis. We needed to develop resistance and not conductance scores, however, and so we used values opposite to the conductance values (Table 3.1). As with the IBR analysis, we compared cost models to a model with all landscape values equal to 1. All pairwise least cost distances were derived using the PATHMATRIX 1.1 (Ray 2005) extension in ArcView 3.2 (ESRI, Redlands, CA).

Table 3.1. Conductance and resistance models used for isolation by resistance and least-cost path analysis. Models were derived from habitat suitability (HS) scores derived from ecological niche factor analysis. See text for additional details.

Model	Unsuitable	Marginal	Suitable	Optimal	Barriers
Isolation by Resistance					
Condeq	1	1	1	1	None
Cond1	1-30	31-45	46-80	81-101	None
Cond2*	1	4	6	10	None
Cond3*	1	4	6	10	0-2 HS scores†
Cond4	1-30	31-45	46-80	81-101	Major 4 lane highway*; urban centers
Cond5*	1	4	6	10	4 lane highway; urban centers
Cond6*	1	4	6	10	0-2 HS scores†; 4 lane highway; urban centers
Least-cost path analysis					
Costeq	1	1	1	1	None
Cost1	101-81	80-46	45-31	30-1	None
Cost2*	10	6	4	1	None
Cost3*	10	6	4	1	0-2 HS scores†
Cost4	101-81	80-46	45-31	30-1	Major 4 lane highway*; urban centers
Cost5*	10	6	4	1	4 lane highway; urban centers
Cost6*	10	6	4	1	0-2 HS scores†; 4 lane highway; urban centers

*Gaps in highway barrier were left at major drains and river underpasses
†99% of locations were in habitat with a score greater than 2.

For both the IBR and LCP analysis, we used Mantel's tests (Mantel 1967) to determine if matrices of pairwise individual genetic distances were more highly correlated to landscape-derived resistance values or to resistance values based on an equal landscape. Subsequently, we used partial Mantel's tests (Smouse *et al*. 1986) to determine if there was a significant correlation between genetic distance and landscape-derived resistance values when controlling for straight-line distance (resistance values based on an equal landscape) and *vice versa*. Using SPAGeDi 1.3 (Hardy & Vekemans 2002), we calculated pairwise genetic differentiation between individuals using both Loiselle's kinship coefficient (Loiselle *et al*. 1995), as it has been shown to be the best estimator in comparative tests (Vekemans & Hardy 2004), and Rousset's 'a' genetic distance (Rousset 2000) because it does not rely on a reference population and is analogous to $F_{ST}/(1-F_{ST})$ (Rousset 2000) and so more appropriate for larger scales (Calderon *et al*. 2007).

We calculated Mantel's and partial Mantel's correlation coefficients (r) using the *ecodist 1.2.2* package (Goslee & Urban 2007) in R (R Development Core Team 2009). Significance was determined with 9999 permutations and 95% bootstrap confidence intervals were determined with 1000 iterations.

Spatial autocorrelation analysis

Across southwestern Ontario where we had detailed habitat maps (Fig. 3.1), we also compared the results of spatial autocorrelation analysis using straight-line distances and resistance values. Because there are no direct tests available to compare these two approaches, we compared the scale of spatial genetic structure (i.e. geographic distance or resistance where the autocorrelation function crosses the x-axis) using both straight-line

and resistance values. We expected that the scale of spatial genetic structure would closely match genetic populations (e.g. individuals spaced > than the scale of positive autocorrelation would be assigned to separate genetic clusters). We determined the scale of spatial genetic structure using both straight-line distances and resistance scores (using the best model from the Mantel's analysis) by calculating Loiselle's kinship coefficient (Loiselle *et al*. 1995) for all pairwise comparisons within increasing spatial distances and resistance categories. Spatial categories were based on an even distribution of the number of pairwise comparisons within 25 categories and calculations were made using SPAGeDi 1.3 (Hardy & Vekemans 2002). We estimated the scale of autocorrelation by determining the distance and resistance values where the kinship coefficient dropped to or below zero (Sokal 1979; Epperson & Li 1997). We subsequently compared the results with assignment tests by mapping connections between individuals that were spaced less than the scale of the spatial genetic structure using straight-line and resistance values. If using resistance values provided a more 'biologically realistic' measure of the scale of genetic structure, then we expected fewer lines to cross between genetic clusters identified through assignment tests.

Results

Microsatellite Screening

After sequential Bonferroni correction (Rice 1989) we found no evidence for deviations from HWE for any loci in any of the populations. Using the 16 geographically defined populations, we found two pairs of loci to be in linkage disequilibrium (FS82 & FS77; FS33 & FS67) for two populations (Pelee Island and Bass Island). Because these trends were not seen in any other populations, both loci were retained.

MICRO-CHECKER found no evidence of scoring errors, but did imply null alleles for three loci (FS77, FS67, FS52) for three different populations. Again, because there was no consistent trend across populations for any of the loci and because no individual sample ever failed to amplify for a particular locus, null alleles are not a pervasive problem and we retained all loci for analysis.

Assignment tests

Results of our BAPS analysis indicated that the most likely number of clusters was 8. The probabilities for 8 genetic clusters were 1, 1, 0.76 and 0.91 when setting max k to 10, 15, 20 and 25, respectively. Based on a bar plot of the Q-matrix there were five groups of two or more of our original 17 sampling locales: GR1 - Ojiway/Lasalle and Holiday Beach, GR2 - Ruscom, Big Creek, Lambton, and Chatham, GR3 - Rondeau and Sheldon Marsh, GR4 - Maumee Bay, Bass Islands, Kelly's Island and Pelee Island, and GR5 - Point Pelee and Hillman Marsh. These groups were diagnosed in all analyses (Fig. 3.2) and, thus, we consider these groups as genetic clusters and defined migrants as individuals not assigned to these. In the BAPS non-admixture analysis most individuals had a high probability of belonging to their own genetic cluster with only 14 individuals showing evidence of being a migrant and 7 classified as unknown ancestry (<0.80 probability to any cluster). Admixture analysis produced very similar results to the mixture analysis with only 9 individuals showing significant evidence ($\alpha = 0.05$) of mixed ancestry (Fig. 3.2a).

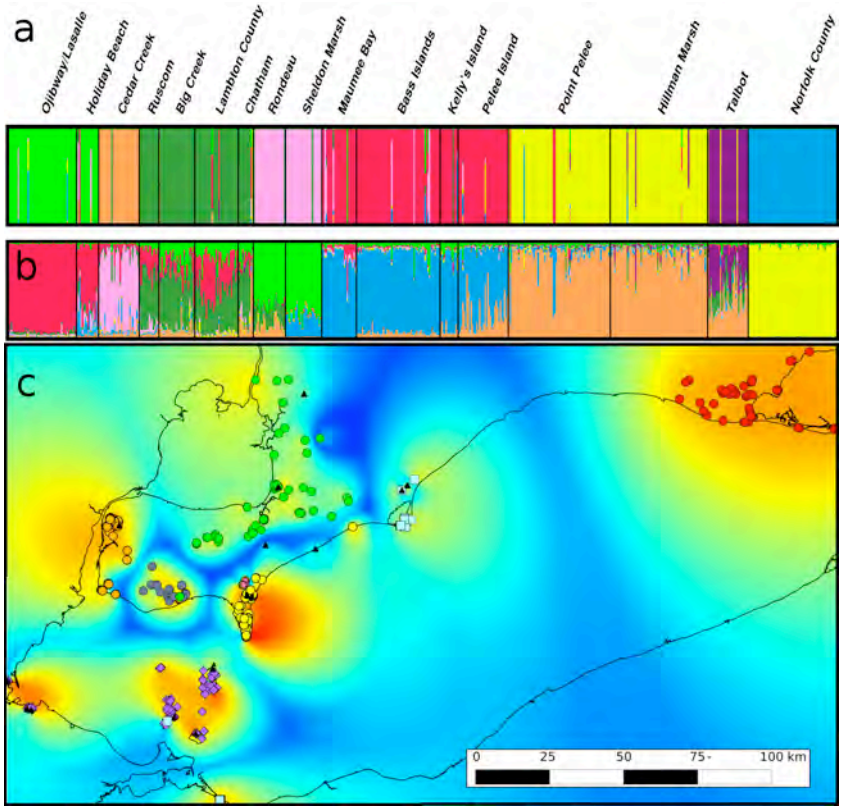

Figure 3.2. Bar plots representing admixture coefficients for eastern foxsnakes from a spatial assignment test performed in a) BAPS 5.1. b) TESS 2.3 (c) The geographical representation of admixture coefficients through spatial kiging with low (cool colours) to high (hot colours) representing mean (TESS and BAPS) admixture proportions. Individuals are classed from the non-admixture analysis in BAPS and TESS with different colour and/or shapes representing different clusters. Black triangles represent individuals where there was a discrepancy between the two programs or neither program assigned the individual to a cluster with > 80% probability. See Fig. 3.1 for spatial reference.

For our TESS analysis the DIC values and the Q-matrix stabilized with the number of clusters also equal to eight. After running 60 replicates with k = 8 we averaged the top 30% (17 clusters) in CLUMPP (Jakobsson & Rosenberg 2007). We chose the top 30% because all of the DIC values were similar up until that point and then increased. The genetic clusters were also similar to the BAPS analysis with the same groupings of populations. There was more uncertainty in the bar plot with 45 individuals classed as having unknown ancestry and 15 identified migrants (7 of those were the same as in the BAPS analysis). When using the admixture analysis, the DIC values for 8 clusters dropped considerably (non-admixture lowest run = 29 431, admixture lowest run = 28 723) suggesting that the admixture model had a better fit. From the 60 replicates, we only imported the top 10 models into CLUMPP because of a large increase in DIC values after the first 10 clusters. The level of admixture suggested by TESS was greater than suggested by BAPS (Fig. 3.2b). Using the non-spatial admixture analysis in STRUCUTRE produced similar results, but only identified 7 genetic clusters (see Appendix 4).

Differentiation between genetic clusters was highly significant ($p < 0.001$) for all pairwise F_{ST} comparisons and ranged from 0.04 to 0.28 (Table 3.3). GR2 (0.09) and GR4 (0.09) had the lowest mean pairwise F_{ST} values and Cedar (0.17) and Norfolk (0.20) populations had the greatest mean F_{ST} values. Genetic diversity within genetic clusters was similar (Table 3.2) with the exception of the Norfolk county cluster, which had lower allelic richness and expected heterozygosity than the other populations.

Spatial kriging

Because there were differences in the level of admixture in the BAPS and TESS analyses, we derived separate kriging surface maps for each and then combined the maps by calculating the mean pixel values. This combined surface map and non-admixture genetic assignments identified a number of boundary regions on the landscape (Fig. 3.2c). All the island populations were grouped together with the mainland population in far northwestern Ohio and southeastern Michigan. The rest of the mainland populations were grouped separately from island populations in Ontario and Ohio, but the differences were not as sharp as with some of the mainland populations (Fig. 3.2c). Seven of the 8 genetic populations were distributed across mainland Ontario with steep differences in admixture proportions between most of the clusters.

Admixture proportions varied significantly among the habitat suitability classes ($F_{4,18439} = 3059$, $p < 0.001$) and Tukey HSD tests revealed that the three highest suitability (marginal, suitable, optimal) classes were significantly higher than the two lowest classes (barrier and unsuitable; Fig. 3.3a). Overlaying the barrier habitat class over the admixture proportions map demonstrated that most regions with low admixture proportions consisted of this barrier habitat (Fig 3.3b).

Table 3.2. Sample size, expected heterozygosity (H_e), mean number of alleles (MNA), allelic richness (AR) and F_{IS} for genetic clusters of eastern foxsnakes (Fig. 3.2) in southwestern Ontario and northwestern Ohio. Standard deviation is given in brackets.

	N	H_e	MNA	AR	F_{IS}
GR1[A]	62	0.60 (0.13)	4.33 (1.61)	4.06 (1.34)	0.02 (0.13)
Cedar	28	0.53 (0.14)	4.00 (0.95)	3.99 (0.95)	0.07 (0.16)
GR2[B]	78	0.63 (0.12)	5.42 (1.62)	4.56 (1.11)	0.12 (0.06)
GR3[C]	47	0.50 (0.17)	4.08 (1.51)	3.73 (1.29)	0.03 (0.17)
GR4[D]	126	0.61 (0.13)	5.33 (1.83)	4.55 (1.40)	0.05 (0.04)
GR5[E]	141	0.53 (0.21)	4.92 (1.73)	4.19 (1.49)	0.02 (0.05)
Talbot	28	0.58 (0.16)	3.83 (1.40)	3.82 (1.40)	-0.01 (0.15)
Norfolk	79	0.31 (0.19)	3.25 (1.29)	2.88 (0.99)	0.11 (0.10)

[A]Ojibway and Holiday Beach populations, [B]Ruscom, Big Creek, Lambton, and Chatham, [C]Rondeau and Sheldon Marsh populations, [D]Maumee Bay, Bass Islands, Kelly's Island and Pelee Island populations [E]Point Pelee and Hillman Marsh populations

Table 3.3. Pairwise F_{ST} values (bottom) and Joust D differentiation values (top) between genetic clusters (see Fig 1 for population distribution and Fig 2 for cluster results) of eastern foxsnakes in southwestern Ontario and northwestern Ohio. All pairwise values were highly significant ($p < 0.001$).

	GR1	Cedar	GR2	GR3	GR4	GR5	Talbot	Norfolk
GR1[A]		0.18	0.03	0.11	0.07	0.08	0.17	0.11
Cedar	0.18		0.17	0.15	0.17	0.18	0.16	0.20
GR2[B]	0.04	0.15		0.06	0.04	0.09	0.11	0.14
GR3[C]	0.10	0.21	0.06		0.05	0.09	0.16	0.09
GR4[D]	0.07	0.15	0.05	0.09		0.04	0.12	0.14
GR5[E]	0.10	0.18	0.10	0.14	0.05		0.08	0.09
Talbot	0.13	0.16	0.09	0.16	0.09	0.08		0.18
Norfolk	0.19	0.36	0.20	0.22	0.20	0.17	0.28	
Mean Fst	0.10	0.17	0.09	0.12	0.09	0.10	0.12	0.20

[A]Ojibway and Holiday Beach populations, [B]Ruscom, Big Creek, Lambton, and Chatham, [C]Rondeau and Sheldon Marsh populations, [D]Maumee Bay, Bass Islands, Kelly's Island and Pelee Island populations [E]Point Pelee and Hillman Marsh populations

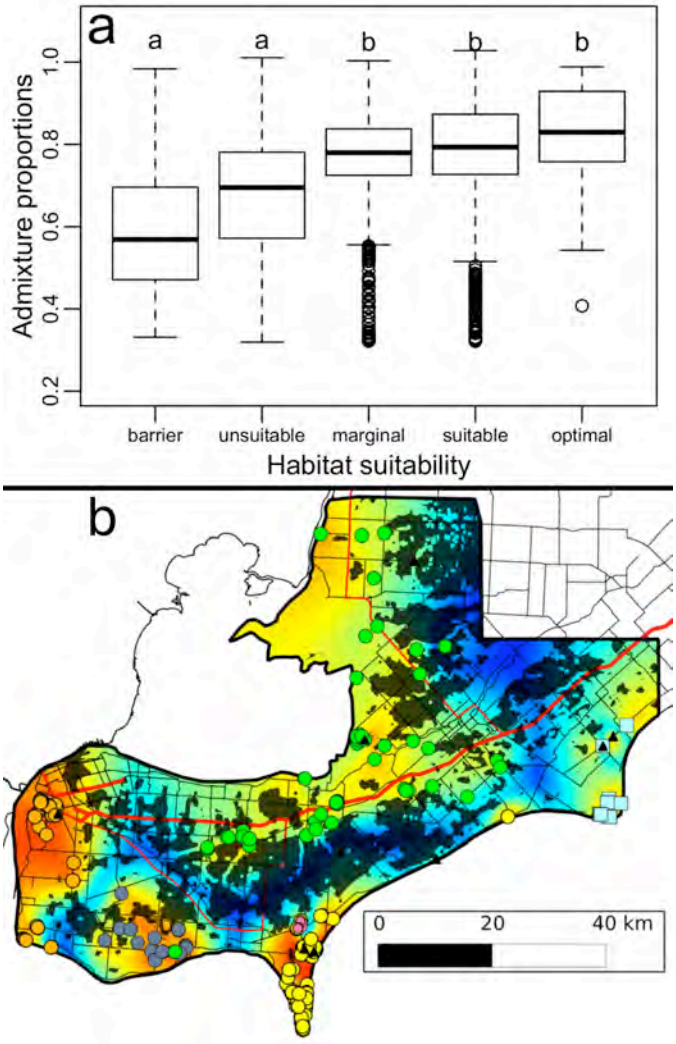

Figure 3.3. a) Box plots of differences in admixture proportions (derived from BAPS and TESS assignment tests and extrapolated onto the landscape using surface interpolation) within habitat suitability classes. Boxes with different symbols are significantly different. (b) Barrier habitat suitability class overlaid on the geographical representation of admixture proportions (see Fig. 3.2 and text for details).

Isolation by resistance and least-cost analysis

When using both Loiselle's kinship coefficient and Rousset's 'a' in the IBR analysis, the trends were similar. All Mantel's tests, including an analysis with an equal landscape (i.e. simple isolation by distance), revealed matrix correlations that were highly significant ($p > 0.001$). Models that used habitat suitability scores (with and without barriers), however, had significantly higher correlations (non-overlapping 95% confidence intervals) than the model only considering an equal landscape (Fig. 3.4a). Using habitat groups (without the barrier class) did not significantly increase the correlation over the correlations simply using classes 1-100 (Cond1 versus Cond2 and Cond4 versus Cond5; Fig. 3.4a). When including the barrier class set as an absolute barrier (zero conductivity for habitat suitability scores ≥ 2) (Cond3 and Cond6; Fig. 3.4a) the Mantel's r was higher than all other models (significantly higher for Loiselle's kinship, but not for Rousset's distance). We found similar correlations between models with urban areas and a 4-lane highway set as absolute barriers and models without these barriers (Fig. 3.4a).

Similar patterns were found with LCP analysis with a few minor differences. When using Loiselle's kinship coefficient, Cost1, Cost2, Cost4 and Cost5 had overlapping confidence intervals with the equal landscape model. As with the IBR models, models with Cost3 and Cost6 had the highest Mantel's r values (Fig. 3.4b). The 95 % bootstrap confidence intervals of these models did not overlap with the equal landscape model and were similar to the IBR values. When using Rousset's 'a' distance

all cost models were greater than the equal landscape and were very similar to the values with the IBR analysis (Fig. 3.4b).

Partial Mantel's tests using the IBR values of the best habitat models (Cond3 and Cond6) and straight-line distance (Condeq model), confirmed the importance of landscape in explaining differentiation patterns between individuals. The correlations between pairwise Rousset's 'a' genetic distance matrices and landscape derived resistance matrices (Cond3 and Cond6), while controlling for straight-line distance (Condeq), were all significantly positively correlated, as expected (Table 3.4). None of the partial Mantel's tests, however, showed a significant positive correlation when comparing Rousset's 'a' to straight-line distance (Condeq), when controlling for landscape derived resistance matrices (Cond3 and Cond6) (Table 3.4). All partial Mantel's tests were significant (negative correlation expected) when using Loiselle's kinship coefficient, but were significantly higher when comparing genetic distance to Cond3 and Cond6 and controlling for straight-line distance (Condeq) (Table 3.4).

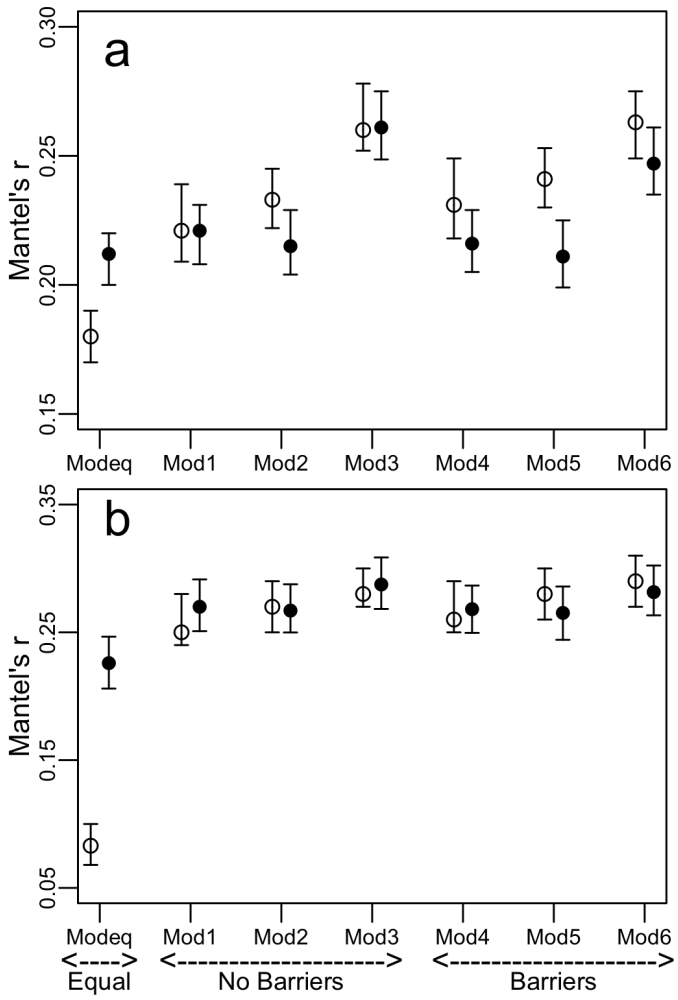

Figure 3.4. Absolute values of Mantel's correlation coefficients (with 95% bootstrap confidence intervals) comparing matrices of pairwise genetic distance for a) Loiselle's kinship coefficient and b) Rousset's 'a' genetic distance. Resistance values were derived from isolation by resistance (open circles) and least-cost (closed circles) models. See table 1 and text for details on models. Modeq and Mod1 through Mod6 are the Cond (IBR data) and Cost (LCP data) models in Table 3.1. See Table 3.1 and text for additional details.

Table 3.4. Results of partial Mantels test comparing matrices of pairwise genetic distance (Rousset's 'a' (Rou 'a'); Loiselle's kinship (Kin)) and resistance values derived from isolation by resistance. Condeq model included an equal landscape (all values =1) and therefore analogous to straight-line distance. See Table 3.1 and text for details on additional models.

Correlation	Controlled	Mantel's r	*P-value > 0
Rou 'a' x Condeq	Cond3	-0.06	0.98
Rou 'a' x Condeq	Cond6	-0.06	0.98
Rou 'a' x Cond3	Condeq	0.28	0.0001
Rou 'a' x Cond6	Condeq	0.29	0.0001
Correlation	Controlled	Mantel's r	†P-value < 0
Kin x Condeq	Cond3	-0.07	0.0001
Kin x Condeq	Cond6	-0.07	0.0001
Kin x Cost 3	Condeq	-0.21	0.0001
Kin x Cost 6	Condeq	-0.20	0.0001

*Rousset's a should increase with increasing distance
†Loiselles kinship coefficient should decrease with distance

Spatial autocorrelation analysis

Using Euclidean distances, the scale of spatial autocorrelation steadily declines and the kinship coefficient drops below zero between 16.2 km and 20.9 km; thereafter the kinship coefficient declines and remains below zero (Fig 3.5a). We therefore considered the scale of spatial genetic structure to be 18.5 km (mid point between last category with relatedness > 0 and first point < 0). When using pairwise resistance from the values derived from the Cond3 model (provided best results in the Mantel's analysis), there was a sharp decline with the kinship coefficient dropping below zero between resistance values of 2.04 and 3.78 (Fig 3.5b) and we thus considered the scale of genetic structure to be 2.91 (mid point between 2.04 and 3.78). We subsequently compared plots with lines connecting individuals greater than 18.5 km apart (Fig. 3.5c) and with lines connecting individuals that had greater than 2.91 ohms between them (Fig. 3.5d) to the assignment results. All genetic clusters were connected when using straight lines distances, but when using resistance values only individuals from the Cedar and Holiday Beach populations and Talbot and Hillman are connected despite being classed in different genetic clusters.

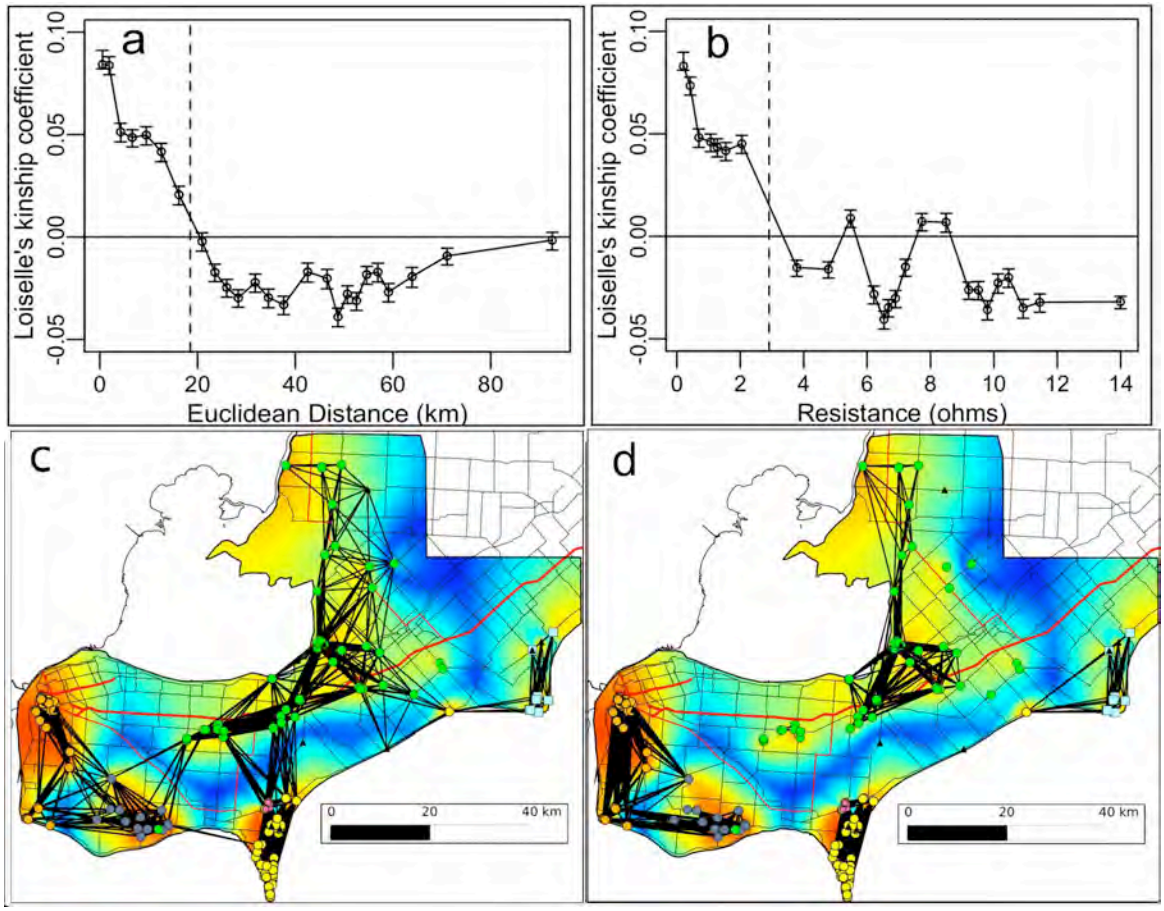

Figure 3.5. Spatial autocorrelation correlograms of Loiselle's kinship coefficient with a) straight-line geographic distance, and b) resistance values from CIRCUITSCAPE using the cond3 model (Table 3.1). Spatial scale of positive autocorrelation was determined as the mid-point distance or resistance between the kinship coefficients above and below zero and is marked with a dotted line. Connecting individuals (black lines) that are < the scale of positive autocorrelation, matches better when using (d) resistance values than (c) geographic distances. See Fig. 3.1 for spatial reference and Fig. 3.2 for assignment results.

Discussion

Role of landscape features on dispersal and population structure

As expected from the preliminary work presented by DiLeo *et al*. (2010), we found striking genetic population structure across fine geographic scales (tens of kilometers) for eastern foxsnakes. Using additional samples (589 samples versus 114 samples) and populations (Lake Erie island populations, Norfolk population) we found an additional 3 genetic clusters not identified in DiLeo *et al*. (2010). Our main goal here was to use the results of habitat suitability modeling to determine if habitat distribution and quality has impacted dispersal patterns, leading to this structure. A pilot study based on 40 eastern foxsnakes from across this region showed that all possessed identical haplotypes for 700 bp of the mtDNA cytochrome *b* region (Row & Lougheed, unpublished data). Therefore deep historical factors (e.g. separate glacial refugia and subsequent contact zones, which are common in this area (Austin *et al*. 2002) were unlikely to confound our analyses aimed at evaluating the effects of habitat distribution on dispersal.

The majority of landscape genetic studies to date have used a series of models based on broad notions of habitat use to determine which habitat types (e.g. forest cover, marsh distribution) or landscape features impact genetic differentiation between individuals or populations (e.g. Lee-Yaw *et al*. 2009; Schwartz *et al*. 2009; Quéméré *et al*. 2010). By combining our genetic results with explicit habitat suitability modeling we objectively established the effects of habitat distribution and quality on population structure and dispersal.

Our Bayesian assignment tests revealed that 7 of 8 genetic clusters in across southwestern Ontario are located where habitat for foxsnakes has been significantly reduced and fragmented. Boundary regions between these clusters were comprised of low suitability habitat demonstrating that low quality habitat is likely restricting gene flow between these clusters. Supporting these results, both IBR and LCP analysis found that matrices of individual genetic distance were significantly more correlated with matrices of resistance values, derived from habitat suitability scores (higher suitability scores = lower resistance), than models with an undifferentiated landscape (i.e. straight-line distance). Further, models with very low suitable habitat set as absolute barriers to any movement had the highest correlation coefficients suggesting that individuals are unwilling or unable to travel through and/or populations are not present in this low quality habitat.

Although, much of the genetic structure across southwestern Ontario could be explained by a lack of suitable habitat, in some cases other factors appear to have played a role. For example, the Talbot population was differentiated from the Point Pelee/Hillman population despite being connected by a significant swath of suitable habitat. These populations, however, are separated by a busy 2-lane highway implying that the highway is a significant impediment to movement. Two road kills found on this road were assigned to the different genetic clusters suggesting without this barrier dispersal would regularly occur between these populations. Other populations were separated by a major 4-lane freeway (Ontario Provincial Highway 401) but were not genetically differentiated. However, underpasses for large creeks and agricultural drains with riparian habitat passing under the highway near these populations likely serve as

conduits for movement of foxsnakes, whereas these are not as prevalent along this smaller highway.

Island biogeography has been cited in the interpretation of genetic population structure for many species (e.g. Kozakiewicz *et al*. 2009; Sebastian *et al*. 2009), including snakes species in this region (King & Lawson 2001). Despite the fact that lake barriers have been in place much longer than current habitat distribution patterns and anthropogenic landscape features, they do not appear to be acting as strong barriers for foxsnakes. We found no differentiation between island populations using assignment tests, but did find some differentiation between island populations and neighbouring mainland populations. King and Lawson (2001) found lower F_{ST} values between populations of garter snakes (*Thamnophis sirtalis*) separated by terrestrial habitats than island populations separated by comparable distances of water. In our study, some of the highest F_{ST} values were populations separated by terrestrial habitats and the island population cluster was one of the least differentiated from other populations (tied with the same mean pairwise F_{ST} values as the largest (by area) mainland cluster) despite not being a central population. Foxsnakes can swim long distances over water (MacKinnon *et al*. 2006) and so this lack of differentiation over water is not surprising, but suggests that individuals are not as willing and/or able to travel across large patches of unsuitable terrestrial habitat and roads as they are willing to traverse open water. On suprising result was the grouping of the Rondeau (southwestern Ontario) and Sheldon Marsh (Ohio) populations into as single genetic cluster. More research would be require to determine if this is the result of natural (e.g. lake currents) or anthropogenic (e.g. translocation) factors.

Over 80% of the terrestrial landscape in our study area has been converted to agriculture and because foxsnakes avoid agricultural fields, low suitability habitat was mainly made up of this landcover type. Habitat fragmentation, conversion and isolation across this region, therefore, seem to have played a major role in restricting gene flow and shaping the mainland population structure for foxsnakes. Habitat fragmentation has been shown to reduce dispersal and affect population structure for a number of terrestrial species (e.g. Cegelski *et al*. 2003), but an increasing number of studies document large effects of fragmentation on population structure and genetic diversity of terrestrial squamates (Berry *et al*. 2005; Jansen *et al*. 2008; Marshall *et al*. 2009; Clark *et al*. 2010; Dubey & Shine 2010). Squamates may be particularly impacted by habitat loss and fragmentation possibly due to their thermoregulatory requirements (Blouin-Demers & Weatherhead 2002; Row & Blouin-Demers 2006). Our results also suggest, however, that habitat corridors may be an effective method for maintaining and improving genetic connectivity for eastern foxsnakes. For example, the strongest barriers appear to be large swaths of very low suitable habitat, and even marginal habitat appears to maintain connections between populations (e.g. Ruscom, Big Creek, Chatham, Lambton populations and Norfolk population; Fig. 3.2), despite extensive habitat fragmentation. Similar studies are required on additional terrestrial squamates to determine if this is specific to foxsnakes or a more common attribute. Foxsnakes are regularly found along riparian habitat and large drainage ditches, which may make them particularly suited to habitat corridors.

Isolation by resistance versus least-cost analysis

Initial tests comparing IBR and LCP analysis demonstrated that IBR produced significantly better results (higher Mantel correlation coefficients) than LCPs for simulations (McRae 2006) and coarse scale (5 km and 50 km resolution) empirical datasets (McRae & Beier 2007). IBR was originally developed for population analysis, but with an individual based dataset. Schwartz *et al*. (2009) found similar results between the two methods, but needed to decrease the resolution of the IBR analysis due to computational constraints of CIRCUITSCAPE. We also found similar results between the two methods with an individual based dataset, both finding a significant result and selecting the same model with the highest Mantel's correlation coefficient. Due to the extensive fragmentation across this region, there may be few possible habitat corridors and thus the scenario we present may not be a rigorous test of these methods as dispersal is forced through a small portion of the total region. Certainly, we second the view of Schwartz *et al*. (2009) that more simulation and empirical studies are required to fully compare these two methods. For conservation purposes IBR has the added benefit of mapping and quantifying all habitat corridors instead of a single least cost path, which can be useful in conservation planning (McRae *et al*. 2008).

Resistance values in spatial autocorrelation analysis

We expected that the scale of spatial genetic structure would closely match genetic populations identified through assignment tests (i.e. individuals separated by greater than the scale of autocorrelation would be grouped in separate genetic clusters). We found significant evidence for spatial genetic structure using both straight-line geographic distances and resistance values derived from CIRCUITSCAPE (Fig. 3.5), but

only when using resistance values did the results match with assignment tests. Individuals separated by < 2.91 ohms (the extent of spatial genetic structure using resistance values) were generally grouped into the same genetic clusters and individuals separated by > 2.91 were from different clusters (Fig. 3.5). This was not the case when mapping individuals separated by 18.5 km (the extent of spatial genetic structure using straight-line distances) (Fig. 3.5) implying that using resistance values may be more biologically realistic. We suggest that more empirical studies compare results of spatial autocorrelation analysis using both straight-line and resistance values. Further, simulation studies on complex landscapes may better establish the relationship between spatial genetic scale and genetic populations identified through assignment tests and determine the benefits of using resistance values when comparing between sexes or groups.

Conclusions

The importance of landscape variables in shaping dispersal patterns and population genetic structure is becoming increasingly clear for a variety of taxa (Cegelski et al. 2003; Berry et al. 2005; Lee-Yaw et al. 2009). Combining well-derived ecological and spatial techniques (e.g. habitat suitability modeling) with detailed surveys of genetic population structure is a promising method to understand how landscape features and habitat distribution impacts population structure, but has not been well utilized in the literature. Eastern foxsnakes (*Mintoinus gloydi*) have persisted to this point across a heavily fragmented region despite being marsh and prairie specialists. Through habitat suitability modeling and genetic analysis we have demonstrated that habitat degradation and fragmentation limit dispersal for foxsnakes, which has had a strong effect on the genetic population structure across this region. Without active efforts to halt habitat

modification, or restore portions of the large swaths of very unsuitable habitat that we identify here as impediments to dispersal, it is likely that isolation among these populations will remain or increase with clear negative consequences for persistence of foxsnakes across this region.

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Literature Cited

Adriaensen F, Chardon JP, Deblust G (2003) The application of 'least-cost' modelling as a functional landscape model. *Landscape and Urban Planning,* **64,** 233–247.

Austin JD, Lougheed SC, Neidrauer L, Chek AA, Boag PT (2002) Cryptic lineages in a small frog: the post-glacial history of the spring peeper, *Pseudacris crucifer* (Anura: Hylidae). *Molecular Phylogenetics and Evolution,* **25,** 316–329.

Balkenhol N, Waits LP, Dezzani R (2009) Statistical approaches in landscape genetics: an evaluation of methods for linking landscape and genetic data. *Ecography,* **32,** 818–830.

Beck N, Peakall R, Heinsohn R (2008) Social constraint and an absence of sex-biased dispersal drive fine-scale genetic structure in white-winged choughs. *Molecular Ecology,* **17,** 4346–4358.

Berry O, Tocher MD, Gleeson DM, Sarre SD (2005) Effect of vegetation matrix on animal dispersal: genetic evidence from a study of endangered skinks. *Conservation Biology,* **19,** 855–864.

Besag J (1975) Statistical analysis of non-lattice data. *The statistician,* **24,** 179–195.

Blouin-Demers G, Gibbs HL (2003) Isolation and characterization of microsatellite loci in the black rat snake (*Elaphe obsoleta*). *Molecular Ecology Notes,* **3,** 98–99.

Blouin-Demers G, Weatherhead PJ (2002) Habitat-specific behavioural thermoregulation by black rat snakes (*Elaphe obsoleta obsoleta*). *Oikos,* **97,** 59–68.

Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *BioTechniques,* **31,** 24–6, 28.

Bowne DR, Bowers MA (2004) Interpatch movements in spatially structured populations: a literature review. *Landscape Ecology,* **19,** 1–20.

Calderon I, Ortega N, Duran S, Becerro M, Pascual M, Turon X (2007) Finding the relevant scale: clonality and genetic structure in a marine invertebrate (*Crambe crambe*, Porifera). *Molecular ecology,* **16,** 1799–1810.

Castric V, Bonney F, Bernatchez L (2001) Landscape structure and hierarchical genetic diversity in the brook charr, *Salvelinus fontinalis*. *Evolution,* **55,** 1016–1028.

Cegelski CC, Waits LP, Anderson NJ (2003) Assessing population structure and gene flow in Montana wolverines (*Gulo gulo*) using assignment-based approaches. *Molecular Ecology,* **12,** 2907–2918.

Chen C, Durand E, Forbes F, François O (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Molecular Ecology Notes,* **7,** 747–756.

Clark R, Brown W, Stechert R, Zamudio K (2010) Roads, interrupted dispersal, and denetic diversity in timber rattlesnakes. *Conservation Biology,* **24,** 1–11.

Corander J, Marttinen P (2006) Bayesian identification of admixture events using multilocus molecular markers. *Molecular Ecology,* **15,** 2833–2843.

Corander J, Sirén J, Arjas E (2008) Bayesian spatial modeling of genetic population structure. *Computational Statistics,* **23,** 111–129.

Crawford NG (2010) SMOGD: software for the measurement of genetic diversity. *Molecular Ecology Resources,* **10,** 556–557.

Cushman SA, Mckelvey KS, Hayden J, Schwartz MK (2006) Gene flow in complex landscapes: testing multiple hypotheses with causal modeling. *The American Naturalist,* **168,** 486–499.

Dieringer D, Schlotterer C (2003) Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes,* **3,** 167–169.

DiLeo M, Row JR, Lougheed SC (2010) Discordant patterns of population structure for two co-distributed snake species across a fragmented Ontario landscape. *Diversity and Distributions,* **16,** 571–581.

Dubey S, Shine R (2010) Restricted dispersal and genetic diversity in populations of an endangered montane lizard (*Eulamprus leuraensis*, Scincidae). *Molecular Ecology,* **19,** 886–897.

Durand E, Jay F, Gaggiotti OE, Francois O (2009) Spatial inference of admixture proportions and secondary contact zones. *Molecular Biology and Evolution,* **26,** 1963–1973.

Epperson BK, Li TQ (1997) Gene dispersal and spatial genetic structure. *Evolution,* **51,** 672–681.

Fahrig L (2007) Non-optimal animal movement in human-altered landscapes. *Functional Ecology,* **21,** 1003–1015.

Frankham R, Ballou JD, Briscoe DA (2002) Population fragmentation. *Introduction to conservation genetics*, Cambridge University Press, Cambridge, UK, pp. 309-334

Frantz A, Cellina S, Krier A, Schley L, Burke T (2009) Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: clusters or isolation by distance? *Journal of Applied Ecology,* **46,** 493–505.

Goslee SC, Urban DL (2007) The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software,* **22,** 1–19.

Greenwood PJ, Harvey PH (1982) The natal and breeding dispersal of birds. *Annual Review of Ecology and Systematics,* **13,** 1–21.

Guillot G, Leblois R, Coulon A, Frantz A (2009) Statistical methods in spatial genetics. *Molecular Ecology,* **18,** 4734–4756.

Guillot G, Mortier F, Estoup A (2005) Geneland: a computer package for landscape genetics. *Molecular Ecology Notes,* **5,** 712–715.

Hardy OJ, Pearcy M, Aron S (2008) Small-scale spatial genetic structure in an ant species with sex-biased dispersal. *Biological Journal of the Linnean Society,* **93,** 465–473.

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes,* **2,** 618–620.

Hirzel AH, Hausser J, Chessel D, Perrin N (2002) Ecolocial-niche factor analysis: how to compute habitat-suitability maps without absence data? *Ecology,* **83,** 2027–2036.

Hirzel AH, Le Lay G, Helfer V, Randin C (2006) Evaluating the ability of habitat suitability models to predict species presences. *Ecological Modelling,* **199,** 142–152.

Hurlbert SH (1971) The nonconcept of species diversity: a critique and alternative parameters. *Ecology,* **52,** 577–586.

Jakobsson M, Rosenberg N (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics,* **23,** 1801–1806.

Jansen KP, Mushinsky HR, Karl SA (2008) Population genetics of the mangrove salt marsh snake, *Nerodia clarkii compressicauda*, in a linear, fragmented habitat. *Conservation Genetics,* **9,** 401–410.

Jost L (2008) G_{ST} and its relatives do not measure differentiation. *Molecular Ecology,* **17,** 4015–4026.

King RB, Lawson R (2001) Patterns of population subdivision and gene flow in three sympatric natricine snakes. *Copeia,* **2001,** 602–614.

Kozakiewicz M, Gortat T, Panagiotopoulou H, Gryczynskasiemiatkowska A, Rutkowski R, Kozakiewicz A, Abramowicz K (2009) The spatial genetic structure of bank vole (*Myodes glareolus*) and yellow-necked mouse (*Apodemus flavicollis*) populations: The effect of distance and habitat barriers. *Animal Biology,* **59,** 169–187.

Lee-Yaw J, Davidson A, McRae B, Green D (2009) Do landscape processes predict phylogeographic patterns in the wood frog? *Molecular Ecology,* **18,** 1863–1874.

Loiselle BA, Sork VL, Nason J, Graham C (1995) Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany,* **82,** 1420–1425.

MacKinnon CA, Lawson A, Stevens ED, Brooks RJ (2006) Body temperature fluctuations in free-ranging eastern foxsnakes (*Elaphe gloydi*) during cold-water swimming. *Canadian Journal of Zoology,* **84,** 9–19.

Manel S, Gaggiotti OE, Waples RS (2005) Assignment methods: matching biological questions with appropriate techniques. *Trends in Ecology & Evolution,* **20,** 136–142.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution,* **18,** 189–197.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Res,* **27,** 209–220.

Marshall J, Kingsbury B, Minchella D (2009) Microsatellite variation, population structure, and bottlenecks in the threatened copperbelly water snake. *Conservation Genetics,* **10,** 465–476.

McRae BH (2006) Isolation by resistance. *Evolution,* **60,** 1551–1561.

McRae BH, Beier P (2007) Circuit theory predicts gene flow in plant and animal populations. *Proceedings of the National Academy of Sciences,* **104,** 19885–19890.

McRae BH, Dickson BG, Keitt TH, Shah VB (2008) Using circuit theory to model connectivity in ecology, evolution, and conservation. *Ecology,* **89,** 2712–2724.

Murphy MA, Evans JS, Cushman SA, Storfer A (2008) Representing genetic variation as continuous surfaces: an approach for identifying spatial dependency in landscape genetic studies. *Ecography,* **31,** 685–697.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics,* **89,** 583–590.

Pierson J, Allendorf F, Saab V, Drapeau P, Schwartz M (2010) Do male and female black-backed woodpeckers respond differently to gaps in habitat? *Evolutionary Applications,* **3,** 263–278.

Piertney SB, Maccoll ADC, Bacon PJ, Dallas JF (1998) Local genetic structure in red grouse (*Lagopus lagopus scoticus*): an analysis using microsatellite DNA. *Molecular Ecology,* **7,** 1645–1654.

Postma E, Van Noordwijk AJ (2005) Gene flow maintains a large genetic difference in clutch size at a small spatial scale. *Nature,* **433,** 65–68.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics,* **155,** 945–959.

Quéméré E, Crouau-Roy B, Rabarivola C, Louis Jr E, Chikhi L (2010) Landscape genetics of an endangered lemur (*Propithecus tattersalli*) within its entire fragmented range. *Molecular Ecology,* **19,** 1606–1621.

R Development Core Team (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Ray N (2005) PATHMATRIX: a geographical information system tool to compute effective distances among samples. *Molecular Ecology Notes,* **5,** 177–180.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of heredity,* **86,** 248–249.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution,* **43,** 223–225.

Ripley BD (1981) *Spatial Statistics*. John Wiley & Sons, New Jersey

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes,* **4,** 137–138.

Rousset F (2000) Genetic differentiation between individuals. *Journal of Evolutionary Biology,* **13,** 58–62.

Row JR, Blouin-Demers G (2006) Thermal quality influences habitat selection at multiple spatial scales in milksnakes. *Ecoscience,* **13,** 443–450.

Row JR, Sun Z, Cliffe C, Lougheed SC (2008) Isolation and characterization of microsatellite loci for eastern foxsnakes (*Elaphe gloydi*). *Molecular Ecology Resources,* **8,** 965–967.

Schwaner TD, Sarre SD (1988) Body size of tiger snakes in southern Australia, with particular reference to *Notechis ater serventyi* (Elapidae) on Chappell Island. *Journal of Herpetology,* **22,** 24–33.

Schwartz MK, Copeland JP, Anderson NJ, Squires JR, Inman RM, Mckelvey KS, Pilgrim KL, Waits LP, Cushman SA (2009) Wolverine gene flow across a narrow climatic niche. *Ecology,* **90,** 3222–3232.

Schweiger O, Frenzel M, Durka W (2004) Spatial genetic structure in a metapopulation of the land snail *Cepaea nemoralis* (Gastropoda: Helicidae). *Molecular Ecology,* **13,** 3645–3655.

Sebastian S, Scott G, Deborah L, Michael R (2009) Progressive colonization and restricted gene flow shape island-dependent population structure in Galápagos marine iguanas (*Amblyrhynchus cristatus*). *BMC Evolutionary Biology,* **9,** 1–18.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science,* **236,** 787–792.

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution,* **47,** 264–279.

Slatkin M, Arter HE (1991) Spatial autocorrelation methods in population genetics. *American Naturalist,* **138,** 499–517.

Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Biology,* **35,** 627–632.

Sokal RR (1979) Testing statistical significance of geographic variation patterns. *Systematic Zoology,* **28,** 227–232.

Tzika AC, Koenig S, Miller R, Garcia G, Remy C, Milinkovitch MC (2008) Population structure of an endemic vulnerable species, the Jamaican boa (*Epicrates subflavus*). *Molecular ecology,* **17,** 533–544.

van Oosterhout C, Hutchhinson WF, Wills DPM, Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes,* **4,** 535–538.

Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology,* **13,** 921–935.

Wang Y, Yang K, Bridgman C, Lin L (2008) Habitat suitability modelling to correlate gene flow with landscape connectivity. *Landscape Ecology,* **23,** 989–1000.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution,* **38,** 1358–1370.

Whitaker JR (1938) Agricultural gradients in southern Ontario. *Economic Geography,* **14,** 109–120.

Wright S (1943) Isolation by distance. *Genetics,* **28,** 114–138.

Wright S (1948) On the roles of directed and random changes in gene frequency in the genetics of populations. *Evolution,* **2,** 279–294.

Zalewski A, Piertney SB, Zalewska H, Lambin X (2009) Landscape barriers reduce gene flow in an invasive carnivore: geographical and local genetic structure of American mink in Scotland. *Molecular Ecology,* **18,** 1601–1615.

Chapter 4: Approximate Bayesian computation reveals the origins of genetic diversity and population structure of foxsnakes

Abstract

Due to difficulty in disentangling the effects of many different processes acting across varying spatial and temporal scales on patterns of variation, there has been a move to embed phylogeography within a more rigorous hypothesis-testing framework. Here we quantify the patterns of genetic diversity and genetic population structure using both mitochondrial DNA (101 cytochrome *b* sequences) and DNA microsatellites (816 individuals, 12 loci) and use Approximate Bayesian computation to test competing models of the demographic history of a North American temperate reptile, the foxsnake (*Patherophis* spp.). We hypothesized that fragmented eastern foxsnake populations represented relicts from the mid-Holocene when populations were larger and more connected due an eastward extension of the prairie peninsula and the warmer temperatures of the Hypsithermal. Supporting our predictions, we found that a model with large populations that underwent large drops in population size and subsequent splitting events had more support than models with small founding populations expanding to stable sizes. Based on timing, the most likely cause of the decline was the cooling of temperatures and infilling of deciduous forest since the Hypisthermal. On a smaller scale, our evidence suggested anthropogenic habitat loss has also caused decline and fragmentation. Regional eastern foxsnake populations, but not western foxsnake populations showed a significant decline in genetic diversity, likely due to larger drops in population size and greater fragmentation. In contrast to our microsatellite results, mitochondrial DNA structure did not show evidence of fragmented populations largely because the majority of foxsnakes had an identical haloptype, perhaps implying a past bottleneck or selective sweep.

Introduction

Quantifying and understanding the mechanisms underpinning geographic variation within species are fundamental to our most basic understanding of evolution and ultimately speciation (Gould & Johnston, 1972). Contemporary patterns of genetic diversity, and the distributions of organisms themselves, reflect both the legacy of historical factors like Pleistocene range fragmentation (e.g. Schoville & Roderick, 2009; Aldenhoven *et al*., 2010; Qu *et al*., 2010) and past demographic events (e.g. population and range expansion, population bottlenecks; Austin *et al*., 2002; Howes *et al*., 2006), and also the influence of more recent factors (e.g. human-caused range fragmentation and isolation; e.g. Dyer *et al*., 2010). Indeed even distinguishing between what constitutes "historical" versus "contemporary" is fraught with difficulty and distinction between the two terms is inconsistent in the literature (Eckert *et al*., 2008). Regardless of the widespread recognition that all of these factors may play important roles in shaping contemporary genetic patterns, it has proven challenging to disentangle their respective contributions (Costello *et al*., 2003; Zellmer & Knowles, 2009). For example, traditional phylogeographic approaches (Avise *et al*., 1987) deduce the relative contributions of past population processes through *post hoc* tests of an association between inferred genealogical patterns and geography (e.g. nested clade analysis; Templeton, 1998). However, because genetic variation results from the interaction of many different factors acting across different spatial and temporal scales, these *post hoc* forms of analysis can lead to spuriously attributing causation to a historical factor (Panchal & Beaumont, 2007).

There has been a recent move to embed phylogeography within a more rigorous hypothesis-testing framework, which allows for both tests of competing models that are

articulated *a priori* and formal tests of certainty (Knowles & Maddison, 2002; Beaumont *et al*., 2010; Knowles & Alvarado-Serrano, 2010). Approximate Bayesian computation (ABC) coupled with coalescent modeling in population genetics (Beaumont *et al*., 2002) is a promising method to accomplish this goal (Bertorelle *et al*., 2010). As with all Bayesian analyses, prior information can be incorporated in the form of prior distributions and competing models can be compared using the marginal densities and by computing Bayes factors (Leuenberger & Wegmann, 2010). These characteristics combined with the ability to test alternate complex and ultimately more realistic demographic scenarios (Bertorelle *et al*., 2010), which are likely the norm for the history of most species, make it an ideal approach for phylogeography. Although the application of ABC analysis to population genetic and phylogeographic questions is quite new (Beaumont *et al*., 2002; Bertorelle *et al*., 2010), it has already proven versatile and has been applied to test alternate demographic (Ray *et al*., 2010) and evolutionary models (Fagundes *et al*., 2007), and also to estimate population parameters such as splitting times, amount of gene flow and effective population sizes (e.g. Estoup & Clegg, 2003; Wegmann & Excoffier, 2010).

Here we use an ABC approach to testing competing models of the demographic history of a North American temperate reptile, the foxsnake (*Pantherophis* spp.). The current northern range of foxsnakes is unusual among terrestrial squamates, as it would have been almost completely covered by ice sheets during the maximum extent of glaciation during the Pleistocene (~70 000 years ago). A relatively large, contemporary geographic range disjunction (see: Conant & Collins, 1991; Fig. 4.1) has caused some speculation over its cause (Morse, 1902; Schmidt, 1938), as well as having taxonomic implications (Conant, 1940; Collins, 1991). Populations on the eastern and western side

of the disjunction are currently recognized as different species, the eastern foxsnake (*Pantherophis gloydi*) and the western foxsnake (*P. vulpinus*), respectively. Foxsnakes are marsh and prairie specialists (Row *et al*., 2010) and it has been suggested that, along with other species with similar habitat preferences, the eastern portion of their range resulted from an expansion of the prairie peninsula (Transeau, 1935) following an eastward postglacial steppe (Schmidt, 1938), which has similar characteristics as a prairie. The proposed maximum extent of the post-glacial steppe (~5000-7000 years ago; Webb, 1981) was during the Hypsithermal Period when temperatures were at a maximum during the Holocene. Evidence to support distributional shifts facilitated by the prairie steppe and warmer temperatures include pollen profiles (King, 1981; Webb, 1981), species distribution patterns (Schmidt, 1938; Smith, 1957), and the existence of snake fossils of other species found at locations north of their current range (Churcher & Karrow, 2008). If the postglacial steppe was responsible for the current eastern extension of their range, the return of deciduous forest combined with cooler temperatures could have subsequently caused local extinctions producing the large disjunction.

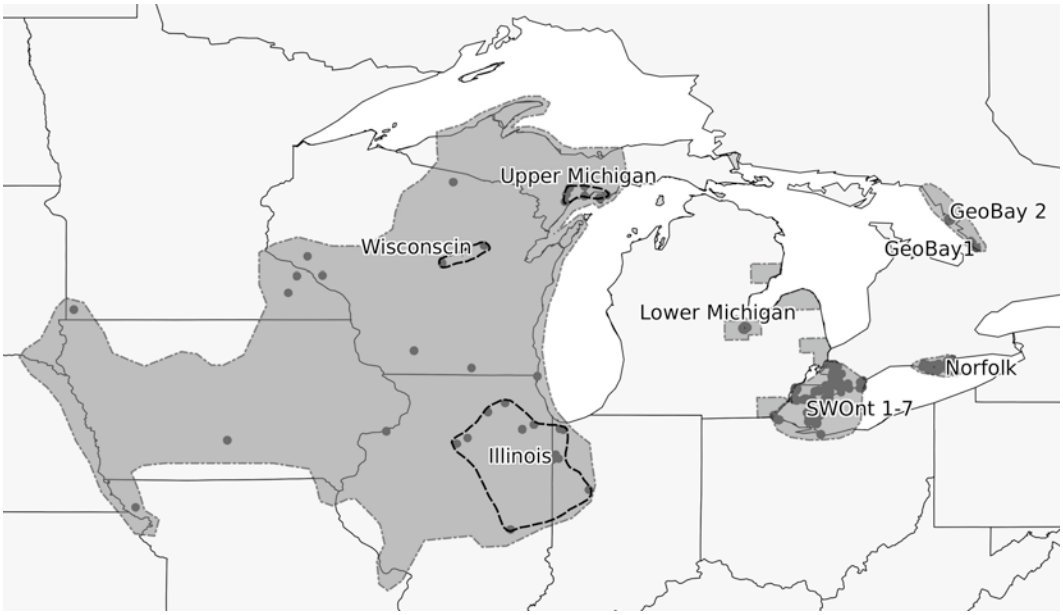

Figure 4.1. Current approximate range of foxsnakes (dark grey) based on Ernst and Barbour (1989) and occurrence records from Michigan and Ontario. Grey dots represent locations of one or more samples used in the analyses. Dashed lines circumscribe western foxsnake locations that were pooled for genetic diversity and differentiation analysis.

Within the range of eastern foxsnakes there are further geographic disjunctions (< 250 km) that possibly pre-dated major European settlement, and also may be due to the aforementioned infilling of deciduous forest into southwestern Ontario. Most of the current range of eastern foxsnakes, however, lies along the shorelines of the Great Lakes (Fig. 4.1). Thus, it is also plausible that the extensive habitat fragmentation due to urban and agricultural development has caused or accentuated these gaps in distribution and caused decline in local populations (Row *et al*., 2010). Western foxsnakes have undoubtedly experienced habitat loss as well, however, this does not appear to have had as great an effect as western foxsnakes are listed as common throughout most of their range, potentially related to differences in the amount of habitat loss, level of fragementation or differences in habitat preferences. This greater degree of isolation and fragmentation of populations presumably would have resulted in greater population structure and reduced genetic diversity in eastern foxsnakes compared to western foxsnakes.

Here, we hypothesize that the fragmented regional eastern foxsnake populations represent relicts from the mid-Holocene when populations were larger and more connected due to the post-glacial steppe and the warmer temperatures of the Hypsithermal. In contrast, it is also possible that these populations were founded through dispersal events, also during the favourable conditions of the mid-Holocene. To test these hypotheses, we first quantify the patterns of genetic diversity and genetic population structure of foxsnakes using both mitochondrial and microsatellite DNA markers. We subsequently use ABC analysis to compare competing population demographic models

that are consistent with these two hypotheses: 1) large populations, which have undergone drops in population size and splitting events, and 2) small founding populations that have split from large populations and subsequently expanded to become stable. We predict that due to the improbability of long distance dispersal events of snakes, models consistent with Hypothesis 1 will have greater support. Because it is also possible for European settlement to have caused population declines and splitting events, we include this possibility in the priors of our demographic models for Hypothesis 1 and determine the more likely scenario by comparing parameter values from our Bayesian analyses. Throughout the ABC analysis, we take a hierarchical approach, first focusing on single regional populations, then building to ultimately include models encompassing the entire foxsnake range.

Methods

Genetic Sampling

Over seasons when foxsnakes were active for years 2006 – 2009, we assembled 833 (70 western foxsnakes; 746 eastern foxsnakes) samples across the range of each taxon (Fig. 4.1, Appendix 3). Samples collected by us were small blood samples (~200 ml stored in 95% ethanol) taken from the caudal vein of hand-captured individuals or from tissue samples collected from road kills. Samples were also acquired from researchers working in other regions. We extracted DNA from blood and tissue using QIAGEN (Venlo, Netherlands) DNeasy blood and tissue kits following the manufacturer's protocols.

Mitochondrial Sequencing

Crother *et al*. (unpublished manuscript) amplified and sequenced mitochondrial DNA (mtDNA) from a subset of individuals for a 1154 bp segment of the cytochrome *b* region using H16064 and L14910 primers (Burbrink *et al*., 2000), and used these sequences to design a set of species-specific primers (Cytb-F & Cytb-R). Using these primers we amplified a 700 bp segment from 43 western and 58 eastern foxsnakes and combined these with 11 western foxsnake sequences amplified by Crother *et al* (unpublished manuscript). Polymerase chain reaction (PCR) reaction cocktails consisted of 10 ng of genomic DNA, 1X Taq buffer with $(NH_4)_2SO_4$ (Fermentas), 0.5 μM forward and reverse primer, 0.1 mM of each nucleotide, 0.25 U of DNA polymerase Taq (Fermentas) and 2.5 mM of $MgCl_2$. PCRs were done in a GeneAmp 9700 or 2700 (Applied Biosystems) using the cycling profile: 7 min denaturation at 94°C; 35 cycles of 45s at 94°C, 60s at 50°C and 90s at 72°C; and a final extension of 72°C for 7 min. DNA sequences were aligned and edited using ClustalX 2.0 (Larkin *et al*., 2007) and Seaview 4.2 (Galtier *et al*., 1996).

Microsatellite Genotyping

All samples were genotyped for the 11 microsatellite loci (FS24, FS50, FS33, FS52, FS67, FS82, FS77, FS63, FS09B, FS42B, FSV16B) developed specifically for this species (Row *et al*., 2008) and one additional locus (EOB10) developed for eastern ratsnakes (*Pantherophis obsoleta*) (Blouin-Demers & Gibbs, 2003) following the methods outlined in Row *et al*. (2008) and DiLeo *et al*. (2010). Neither deviations from Hardy-Weinberg Equilibrium (HWE) nor linkage disequilibrium (Row *et al*., 2008) were evident, nor were null alleles prevalent (DiLeo *et al*., 2010; Row *et al*., 2010).

Mitochondrial Structure and Diversity

The glacial periods of the Pleistocene have had a large impact on the genetic structure of temperate North American herpetofauna (e.g. Austin *et al*., 2002; Zamudio & Savage, 2003; Howes *et al*., 2006; Placyk Jr *et al*., 2007). Phylogenetic analyses often revealed deep phylogenetic splits between mitochondrial clades within contemporary species' ranges, likely resulting from initial divergence in allopatric glacial refugia followed by post-glacial expansion into secondary contact (Austin *et al*., 2002; Gibbs *et al*., 2006). We estimated an mtDNA genealogy in eastern and western foxsnakes by first identifying unique haplotypes and estimating phylogenetic relationships among them using MRBAYES 3.1.2 (Ronquist & Huelsenbeck, 2003), with the corn snake (*Pantherophis guttatus*) (Genbank accession # DQ902111) as the outgroup. We first determined the most appropriate model of evolution (HKY model) using MRMODELTEST 2.0 (Nylander, 2004) and the Akaike information criterion (AIC). We ran two independent runs of 1.0 x10^6 Metropolis-coupled MCMC iterations (until the standard deviation of the split frequencies was < 0.01) with 4 incrementally heated Markov chains specifying the HKY model, but with parameters estimated as part of the Bayesian analysis. The run was sampled every 100 iterations and we discarded the first 2500 of these (250000 generations total) as burnin. We confirmed convergence by, 1) examining a plot of the log probability versus generation to ensure stationarity, and 2) ensuring the Potential Scale Reduction Factors (Gelman & Rubin, 1992) were all close to one. We calculated the number of variable sites, parsimony informative sites and sequence divergence between clades using MEGA 4.0 (Tamura *et al*., 2007).

Microsatellite Structure and Diversity

We examined more recent patterns of genetic population structure and diversity using microsatellites, which typically have a higher mutation rate than mtDNA, are often hypervariable, and have been shown to be excellent for resolving structure at fine temporal and spatial scales (Sunnucks, 2000).

Genetic population structure

We first quantified genetic population structure using assignment tests, which identify the number of genetic clusters in a given dataset and probabilistically assign individuals to their population of origin based on Hardy Weinberg and linkage equilibrium (Manel *et al*., 2005). The number of genotyped samples for eastern foxsnakes heavily exceeded our western foxsnake samples. To minimize the impact of this difference in sampling intensity, we sub-sampled our eastern foxsnakes samples and included only 10 random samples per geographic population when we had large sample numbers. This sub-sampling lead to dataset comprised of 134 eastern foxsnake and 70 western foxsnake samples, which we used in a non-spatial admixture analysis in STRUCTURE 2.3.3 (Pritchard *et al*., 2000). We ran 200,000 (100,000 burn-in) MCMC iterations 100 times for each of k=1 to k=10 using correlated allele frequencies and default parameters. The top 10 models for each k were averaged in CLUMPP 1.2 (Jakobsson & Rosenberg, 2007) and displayed using DISTRUCT 1.1 (Rosenberg, 2004).

We also summarized genetic structure using a principal component analysis (PCA) on the microsatellite genotypes, because this method makes no assumptions (e.g. Hardy Weinberg, linkage equilibrium) of the data set (Reviewed in: Jombart *et al*., 2008).

The analysis was conducted with the *adegenet* package (Jombart, 2008) in R (R Development Core Team, 2009).

Genetic Differentiation

We determined the distribution of genetic variation within regional populations using a hierarchical analysis of molecular variance (AMOVA) (Michalakis & Excoffier, 1996) in Arlequin 3.5 (Excoffier & Lischer, 2010). Significance was determined with 9999 permutations. In the analysis we included 5 regional populations and 14 local populations: western (Illinois; Wisconsin; Upper Michigan), Lower Michigan, southwestern Ontario (SWont1 – SWont7), Norfolk and Georgian Bay (Geo Bay 1 & Geo Bay 2) (Fig. 4.1; Appendix 3). For western foxsnakes, population groupings for both the genetic differentiation and diversity analysis were made based on geographic locations where we had clusters of samples. Eastern foxsnake populations in southwestern Ontario were based on previous spatial assignment tests (Row *et al*., 2010) or defined as geographic clusters of individuals for those samples outside of the southwestern Ontario regional population. Pairwise F_{ST} (Weir & Cockerham, 1984) and JOUST D differentiation (Jost, 2008) values between local populations were also calculated.

Diversity

We summarized patterns of genetic diversity within the populations identified in the preceding section by calculating expected heterozygosity (H_e - corrected for sample size; Nei, 1978), mean number of alleles, mean $F_{IS,}$ and standardized allelic richness (Hurlbert, 1971) using Microsatellite analyzer 4.05 (Dieringer & Schlotterer, 2003). We determined if there were significant differences between populations using a rank-based Freidman test (unreplicated block design) and Wilcoxon-Nemenyi-McDonald-Thompson

post hoc test (Zar, 1996; Hollander & Wolfe, 1999) with sequential Bonferroni correction (Rice, 1989).

Demographic modeling with Approximate Bayesian computation

Briefly, for ABC analysis, genetic datasets are generated from coalescent simulations using population parameters, drawn from a prior distribution, under a specified model. For each simulation, summary statistics (e.g. allelic range, number of alleles, F_{st}) are calculated and the Euclidean distance (using the multivariate space of the summary statistics) between the generated and actual summary statistics is calculated. Models can be compared and parameters estimated by retaining a proportion, N, of the simulations with the lowest Euclidean distance (e.g. Ray *et al*., 2010) or the simulations that are below (in Euclidean distance) a set threshold (e.g. Fagundes *et al*., 2007). We used ABCtoolbox (Wegmann *et al*., 2010), which has 4 programs: SIMCOAL 2.0 (Laval & Excoffier, 2004), arlsumstat (Excoffier & Lischer, 2010), ABCsampler and ABCestimator. Together these programs: 1) generate coalescent simulations, 2) calculate summary statistics, 3) calculate Euclidean distances and retain the generated simulations with the lowest distance in multivariate space to the actual dataset, and 4) perform a post sampling regression adjustment and estimate the posterior distribution. When included as a summary statistic, we used a modified python script of the program SMOGD 1.2.5 (Crawford, 2010) to calculate pairwise Joust D. Details on the model choice and parameter estimation are provided below.

For the simulations, we set the number of loci and sample sizes to those of the actual dataset and microsatellite diversity was generated under a strict stepwise mutational model (SMM). Because two microsatellite loci (EOB10 and FS09) had large

gaps in repeat number implying that they may not follow a SMM they were excluded from the ABC analysis. A maximum of 50 individuals were chosen from any given population to reduce computing time. Although ABCtoolbox allows one to incorporate different types of genetic markers, we excluded mtDNA sequence data due to the low variation. Unless stated otherwise, 5×10^5 simulations were run for each model and the 5000 simulations with the lowest Euclidean distance were retained for model testing and parameter estimation.

Because large-scale demographic models that include all populations would have large numbers of parameters so as to make calculations too computationally intensive, we used a hierarchical approach. We first modeled regional populations separately, to allow us to more confidently fix or narrow the range of priors for parameters in the range wide models. Model descriptions and model parameters are described in turn below. For the Georgian Bay region we had samples from 2 locations separated by ~50 km. To simplify the models, for all ABC analyses, we only used samples from the more southerly population (Geo Bay 1 - Fig. 4.1; Appendix 3), where we had a larger sample size.

Model Choice

Following the selection of the datasets with the lowest Euclidean distances to the actual summary statistics, we estimated the fit and compared competing historical-demographic models using three different methods. First, we used ABCtoolbox to calculate the distribution of marginal densities of the retained simulations and output a P value as the proportion of the retained simulations with lower marginal densities (i.e. low P value indicates an inability of the model to produce the observed summary statistics; Wegmann *et al*., 2010). Second, we calculated the Bayes factor (marginal density of

model A / marginal density of model B) as the probability of one model versus another (Wegmann *et al*., 2009; Wegmann *et al*., 2010). Third, following Pritchard *et al*. (1999), we combined the 5000 simulations with the lowest Euclidean distances for each model (15 000 total) and then estimated the relative probability of each model as the proportion of simulations that were included the top 1000 models (of the 15 000) with the lowest overall Euclidean distances.

Parameter estimation

To estimate population parameters we applied a General Linear Model (ABC-GLM) post sampling regression adjustment to the 5000 retained simulations (Leuenberger & Wegmann, 2010), as implemented in ABC estimator. The regression adjustment assumes a linear model within a narrowed prior based on the retained simulations, and calculates the density at 100 evenly spaced points along the parameter values, to generate the posterior distribution. We report the mode and 90% highest posterior density (HPD) interval as an estimate of that population parameter. The potential of the parameter to be correctly estimated by the summary statistics was summarized by calculating the coefficient of determination, R^2, of a multiple regression of the parameter against all summary statistics, using all of the simulated datasets (Neuenschwander *et al*., 2008; Ray *et al*., 2010). Neuenschwander *et al*. (2008) suggested parameters with an R^2 of less than 10% are unreliable, because the summary statistics explain little of their variability.

Population-scale analysis

For each of the three eastern foxsnake regional populations (Lower Michigan, Georgian Bay, Norfolk County) and the Illinois population of western foxsnakes, we compared three demographic models: 1) *Drop* – a large population underwent a

instantaneous diminution in size to its current population size, *2) Decline* – a large population underwent an exponential decline to its current population size, and 3) *Stable* – a small founding population expanded to a present-day stable configuration (Fig. 4.2a). We refined prior distributions by testing models with different prior distributions and comparing the marginal density between models (Table A4.1; Appendix 5). In the *Drop* and *Decline* population models, prior distributions on the timing of the drop or decline were wide enough to allow the reduction in population size to be a result of reforestation of northern Ohio and southern Ontario and cooler temperatures after the Hypsithermal (~2000-8000 years before present) or to have resulted from human habitat loss and fragmentation (10-150 years before present). We determine the more likely scenario by comparing the estimated parameter values from the selected model. In the *Stable* models, priors on the founding event included the Hypsithermal, when increased temperatures and the postglacial steppe conditions would have been optimal for foxsnakes. Separate priors were used for eastern and western foxsnake populations because of different expectations (e.g. expect southwestern populations to have been established further in the past as predicted by a south to north postglacial colonization history) and marginal densities during testing. For this smaller scale analysis we used the mean and standard deviation (calculated over loci) for four summary statistics: number of alleles, heterozygosity, modified Garza-Williamson index (Garza & Williamson, 2001; Excoffier *et al*., 2005) and allelic range, thus a total of eight statistics for model comparison.

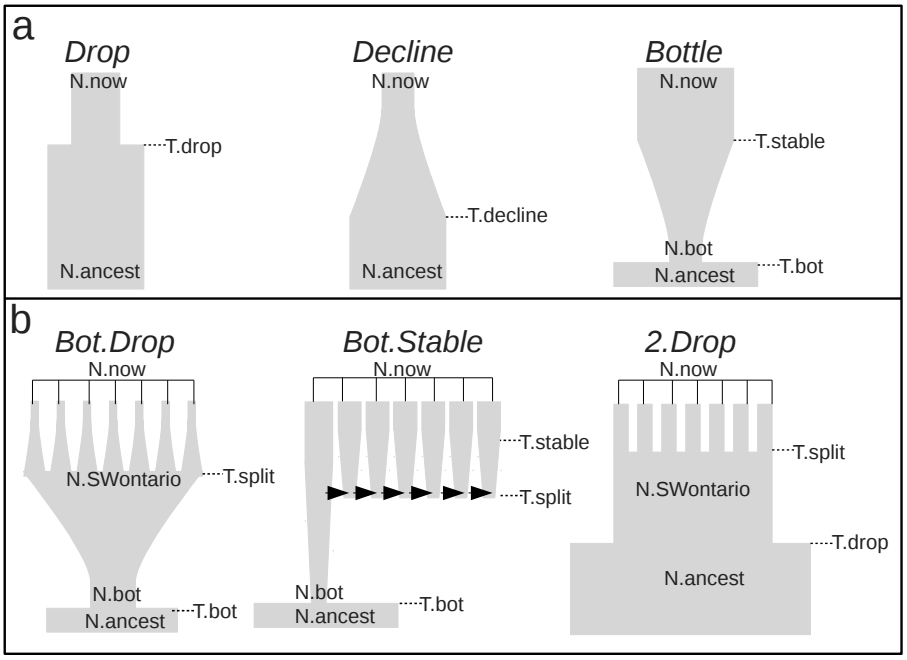

Figure 4.2. Population demographic models used in Approximate Bayesian computation analysis for a) single populations (Illinois, Georgian Bay 1, Lower Michigan, Norfolk; Fig. 4.1), and b) southwestern Ontario where a number of genetic clusters have been identified (Row *et al*., 2010). Additional details of models and parameters (T.Drop = time of population drop, T.Decline = time of exponential decline, T.stable = time since population has become stable, T.split = time of population split, N.Now = current population size, N.SWontario = size of combined population in southwestern Ontario, N.Ancest = ancestral population size, N.bot = size of population bottleneck) can be found in the text.

Because southwestern Ontario is comprised of seven genetic clusters identified previously through spatial assignment tests (Row *et al*., 2010), we tested more complex models representing alternative possible demographic histories (Fig. 4.2b, Table A4.2; Appendix 5): 1) *Bot.Drop* – a small population founded southwestern Ontario, expanded to a large population and subsequently split into 7 populations, which all underwent exponential decline into their current population sizes, 2) *Bot.Stable* – the 7 populations were sequentially colonized from a small population and exponentially expanded into stable populations, and 3) *2.Drop* – a large population dropped to a smaller population, (i.e. split from the other regional populations) and then the numbers dropped again and split into the current 7 populations. For each population we used the 8 summary statistics listed above, but added pairwise Joust's D (Jost, 2008) as a metric of differentiation between populations. With the inclusion of 7 populations and Joust's D the total number of summary statistics is large (77 statistics), which can lead to statistical noise and make posterior parameter estimation difficult (Joyce & Marjoram, 2008). Following the methods of Wegmann *et al*. (2009) and using an R (R Development Core Team, 2009) script provided with ABCtoolbox, we therefore reduced the statistical summary space using a Partial Least Squares (PLS) approach to include uncorrelated orthogonal components that explain the largest amount of variation in the parameter set. The number of PLS components to include was chosen by visually determining when additional components did not reduce the root mean square error of the parameters.

Range-Wide Scale

At the range-wide scale, the Wisconsin and Upper Michigan populations were combined because they had low sample sizes and the STRUCTURE analysis suggested they belonged to the same genetic cluster. At this scale we tested three models that included 12 populations (Illinois, Wisconscin Upper Michigan, southwestern Ontario 1-7, Lower Mich, Norfolk, Georgian Bay 1). In this large-scale model, we did not attempt to model either individual population sizes for the southwestern Ontario populations or the splitting time. Instead population sizes were set to be gamma distributed as Gamma (8,8/X), where 'X' is the average of southwestern Ontario population sizes derived from our earlier analyses. Using the gamma distribution with the prior distribution of the mean (400-2000), the population sizes and population size variation observed in the small-scale southwestern Ontario population models, were possible. The merging of the southwestern Ontario was set to 10-80 generations, which was not constrained to match the values found in the southwestern Ontario population model, but rather allowed recent coalescent events to occur within each population (Ray *et al*., 2010).

At this scale we tested three different models that we think best reflect possible historical demographic scenarios, based on our current knowledge of regional post-Pleistocene events and the species' ecology: 1) *Bot.Decline* – after a population bottleneck foxsnakes expanded exponentially to a large population representing their current range. Consistent with the forest infill hypothesis, populations then began to drop and fragment (Fig. 4.3a) 2) *Colonize* - after a population bottleneck, foxsnake populations colonized their current range through sequential founder populations and subsequent population expansions (Fig. 4.3b), and 3) *Decline* – a variant of the *Bot.Decline* model

where there is no initial bottleneck for foxsnake populations (Fig. 4.3c) (Table A4.3; Appendix 5). Summary statistics were the same as for the southwestern Ontario population models. Because we were not attempting to estimate divergence times of the southwestern Ontario populations in this model, we combined the local southwestern Ontario populations into one regional population before calculating the pairwise Joust D differentiation. For these models we have ignored gene flow, a simplification that we discuss later.

Results

Mitochondrial Structure and Diversity

Of the 113 cytochrome *b* mtDNA sequences within the ingroup, there were only 11 unique haplotypes and 18 variable sites, 10 of which were parsimony informative. The majority of individuals (73%), including all but one eastern foxsnake, had one haplotype. The Bayesian analysis suggested two genetic lineages and a weakly supported polytomy in the eastern clade (Fig. 4.4). Based on the distribution of haplotypes there is some suggestion of an eastern and western split of the 2 major lineages, but sample sizes from the western portion of the range are too low to make a definitive statement. Raw sequence divergences were 1.5% between the western and eastern clades (Fig. 4.4).

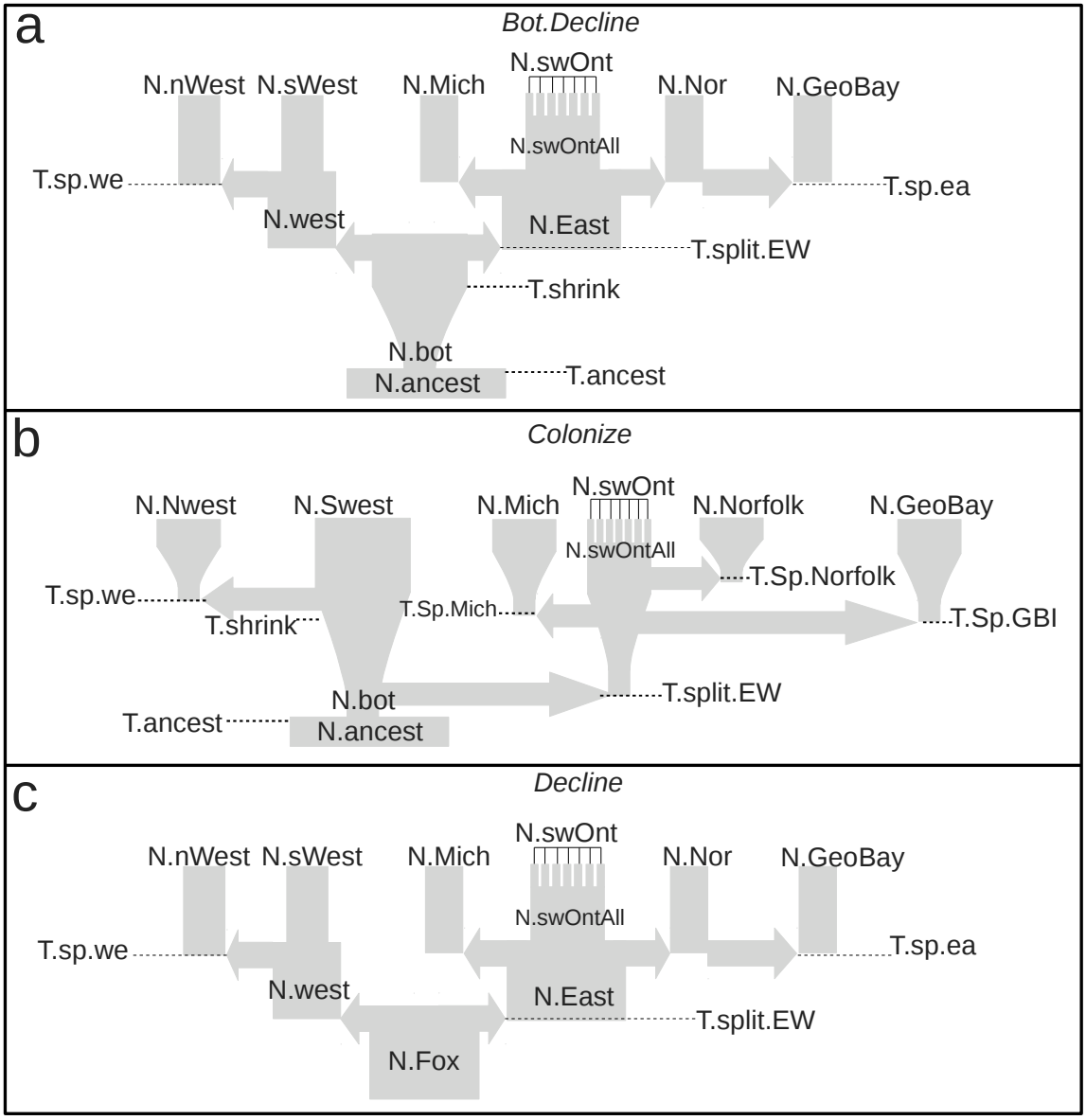

Figure 4.3. Three possible colonization models of foxsnakes into their current range and used in the Approximate Bayesian computation analysis. Additional details of models and parameters can be found in Table 4.6 and in the text.

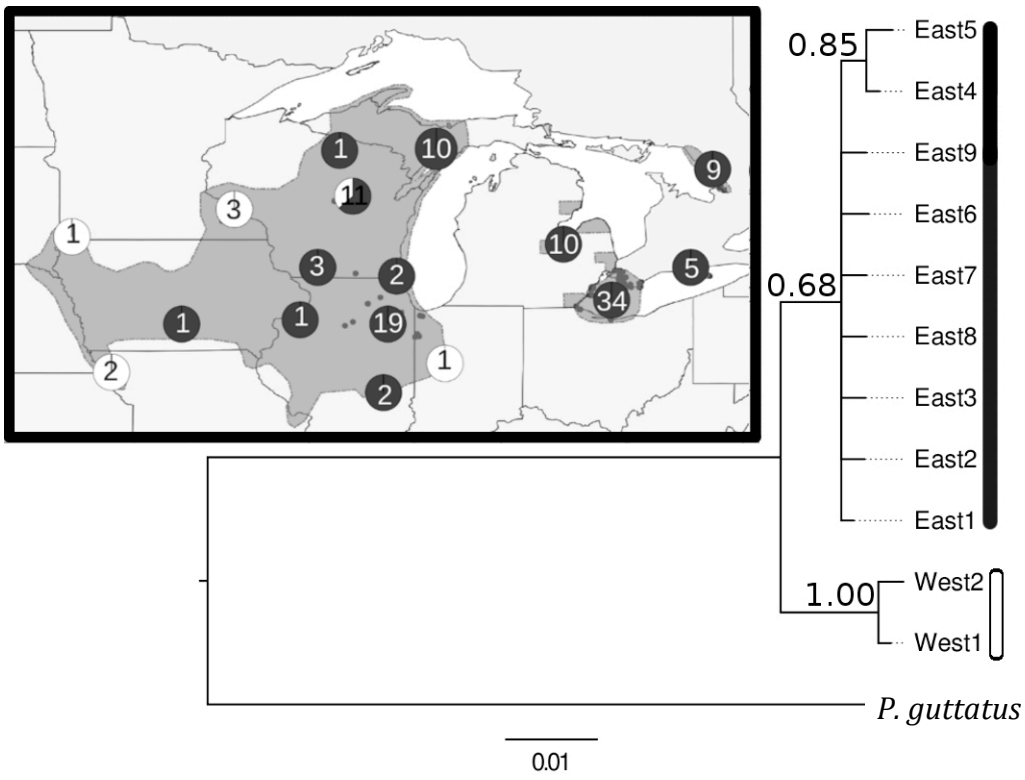

Figure 4.4. Bayesian phylogram from analysis of 11 unique mtDNA haplotypes (using HKY mutational model) of 710 bp of cytochrome *b* from across the range of eastern and western foxsnakes (n= 113 foxsnakes). Bayesian posterior probabilities are shown for all resolved nodes. The geographic distribution of the two major clades is shown on the map with pie charts summarizing the proportions of western and eastern haplotypes at each locale. Sample sizes are listed inside the pie chart.

Microsatellite Structure and Diversity

Population Structure

The log probability of data from STRUCTURE reached a plateau around k = 6 to k = 8, suggesting that the most likely number of clusters was within that range. The first major identified cluster (k = 2) was defined by a clear split between eastern and western foxsnakes (Fig. 4.5). This division remained for all values of k and suggested very little admixture between western foxsnakes and any of the eastern foxsnake populations. Overall there was clearly more genetic structure within eastern foxsnakes, with both the Georgian Bay (at k = 3) and Lower Michigan regional populations (at k = 4) separating from the other populations with little suggested admixture. The remaining clusters (defined at k=5 to k=8) were less clear with some admixture between the Norfolk regional population and the other southwestern Ontario populations. Using spatial clustering and the full southwestern Ontario dataset Row *et al*. (2010), the Norfolk population was clearly separated from the southwestern Ontario populations. The appearance of additional clusters within southwestern Ontario and within western foxsnakes was present when k was set to the highest values.

The first three components of the PCA only explained a total of 31% (axis 1 = 14%, axis 2 = 11%, axis 3 = 6%) of the total variation, but clearly separated the 3 regional populations of eastern foxsnakes from the western foxsnakes (Fig. 4.6). Similar to the STRUCTURE analysis, the first axis, which explained the highest percentage of the variation, separated western foxsnakes from the eastern foxsnake populations. On the second axis, the Georgian Bay population forms a distinct cluster from all others (Fig. 4.6).

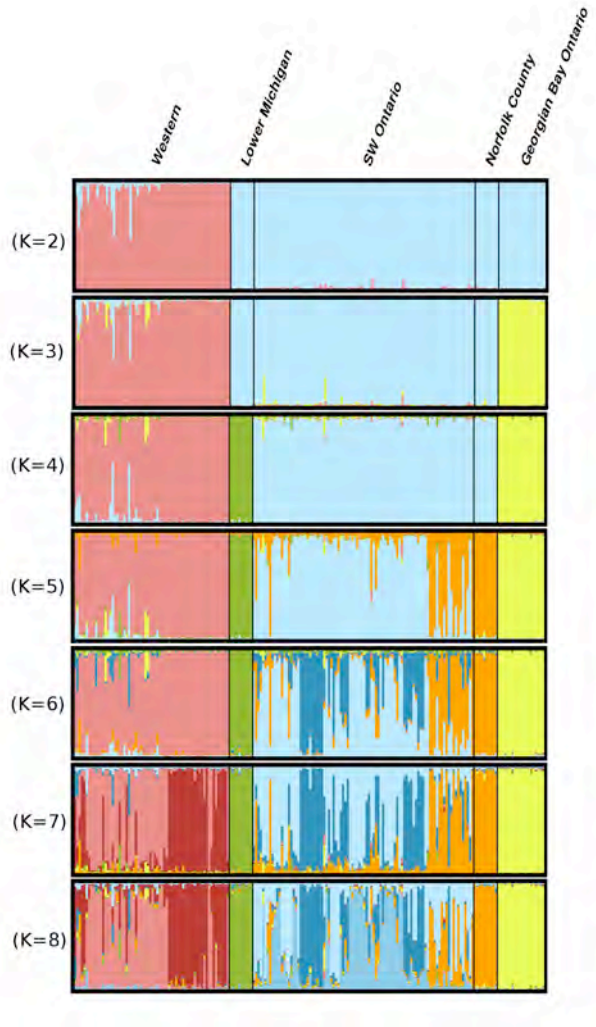

Figure 4.5. Bar plots representing admixture coefficients for eastern and western foxsnakes from assignment test analyses performed in STRUCTURE 2.3.3. The top 10 runs (highest log probability of data) from 100 replicates were averaged in CLUMPP 1.2 and displayed with DISTRUCT 1.1 for each of k = 2 through k = 8. See Fig. 4.1 and text for description of populations.

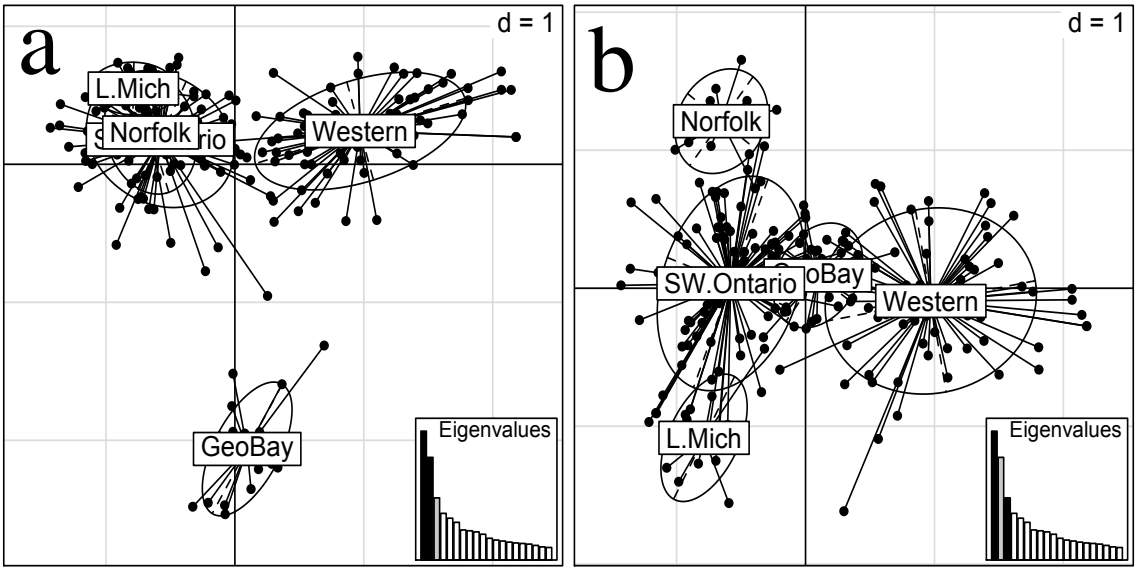

Figure 4.6. Biplots of individual genotypes for a) PCA axis 1 (x-axis) versus PCA axis 2 (y-axis) and, b) PCA axis 1 (x-axis) versus PCA axis 3 (y-axis). Shown are 95% inertia ellipses of populations represented by black ovals for with black dots representing genotypes and black lines extending to the centroids of the respective populations (see text and Fig. 4.1 for distribution of samples). Inset shows bar chart of the eigenvalues with corresponding components in black. Grid distance (d) corresponds to a value of 1.

Genetic Differentiation

The AMOVA revealed that significant amounts of the genetic variation were partitioned among regions (Sum of Squares (SS) = 1129, Percentage of Variation (POV) =26.35, $p < 0.001$), among populations within regions (SS = 357, POV = 8.03, $p < 0.001$) and within populations (SS = 4372, POV = 65.70, $p < 0.001$). Pairwise F_{st} values ranged from 0.07 to 0.10, among western foxsnake populations, and from 0.05 to 0.60 among eastern foxsnake populations (Table 4.1). Between eastern and western populations F_{st} values were between 0.10 and 0.61 (Table 4.1). The Illinois population was most similar to eastern foxsnakes. The patterns with Joust D differentiation were similar to F_{st}. All pairwise F_{st} were significantly different from zero ($p < 0.001$).

Genetic Diversity

F_{IS} values were not significantly different between defined populations (X^2_{13}=18.93, p=0.12), but both allelic richness (X^2_{13}=73.64, $p < 0.001$) and H_e (X^2_{13}=42.52, $p < 0.001$) values varied significantly among populations. The three most isolated eastern foxsnake populations (Georgian Bay 1&2; Lower Michigan; Norfolk) had the lowest allelic richness and were significantly lower than the Illinois population when compared using a Wilcoxon-Nemenyi-McDonald-Thompson *post hoc* test (Zar, 1996; Hollander & Wolfe, 1999) (Table 4.2). Similarly, H_e was lowest in populations in the three isolated regional populations, but only two populations (Georgian Bay 1 and Norfolk) were significantly different from the population with the highest H_e (Illinois).

Table 4.1. Pairwise F_{ST} values (below the diagnonal) and Joust D differentiation values (above the diagonal) between genetic clusters (see Fig. 4.1 for population distributions). All pairwise values were highly significant ($p < 0.001$).

	Ill	Wisc	U.Mi	ON1	ON2	ON3	ON4	ON5	ON6	ON7	L. Mi	Nor.	GB1	GB2
Ill		0.07	0.07	0.14	0.20	0.32	0.23	0.26	0.18	0.26	0.38	0.30	0.51	0.45
Wisc	0.07		0.03	0.34	0.41	0.56	0.43	0.51	0.38	0.43	0.59	0.47	0.66	0.62
U.Mi	0.10	0.06		0.22	0.35	0.49	0.34	0.50	0.32	0.35	0.54	0.34	0.66	0.66
ON1	0.11	0.25	0.23		0.07	0.18	0.08	0.16	0.03	0.11	0.24	0.12	0.37	0.43
ON2	0.12	0.26	0.27	0.07		0.17	0.04	0.12	0.04	0.04	0.23	0.14	0.37	0.29
ON3	0.19	0.37	0.39	0.17	0.15		0.19	0.17	0.16	0.15	0.22	0.20	0.37	0.35
ON4	0.15	0.31	0.30	0.11	0.05	0.18		0.08	0.10	0.14	0.20	0.09	0.38	0.34
ON5	0.15	0.32	0.34	0.12	0.09	0.16	0.08		0.09	0.17	0.22	0.18	0.47	0.41
ON6	0.10	0.26	0.26	0.05	0.05	0.14	0.09	0.11		0.06	0.22	0.14	0.40	0.40
ON7	0.18	0.35	0.34	0.10	0.10	0.23	0.09	0.16	0.06		0.20	0.10	0.41	0.39
L. Mi	0.32	0.51	0.52	0.32	0.27	0.33	0.29	0.31	0.28	0.33		0.30	0.61	0.51
Nor	0.33	0.53	0.48	0.20	0.20	0.37	0.17	0.29	0.20	0.24	0.53		0.31	0.37
GB1	0.46	0.60	0.61	0.42	0.40	0.54	0.43	0.53	0.42	0.55	0.67	0.60		0.07
GB2	0.21	0.41	0.45	0.25	0.23	0.37	0.27	0.34	0.25	0.40	0.54	0.50	0.20	

Population names are abbreviations from populations described in Fig. 4.1 and Appendix 5.

Table 4.2. Sample size, expected heterozygosity (H_e), mean number of alleles (MNA) and allelic richness (AR) for genetic clusters of eastern foxsnakes (Fig. 4.2) in southwestern Ontario and northwestern Ohio. Standard deviation is given in brackets and populations connected with different letters for H_e and allelic richness were significantly different. F_{is} was not significantly different and MNA was not tested. See text for details of tests and Fig. 4.1 for distribution of populations.

Population	N	H_e	MNA	AR	F_{is}
GeoBay1	119	0.28(0.13)[b]	2.41(0.79)	2.05(0.54)[b]	0.03(0.09)
GeoBay2	41	0.36(0.21)[ab]	2.81(0.75)	2.40(0.66)[b]	-0.02(0.13)
Swont1	62	0.59(0.14)[ab]	4.33(1.61)	3.69(1.07)[ab]	0.01(0.13)
SWont2	134	0.61(0.13)[ab]	5.50(1.83)	3.97(1.10)[ab]	0.04(0.05)
SWont3	28	0.52(0.14)[ab]	4.00(1.13)	3.46(0.75)[ab]	0.05(0.16)
SWont4	142	0.53(0.20)[ab]	4.91(1.73)	3.69(1.27)[ab]	0.02(0.05)
SWont5	28	0.58(0.15)[ab]	3.83(1.40)	3.43(1.06)[ab]	-0.01(0.16)
SWont6	84	0.62(0.11)[ab]	5.33(1.72)	3.93(0.87)[ab]	0.12(0.06)
SWont7	47	0.50(0.16)[ab]	4.08(1.50)	3.23(0.94)[ab]	0.03(0.17)
Norfolk	64	0.32(0.19)[b]	3.25(1.28)	2.51(0.80)[b]	0.13(0.11)
L. Mich	33	0.45(0.22)[ab]	2.08(1.08)	2.04(1.04)[b]	0.02(0.18)
Illinois	27	0.74(0.12)[ab]	7.25(1.76)	5.96(1.44)[a]	0.07(0.11)
Wisconsin	12	0.61(0.19)[ab]	4.33(1.40)	4.33(1.61)[a]	0.03(0.14)
U. Mich	12	0.55(0.25)[ab]	3.83(1.80)	3.80(1.75)[a]	0.01(0.18)

Population names are abbreviations from populations described in Fig. 4.1 and Appendix 5.

Demographic modeling with Approximate Bayesian computation

Population-Scale

For the L. Michigan, Georgian Bay and Illinois populations the marginal densities of all three models (*Drop, Decline, Stable*) had P values above 0.05 (Table 4.3). This indicated that the observed marginal densities were within the range of the distribution of marginal densities for the retained simulations, and capable of producing the observed summary statistics. The marginal densities of the *Drop* model, however, were highest for all three populations with Bayes factors of 2.89 and 162.5 for L. Michigan, 2.59 and 316.08 for Georgian Bay and 3.62 and 182282 for Illinois, when comparing the *Drop* model to the *Decline* and *Stable* models, respectively. The marginal density for the Norfolk population had P values that were < 0.05 for all of the models, suggesting none of these models could accurately produce the summary statistics. Examining the posterior distributions for the *Drop* model for L. Michigan and Georgian Bay it appears they both had a significant drop in population size around 430 and 300 generations in the past, respectively (Table 4.4). Current population sizes were larger for L. Michigan (mode of 774 individuals) than for the Georgian Bay population (mode of 392 individuals) (Table 4.4). The Illinois drop in population size appeared to occur much earlier (2093 generations in the past) and resulted in a larger current population size (10754) (Table 4.4). But the Illinois population stretches over a much larger area (Fig. 4.1) so these populations should not be directly compared.

Table 4.3. Comparison of Approximate Bayesian computation models using marginal densities, probabilities (low P value indicates an inability of the model to produce the observed summary statistics) and relative probabilities. Models are presented graphically in Fig. 4.2 and described in more detail in the text.

Population	#PLS	Model	Mar. Density	P value	Rel. Prob.
Michigan	NA	*Drop*	2.6×10^{-1}	0.71	0.74
Michigan	NA	*Decline*	9.9×10^{-2}	0.71	0.26
Michigan	NA	*Bottle*	1.6×10^{-3}	0.41	0.02
GBI	NA	*Drop*	4.1×10^{-3}	0.69	0.59
GBI	NA	*Decline*	1.5×10^{-3}	0.15	0.39
GBI	NA	*Bottle*	1.3×10^{-5}	0.07	0.02
Norfolk	NA	*Drop*	1.7×10^{-4}	0.01	0.50
Norfolk	NA	*Decline*	7.9×10^{-6}	<0.001	0.46
Norfolk	NA	*Bottle*	1.7×10^{-6}	0.01	0.04
Illinois	NA	*Drop*	12.1	0.99	0.75
Illinois	NA	*Decline*	3.3	0.98	0.25
Illinois	NA	*Bottle*	6.7×10^{-5}	0.98	0
swOnt	10	*Bot.Decline*	5.1×10^{-5}	0.70	0.42
swOnt	10	*Bot.Stable*	1.3×10^{-5}	0.95	0.14
swOnt	10	*2.Drop*	2.4×10^{-4}	0.99	0.44
Full	15	*Bot.Decline*	1.3×10^{-12}	0.003	0.22
Full	15	*Colonize*	1.9×10^{-16}	<0.001	0
Full	15	*Decline*	2.9×10^{-12}	0.01	0.77

Table 4.4. Prior distribution and posterior probabilities (with 90% highest probability density (HPD)) for parameters of the *Drop* single population models (Fig. 4.2a).

Parameter	Population	Mode	90% HPD	R^2
N.now	Michigan	774	100 - 1564	0.49
N.ancest	Michigan	140606	57454 - 200000	0.37
T.drop	Michigan	430	100 - 860	0.31
N.now	GeoBay	392	100 - 878	0.49
N.ancest	GeoBay	83171	166342 - 200000	0.38
T.drop	GeoBay	300	40 - 700	0.30
N.now	S.West	10754	4722 - 17988	0.57
N.ancest	S.West	94948	46464 - 175758	0.29
T.drop	S.West	2093	734 - 2970	0.19

N.now = current population size; N.ancest = ancestral population size, T.drop = time of decline in population size.

For southwestern Ontario, the marginal densities of all three models again had P values above 0.05 (Table 4.3). When comparing the marginal densities of the models, the *2.Drop* had the highest marginal density resulting in Bayes factors of 18.46 and 4.71 when comparing to the *Bot.stable* and *Bot.decline* models, respectively. For the *2.Drop* model, the population size posterior distributions had modes that ranged from 1236 to 3200 and posterior distributions for the timing, suggested a major drop in population size 2106 generations in the past and a drop/split of the southwestern Ontario populations 130 generations in the past (Table 4.5). T.Drop (large drop in population size in the past), however, had an R^2 value of much less than 10% and should be interpreted with caution.

Range-Wide Scale

At the range-wide scale the *Bot.Decline* and *Decline* models both had much higher marginal densities than the *Colonize* model, but there was conflicting evidence over which of the former two models had stronger support (Table 4.3). The marginal density for the *Decline* model was higher leading to a modest Bayes factor of 2.23 when comparing the *Decline* to *Bot.Decline* model, but the relative probability (i.e. proportion of simulations within top 1000 simulations) was higher (0.77) for the *Bot.Decline* model. Neither of these models, however, had P values above 0.05 suggesting they could not produce the observed summary statistics.

This equivocal result could be due to the complexity of the models that we tested and/or the low sample sizes and less intensive sampling coverage that we had for the western foxsnake populations. We therefore used the same generated dataset, but reduced the complexity by only including eastern foxsnake parameters and summary statistics

when calculating Euclidean distances between the generated and actual datasets and in the post sampling regression adjustment. After pruning the models in this way, the P value for the *Decline* (P value = 0.09), but not the *Bot.Decline* (P value = 0.004) model was above 0.05. The marginal Density was also higher for the *Decline* model resulting in a Bayes Factor of 56.77. We therefore estimated the parameters of this simplified *Decline* model (Table 4.6). The posterior probabilities of these suggested that the eastern foxsnake regional populations were split approximately 312 generations (100-594 90% HPD) in the past, which matched well with the timing of the population drop for the L.Michigan and Georgian Bay single population models. Also consistent with the single population models, the population sizes for the L.Michigan and Georgian Bay populations were, 788 and 642 individuals, respectively. For southwestern Ontario, the mean population size (mode = 772) was lower than any of the population sizes estimated, when we ran the southwestern Ontario in the single population model. The gamma distribution (Gamma(8,8/X)) with a population mean of 772 would allow population sizes to vary between 200 and 1600 and so when incorporating the 90% HPD (400-1384), the mean population size would be well within the confidence intervals of the southwestern Ontario population model.

Table 4.5. Prior distribution and posterior probabilities (with 90% highest probability density (HPD) estimate) for parameters *2.Drop* model for southwestern Ontario (Fig. 4.2b).

Parameter	Priors	Mode	90% HPD	R^2
N.SWont1	400-4000	1236	400-2200	0.69
N.SWont2	400-4000	3200	963-2000	0.68
N.SWont3	400-4000	2908	818-1964	0.69
N.SWont4	400-4000	1636	309-1400	0.68
N.SWont5	400-4000	3018	836-1981	0.69
N.SWont6	400-4000	1962	400-1671	0.69
N.SWont7	400-4000	1636	327-1491	0.69
T.split	10-1000	130	10-270	0.73
T.Drop	1000-2500	2106	1243-2469	0.02
N.SWont	2000-20000	3274	2001-8910	0.24
N.ancest	20000-100000	72525	34545-97575	0.33

N.SWont = current population size of populations in southwestern Ontario; N.ancest = ancestral population size before first drop; T.drop = time of first drop in population size; N.SWont = Size of sw Ontario population before splitting; T.Split = time of second population drop and split into current populations.

Table 4.6. Prior distribution and posterior probabilities (with 90% highest probability density (HPD) estimate) for parameters of the simplified *Decline* regional model (see Fig. 4.3c and text for details).

Parameter	Prior	Mode	90%HPD	R^2
N.swOnt	400-2000	772	400 - 1384	0.33
N.Mich	400-2000	788	416 - 1256	0.58
N.Norfolk	400-2000	1450	868 - 1918	0.58
N.GeoBay	400-2000	642	400 - 1046	0.58
N.East	5000-50000	33756	10000- 78220	0.10
N.Fox	20000-200000	158182	50910 - 200000	0.40
T.split.EW	200-2000	1727	1000 - 2000	0.16
T.sp.ea	50-1500	312	100 - 594	0.67

N.swOnt = mean population size of sw Ontario populations; N.Mich = population size of lower Michigan population; N.Norfolk = size of Norfolk population; N.GeoBay = size of Georgian Bay 1 population; N.East = size of eastern foxsnake population before fragmenting; N.Fox size of foxsnake populaton before splitting from western foxsnakes

Discussion

Our microsatellite analysis showed that genetic population structure and population differentiation are much greater in eastern foxsnakes than western foxsnakes, and genetic diversity was lower in isolated peripheral eastern foxsnake populations. Based on ABC analysis these patterns appear to be attributable to large drops in population size, combined with population splits. Given the estimated timing of population size drops and splits, the most likely cause is the infilling of deciduous forest and/or cooler temperatures since the Hypisthermal. Further population drops and fragmentation in southwestern Ontario were also evident and most likely caused by anthropogenic habitat loss and fragmentation.

Sequence data from the cytochrome *b* region of mtDNA showed very little variation and patterns were not consistent with microsatellite analysis or with current fragmented regional populations. All but two eastern foxsnakes (58 total) and most of the western foxsnakes, east of the Mississippi, had an identical haplotype possibly reflecting a selective sweep or founder event in the past.

Genetic Diversity and Genetic population structure

Results from both assignment tests and PCA with microsatellite data showed a clear split between the current designation of eastern and western foxsnakes, with genetic structure more pronounced within eastern foxsnakes. This was expected given that the range of eastern foxsnakes appears to be more fragmented, but implies that the distribution of western foxsnakes is potentially more continuous. This fragmentation and geographical isolation has impacted microsatellite diversity; the isolated eastern foxsnake

regional populations show significantly lower expected heterozygosity and allelic richness than the Illinois foxsnake population. This is consistent with other studies on temperate species that have found that as populations move northward, away from glacial refugia, there is a decrease in genetic diversity (Johansson *et al*., 2006; Howes & Lougheed, 2008). This decline, however, was non-significant between the Illinois population and the Wisconsin and upper Michigan populations. The upper Michigan population is likely as far, or farther, from potential glacial refugia than some of the eastern foxsnake populations that show a significant decline in genetic diversity. Because western foxsnake populations are seemingly more continuously distributed, this lack of decline may be attributed to ongoing gene flow with southern populations, which would contribute to maintenance of genetic diversity (Wright, 1978; Slatkin, 1987), but would not be possible for the isolated eastern populations.

Using mtDNA we found two major clades (1.5% divergence), but in contrast to the patterns found with microsatellites these clades did not correspond to the current designation of eastern and western foxsnakes or to any of the eastern foxsnake regional populations (Fig. 4.4). Using more sequences, Crother *et al*. (unpublished manuscript) suggested the Mississippi as a possible barrier that lead to this divergence. The Mississippi has proven to be a barrier for other species (Burbrink *et al*., 2000; Howes *et al*., 2006) and based on the distribution of haplotypes (Fig. 4.4) this is a possible scenario. Crother *et al*. (unpublished manuscript) and we, however, found eastern and western haplotypes on either side of the Mississippi River, suggesting this is not presently a strong barrier. In the Wisconsin population (Fig. 4.1), we found haplotypes from both clades, but microsatellite assignment tests put these individuals in the same genetic cluster, implying

that the lineages are not reproductively isolated. Other snake species with similar divergences between cytochrome *b* lineages, also show no evidence of assortative mating in zones of contact indirectly implying lack of reproductive isolation between clades (Gibbs *et al*., 2006).

The diversity and structure at cytochrome *b* was generally very low and, within eastern foxsnakes, all except two individuals possessed a single mitochondrial haplotype. The majority of western foxsnakes, east of the Mississippi, also had this same haloptype suggesting a bottleneck or selective sweep prior to split between eastern and western foxsnakes. The cytochrome *b* region of mtDNA has been found to be variable and informative for closely related snake species (Burbrink *et al*., 2000). Preliminary tests also found that cytochrome *b* was more variable than mtDNA control region in foxsnakes (L.Gibbs, unpublished data), suggesting this paucity of diversity in eastern foxsnakes was not simply related to the region of mtDNA that we examined.

Colonization patterns and Approximate Bayesian computation analysis

We are fully aware that, although our models were complex, there remained many simplifications (e.g. no gene flow, combined eastern foxsnake splitting times) that could affect our parameter estimates. Overall, however, all models (both single population and regional models) that included large population declines consistently had better support than population expansion models (i.e. founder effects and population expansion). The current geographic range of foxsnakes was covered by ice sheets > 70 000 years ago and so there is no doubt that ancestral populations expanded into their current range since that time. Our models suggest, however, that ancestral foxsnake populations were larger and more widely distributed, and that subsequent declines and population fragmentation have

had the largest effect in shaping the current microsatellite diversity and structure patterns. Based on herpetofauna distribution patterns, Schmidt (1938) suggested that a post-glacial steppe extended prairie like conditions eastward from the prairie peninsula. This hypothesis has been supported by pollen profiles (King, 1981; Webb, 1981). These prairie conditions, combined with the higher temperatures at the Climatic Optimum ~5000 years ago (Smith, 1957; Churcher & Karrow, 2008) arguably permitted these higher population sizes and/or greater connectivity across the range of eastern and western foxsnakes.

The maximum extent of the eastward extension of prairie conditions has been estimated at approximately 5000 - 7000 years ago with subsequent westward retreat until approximately 2000 years ago (Webb, 1981). Estimating the timing of the demographic parameters relies on the estimated generation time. Based on growth models built for a population in Georgian Bay, the minimum size of observed gravid females and the maximum size of observed females the age at maturity and maximum life span for females were estimated at 4 and 13 years, and 3 and 10 years, for populations in Geogian Bay and southwestern Ontario, resectively (J.R. Row and S.C. Lougheed unpublished data). Assuming a generation time of 7.5 years (midpoint between age at maturity and longevity, averaged for Georgian Bay and southwestern Ontario) the fragmentation of eastern foxsnakes populations occurred approximately 2340 years in the past (90% HPD confidence interval of 750-4455). This estimates seem to preclude the possibility that the geographic disjunctions within eastern foxsnakes were caused by European settlement and would be consistent with existence of the post-glacial steppe and subsequent infilling with deciduous forest coincident with post-Hypsithermal cooling of temperatures. Posterior distributions suggest that the split between eastern and western foxsnakes

occurred approximately 12,952 years in the past (90% confidence interval of 7500 to 15,000 years ago). Again this timing strongly suggests that the disjunctions did not result from European settlement, but would appear to predate the proposed timing of the infilling of deciduous forest. The wide confidence intervals and low R^2 suggest, however, that we may not have significant power to estimate this splitting time with our microsatellite markers alone.

Anthropogenic Habitat Alteration and Conservation implications

Although the large population declines and regional population splits appear to predate major European colonization, there is evidence that agricultural, residential and urban development have further impacted populations across the distribution, but at finer geographic scales. Indeed, Row *et al*. (2010) found that disjunctions between diagnosed genetic clusters in southwestern Ontario correlated well with agricultural fields and road barriers. The timing of the population split in this region (10-270 HPD generations; 75-2025 years) is consistent with the notion that anthropogenically driven habitat fragmentation isolated previously larger and more connected populations of foxsnakes in this region. Results from our ABC analysis also imply that the current population sizes of foxsnakes are much smaller than those in the past, which is especially true for eastern foxsnakes. Although, it appears the largest decline pre-dated extensive European settlement, it is unlikely that the large anthropogenic habitat loss and fragmentation is not having a continued impact on populations, as evidenced by the southwestern Ontario analysis. There is recent evidence of a widespread recent decline in snakes (Reading *et al*., 2010) and small increases in mortality can have large impacts on populations of late maturity species, such as large snakes in temperate climates (Row *et al*., 2007).

Combining these population size estimates with population viability analysis would be beneficial for determining the viability of these remaining populations.

Conclusions

The Approximate Bayesian computing approach (Beaumont *et al*., 2002; Beaumont *et al*., 2010) that we deployed in this study provided a robust hypothesis-testing framework for comparing alternate historical demographic models. Using this analysis we found that major disjunctions evident in the current distribution of foxsnakes predate European colonization and thus cannot be attributed to extensive land alteration that has occurred over the last two centuries. In our hierarchical analysis, results of the single population models and regional population models showed consistent results in terms of splitting times and population sizes. This provided us with confidence in our results, but also suggests that ABC analysis may be robust in situations where there are gaps in sampling distribution. Simulation studies will provide further clarification as to the situations where this would hold true.

All of the timing estimates must be interpreted with some caution as they depend on an accurate estimation of both generation time and mutation rate. Although mutation rate was allow to vary within a reasonable interval (10^{-4} - 10^{-5}) and estimated using our models, generation time will likely vary depending on latitudue and length of active season (Blouin-Demers et al., 2002) and could have a large effect on our estimates of splitting time. Furthmore, the exclusion of parameters such as gene flow, which may have had a role in shaping the patterns of diversity, may also have an effect on all of our parameter estimates. Simulation studies testing the effect of the exclusion or inclusion of

parameters that were not used to derive the 'observed' dataset would be beneficial in assessing the sensitity of the ABC analysis and parameter estimation.

This study provides a firm foundation for future work both on the foxsnake itself, but also on other co-distributed species. Schmidt (1938) used the eastern range extension of 11 prairie herpetofauna species (including 6 snake species) as evidence for the post-glacial steppe. Other studies have since identified similar distibuion patterns in other species of herpetofauna, as well as species of mammals, plants and insects (Thomas, 1951; Smith, 1957; Lloyd, 1967). Many of these species are also associated with aquatic habitats (e.g. turtles and frogs) and likely also benefited from the lake formation and drainage basins from the melting ice caps (Mockford *et al*., 2007). Similar tests of the postglacial expansion of some of these other species would determine if they show similar evidence for declines and if timing and extent of declines are consistent. For comparisons with foxsnakes, a test of the Massasauga rattlesnake (*Sistrurus c. catenatus*) populations would be particularly useful, as their range in Ontario is very similar, including the presence of disjunct populations in southwestern Ontario and the Georgian Bay area. Furthermore identification and inclusion of other genetic markers with slower mutations rates (e.g. longer repeat microsatellite markers, nulear DNA sequences) and additional western foxsnake samples may provide more accurate parameter estimates and insight into deeper historical trends (e.g. split between eastern and western foxsnakes, botteneck during maximum glacial extent).

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Literature Cited

Aldenhoven, J., Miller, M., Corneli, P. & Shapiro, M. 2010. Phylogeography of ninespine sticklebacks (*Pungitius pungitius*) in North America: glacial refugia and the origins of adaptive traits. *Molecular Ecology,* **19:** 4061–4076.

Austin, J.D., Lougheed, S.C., Neidrauer, L., Chek, A.A. & Boag, P.T. 2002. Cryptic lineages in a small frog: the post-glacial history of the spring peeper, *Pseudacris crucifer* (Anura: Hylidae). *Molecular Phylogenetics and Evolution,* **25:** 316–329.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A. & Saunders, N.C. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics,* **18:** 489–522.

Beaumont, M.A., Nielsen, R., Robert, C., Hey, J., Gaggiotti, O., Knowles, L., Estoup, A., Panchal, M., Corander, J. & Hickerson, M. 2010. In defence of model-based inference in phylogeography. *Molecular Ecology,* **19:** 436–446.

Beaumont, M.A., Zhang, W. & Balding, D.J. 2002. Approximate Bayesian computation in population genetics. *Genetics,* **162:** 2025–2035.

Bertorelle, G., Benazzo, A. & Mona, S. 2010. ABC as a flexible framework to estimate demography over space and time: some cons, many pros. *Molecular Ecology,* **19:** 2609–2625.

Blouin-Demers, G. & Gibbs, H.L. 2003. Isolation and characterization of microsatellite loci in the black rat snake (*Elaphe obsoleta*). *Molecular Ecology Notes,* **3:** 98–99.

Blouin-Demers, G., Prior, K.A. & Weatherhead, P. 2002. Comparative demography of black rat snakes (*Elaphe obsoleta*) in Ontario and Maryland. *Journal of Zoology*, **256**: 1–10.

Burbrink, F.T., Lawson, R. & Slowinski, J.B. 2000. Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. *Evolution,* **54:** 2107–2118.

Churcher, C. & Karrow, P. 2008. The Hamilton Bar Fauna: evidence for a Hypsithermal age. *Canadian Journal of Earth Sciences,* **45:** 1487–1500.

Collins, J.T. 1991. Viewpoint: a new taxonomic arrangement for some North American amphibians and reptiles. *Herpetological Review,* **22:** 42–43.

Conant, R. 1940. A new subspecies of the Fox Snake, *Elaphe vulpina* Baird and Girard. *Herpetologica,* **2:** 1–14.

Conant, R. & Collins, J.T. (1991) *A field guide to amphibians and reptiles of eastern and central North America*. Houghton Mifflin Co, Boston, Massachusetts

Costello, A.B., Down, T.E., Pollard, S.M., Pacas, C.J. & Taylor, E.B. 2003. The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species: an examination of microsatellite DNA variation in bull trout, *Salvelinus confluentus* (Pisces: Salmonidae). *Evolution,* **57:** 328–344.

Crawford, N.G. 2010. SMOGD: software for the measurement of genetic diversity. Molecular Ecology Resources, **10:** 556–557.

Dieringer, D. & Schlotterer, C. 2003. Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes,* **3:** 167–169.

DiLeo, M., Row, J.R. & Lougheed, S.C. 2010. Discordant patterns of population structure for two co-distributed snake species across a fragmented Ontario landscape. *Diversity and Distributions,* **16:** 571–581.

Dyer, R., Nason, J. & Garrick, R. 2010. Landscape modelling of gene flow: improved power using conditional genetic distance derived from the topology of population networks. *Molecular Ecology,* **19:** 3746–3759.

Eckert, C.G., Samis, K.E. & Lougheed, S.C. 2008. Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. *Molecular Ecology*, **17:** 1170–1188.

Estoup, A. & Clegg, S. 2003. Bayesian inferences on the recent island colonization history by the bird *Zosterops lateralis lateralis*. *Molecular Ecology,* **12:** 657–674.

Excoffier, L., Estoup, A. & Cornuet, J.M. 2005. Bayesian analysis of an admixture model with mutations and arbitrarily linked markers. *Genetics,* **169:** 1727–1738.

Excoffier, L. & Lischer, H.E.L. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources,* **10:** 564–567.

Fagundes, N.J.R., Ray, N., Beaumont, M., Neuenschwander, S., Salzano, F.M., Bonatto, S.L. & Excoffier, L. 2007. Statistical evaluation of alternative models of human evolution. *Proceedings of the National Academy of Sciences,* **104:** 17614–17619.

Galtier, N., Gouy, M. & Gautier, C. 1996. SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. *Bioinformatics,* **12:** 543–548.

Garza, J.C. & Williamson, E.G. 2001. Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology,* **10:** 305–318.

Gelman, A. & Rubin, D.B. 1992. Inference from iterative simulation using multiple sequences. *Statistical science,* **7:** 457–472.

Gibbs, H.L., Corey, S., Blouin-Demers, G., Prior, K. & Weatherhead, P.J. 2006. Hybridization between mtDNA-defined phylogeographic lineages of black ratsnakes (*Pantherophis sp*.). *Molecular Ecology,* **15:** 3755–3767.

Gould, S.J. & Johnston, R.F. 1972. Geographic variation. *Annual Review of Ecology and Systematics,* **3:** 457–498.

Hollander, M. & Wolfe, D.A. (1999) Nonparametric Statistical Methods, 2nd Edition. New York

Howes, B.J., Lindsay, B. & Lougheed, S.C. 2006. Range-wide phylogeography of a temperate lizard, the five-lined skink (*Eumeces fasciatus*). *Molecular Phylogenetics and Evolution,* **40:** 183–194.

Howes, B.J. & Lougheed, S.C. 2008. Genetic diversity across the range of a temperate lizard. *Journal of Biogeography,* **35:** 1269–1278.

Hurlbert, S.H. 1971. The nonconcept of species diversity: a critique and alternative parameters. *Ecology,* **52:** 577–586.

Jakobsson, M. & Rosenberg, N. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics,* **23:** 1801–1806.

Johansson, M., Primmer, C.R. & Merilae, J. 2006. History vs. current demography: explaining the genetic population structure of the common frog (*Rana temporaria*). *Molecular Ecology,* **15:** 975–983.

Jombart, T. 2008. *adegenet:* a R package for the multivariate analysis of genetic markers. *Bioinformatics,* **24:** 1403–1405.

Jombart, T., Devillard, S., Dufour, A.B. & Pontier, D. 2008. Revealing cryptic spatial patterns in genetic variability by a new multivariate method. *Heredity,* **101:** 92–103.

Jost, L. 2008. G_{ST} and its relatives do not measure differentiation. *Molecular Ecology,* **17:** 4015–4026.

Joyce, P. & Marjoram, P. 2008. Approximately sufficient statistics and Bayesian computation. *Statistical Applications in Genetics and Molecular Biology,* **7:** 26

King, J.E. 1981. Late Quaternary vegetational history of Illinois. *Ecological Monographs,* **51:** 43–62.

Knowles, L.L. & Alvarado-Serrano, D. 2010. Exploring the population genetic consequences of the colonization process with spatio-temporally explicit models: insights from coupled ecological, demographic and genetic models in montane grasshoppers. *Molecular Ecology,* **19:** 3727–3745.

Knowles, L.L. & Maddison, W.P. 2002. Statistical phylogeography. *Molecular Ecology,* **11:** 2623–2635.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., Mcgettigan, P.A., Mcwilliam, H., Valentin, F., Wallace, I.M., Wilm, A. & Lopez, R. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics,* **23:** 2947.

Laval, G. & Excoffier, L. 2004. SIMCOAL 2.0: a program to simulate genomic diversity over large recombining regions in a subdivided population with a complex history. *Bioinformatics,* **20:** 2485–2487.

Leuenberger, C. & Wegmann, D. 2010. Bayesian computation and model selection without likelihoods. *Genetics,* **184:** 243–252.

Lloyd, J. 1967. The Prairie Peninsula and secondary intergradation in Photinus fireflies (Coleoptera: Lampyridae). *The Coleopterists' Bulletin,* **21:** 33–39.

Manel, S., Gaggiotti, O.E. & Waples, R.S. 2005. Assignment methods: matching biological questions with appropriate techniques. *Trends in Ecology & Evolution,* **20:** 136–142.

Michalakis, Y. & Excoffier, L. 1996. A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics,* **142:** 1061–1064.

Mockford, S.W., Herman, T.B., Snyder, M. & Wright, J.M. 2007. Conservation genetics of Blanding's turtle and its application in the identification of evolutionarily significant units. *Conservation Genetics,* **8:** 209–219.

Morse, M. 1902. The range of the fox snake. *Science,* **15:** 1035.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics,* **89:** 583–590.

Neuenschwander, S., Largiadèr, C.R., Ray, N., Currat, M., Vonlanthen, P. & Excoffier, L. 2008. Colonization history of the Swiss Rhine basin by the bullhead (*Cottus gobio*): inference under a Bayesian spatially explicit framework. *Molecular ecology,* **17:** 757–772.

Nylander, J.A.A. 2004. MrModeltest v2. *Program distributed by author,* Evolutionary Biology Centre, Uppsala University.

Panchal, M. & Beaumont, M.A. 2007. The automation and evaluation of nested clade phylogeographic analysis. *Evolution,* **61:** 1466–1480.

Placyk Jr, J.S., Burghardt, G.M., Small, R.L., King, R.B., Casper, G.S. & Robinson, J.W. 2007. Post-glacial recolonization of the Great Lakes region by the common gartersnake (*Thamnophis sirtalis*) inferred from mtDNA sequences. *Molecular Phylogenetics and Evolution,* **43:** 452–467.

Pritchard, J.K., Seielstad, M.T., Perez-Lezaun, A. & Feldman, M.W. 1999. Population growth of human Y chromosomes: a study of Y chromosome microsatellites. *Molecular Biology and Evolution,* **16:** 1791–1798.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics,* **155:** 945–959.

Qu, Y., Lei, F., Zhang, R. & Lu, X. 2010. Comparative phylogeography of five avian species: implications for Pleistocene evolutionary history in the Qinghai–Tibetan plateau. *Molecular Ecology,* **19:** 338–351.

R Development Core Team (2009) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria

Ray, N., Wegmann, D., Fagundes, N.J.R., Wang, S., Ruiz-Linares, A. & Excoffier, L. 2010. A statistical evaluation of models for the initial settlement of the American continent emphasizes the importance of gene flow with Asia. *Molecular Biology and Evolution,* **27:** 337–345.

Reading, C.J., Luiselli, L.M., Akani, G.C., Bonnet, X., Amori, G., Ballouard, J.M., Filippi, E., Naulleau, G., Pearson, D. & Rugiero, L. 2010. Are snake populations in widespread decline? *Biology Letters,* doi:10.1098/rsbl.2010.0373.

Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution,* **43:** 223–225.

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics,* **19:** 1572–1574.

Rosenberg, N.A. 2004. DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes,* **4:** 137–138.

Row, J.R., Blouin-Demers, G. & Lougheed, S.C. 2010. Habitat distribution influences dispersal and fine-scale genetic population structure of eastern foxsnakes (*Mintonius gloydi*) across a fragmented landscape. *Molecular Ecology,* **19:** 5157-5171

Row, J.R., Blouin-Demers, G. & Weatherhead, P.J. 2007. Demographic effects of road mortality in black ratsnakes (*Elaphe obsoleta*). *Biological Conservation,* **137:** 117-124.

Row, J.R., Sun, Z., Cliffe, C. & Lougheed, S.C. 2008. Isolation and characterization of microsatellite loci for eastern foxsnakes (*Elaphe gloydi*). *Molecular Ecology Resources,* **8:** 965–967.

Schmidt, K. 1938. Herpetological evidence for the postglacial eastward extension of the steppe in North America. *Ecology,* **19:** 396–407.

Schoville, S. & Roderick, G. 2009. Alpine biogeography of Parnassian butterflies during Quaternary climate cycles in North America. *Molecular Ecology,* **18:** 3471–3485.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. *Science,* **236:** 787–792.

Smith, P. 1957. An analysis of post-Wisconsin biogeography of the Prairie Peninsula region based on distributional phenomena among terrestrial vertebrate populations. *Ecology,* **38:** 205–218.

Sunnucks, P. 2000. Efficient genetic markers for population biology. *Trends in Ecology & Evolution,* **15:** 199–203.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution,* **24:** 1596–1599.

Templeton, A.R. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology,* **7:** 381–397.

Thomas, E. 1951. Distribution of Ohio animals. *The Ohio Journal of Science,* **51:** 153–167.

Transeay, E.N. 1935. The prairie peninsula. *Ecology,* **63:** 423–437.

Webb, T. 1981. The past 11,000 years of vegetational change in eastern North America. *Bioscience,* **31:** 501–506.

Wegmann, D. & Excoffier, L. 2010. Bayesian Inference of the demographic history of chimpanzees. *Molecular Biology and Evolution,* **27:** 1425–1435.

Wegmann, D., Leuenberger, C. & Excoffier, L. 2009. Efficient approximate Bayesian computation coupled with Markov chain Monte Carlo without likelihood. *Genetics,* **182:** 1207–1218.

Wegmann, D., Leuenberger, C., Neuenschwander, S. & Excoffier, L. 2010. ABCtoolbox: a versatile toolkit for approximate Bayesian computations. *BMC bioinformatics,* **11:** 116.

Weir, B.S. & Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution,* **38:** 1358–1370.

Wright, S. 1978. Evolution and the genetics of populations: variability within and among natural populations. University of Chicago Press, Chicago, IL

Zamudio, K.R. & Savage, W.K. 2003. Historical isolation, range expansion, and secondary contact of two highly divergent mitochondrial lineages in spotted salamanders (*Ambystoma maculatum*). *Evolution,* **57:** 1631–1652.

Zar, J.H. (1996) *Biostatistical analysis*. Prentice Hall, Englewood Cliffs, New Jersy

Zellmer, A.J. & Knowles, L.L. 2009. Disentangling the effects of historic vs. contemporary landscape structure on population genetic divergence. *Molecular Ecology,* **18:** 3593–3602.

Chapter 5: General Discussion

Due to the complex demographic and evolutionary history of most species, it is often difficult to determine the relative contribution of processes acting across different spatial and temporal scales (Eckert *et al*. 2008; Knowles & Alvarado-Serrano 2010). Nonetheless, understanding the causes of spatial distribution of genetic variation has long been a key emphasis in evolutionary biology and underpins many evolutionary models (Mayr 1963; Gould & Johnston 1972). In my thesis I have combined behavioural, ecological and spatial analyses with population genetic and phylogenetic approaches to understand the factors that have shaped population structure and patterns of diversity across the range of eastern and western foxsnakes. It was only through these multi-perspective analyses using different data types, that it became clear the degree to which each of a series of historical and contemporary factors has contributed to shape the patterns of variation observed today. Below I summarize each chapter and discuss its evolutionary significance and conservation implications.

Summary of Chapters

Chapter 2

In the second chapter, we used radio-telemetry and occurrence records to examine the impact of habitat fragmentation on behaviour, habitat use and distribution patterns for foxsnakes across a heavily fragmented region. We first compared habitat use patterns at two locations varying in their habitat patch size and level of disturbance. We predicted that foxsnakes would maintain similar habitat use patterns but restrict movements at the more disturbed site. Supporting our predictions, we found that, although foxsnakes were relatively widespread across this region, they appeared to limit their movements within

usable habitat patches. Occurrence records were found not far from areas of usable habitat, suggesting their current distribution is limited to small isolated populations.

Snakes are the top predators in many ecosystems (Schwaner & Sarre 1988; Tzika *et al*. 2008) and thus can be disproportionately important to community stability (Paine 1969; Duffy 2002). A growing number of population and landscape genetic studies have found that snake populations may be heavily impacted by habitat fragmentation (Row *et al*. 2010; Jansen *et al*. 2008; Clark *et al*. 2010; DiLeo *et al*. 2010). This may be due to strict thermoregulatory needs of terrestrial ectotherms, particularly in temperate climates (Blouin-Demers & Weatherhead 2002; Row & Blouin-Demers 2006). Our study was one of the first to examine the behavioural impact of habitat patch size and fragmentation on snakes. Without more such studies it will be impossible to devise effective management practices that mitigate the effects of habitat loss and avoid small isolated populations, which have an increased risk of local extinction (Saccheri *et al*. 1998).

Chapter 3

In the third chapter, we combined habitat suitability modeling with spatial genetic analysis to test the link between habitat quality and distribution with dispersal patterns and resulting genetic population structure. Supporting our predictions and providing more direct evidence that recent habitat changes can have large effects on population structure, we found that the distribution and quality of habitat correlated with the number, extent and location of genetic clusters. Further, including resistance values, based on habitat quality, improved the fit of isolation by distance models. Lakes also apparently acted as barriers to gene flow between some populations, but the amount of differentiation was not as great as populations separated by low quality terrestrial habitat.

Dispersal and gene flow counteract the diversifying effects of genetic drift and mutation (Slatkin 1987) and therefore can have large effects on how variation is distributed among populations (Postma & van Noordwijk 2005). Although, many studies have recognized the impact of landscape structure on dispersal patterns, to our knowledge ours was one of the first to combine habitat suitability analysis with population genetics (but see: Wang *et al*. 2008). This approach allowed us to more directly test how habitat distribution and quality can impact dispersal and gene flow and not simply which habitat features correlate with patterns of differentiation. More studies combing the well established methods of habitat suitability modeling (reviewed in: Hirzel & Le Lay 2008) with population genetic models, would allow for more ecologically driven landscape quantification and more direct tests of how the amount, distribution and quality of habitat can impact dispersal patterns and population differentiation of different species.

In addition to the inclusion of habitat modeling with population genetics, we tested existing, and introduced new methodology for landscape genetics outlined below:
1) Combining assignment tests with surface interpolation of posterior probabilities or admixture proportions has been used in the literature, but is under-utilized, particularly when three or more clusters are present (Guillot *et al*. 2005; Murphy *et al*. 2008; Durand *et al*. 2009; Pierson *et al*. 2010). We developed a method to identify common boundaries of genetic clusters by combining surface interpolation maps of clusters across a common landscape. This method will assist with identifying barriers on the landscape in species that show significant genetic clustering, as we observed.
2) McRae (2006) found that isolation by distance (IBR) produced better results than least cost path (LCP) analysis when modeling connectivity of populations. We compared the

methods using an individual based dataset and found similar results when using the two methods.

3) Spatial autocorrelation analysis has been widely used in the literature to determine the scale of spatial genetic structure and to make comparisons between groups, particularly with respect to identifying sex-biased dispersal (Beck *et al*. 2008; Dubey *et al*. 2008; Hardy *et al*. 2008). Although resistance values can easily be incorporated into spatial autocorrelation analysis using most spatial autocorrelation software, and would seem more biologically realistic than using straight-line distances, this is rarely tested in natural populations. Here we found that, when using resistance values, the spatial autocorrelation analysis results were more consistent with the assignment test results.

Chapter 4

The entire range of eastern foxsnakes would have been completely covered with ice sheets during the Pleistocene, > 10 000 years ago. In chapter 4, we first quantified patterns of genetic diversity and genetic population structure of foxsnakes using both mitochondrial (mtDNA) and microsatellite DNA markers. We found little variation using mtDNA sequence data, especially within eastern foxsnakes. However, the population structure of microsatellites revealed a clear split between eastern and western foxsnakes and much greater population structure and differentiation among diagnosed genetic clusters within eastern foxsnakes, which corresponded to regional, fragmented and geographically isolated locales where foxsnakes are found. The isolated regional eastern foxsnake populations also showed a significant decline in genetic diversity. This decline was not evident in western foxsnake populations, which based on distribution patterns and assignment tests, appear to be much more continuously distributed.

Using Approximate Bayesian computation (ABC) we compared competing historical-demographic models to determine if the observed genetic patterns were more likely the result of repeated founder effects and population expansion or the result of a previously extensive range that was followed by subsequent retraction and subdivision. Supporting our prediction, we found that both single population models and regional population models that included a large population decline (i.e. corresponding to an extensive range followed by a subsequent retraction) showed the greatest support. The timing of declines and population splits suggested the most likely cause was the infilling of deciduous forest within the present-day range, following the expansion of eastern foxsnakes along a post-glacial steppe some 5-7000 years before present. Recent, human-induced, impacts on population structure were also apparent in the ABC analysis. The confidence intervals for the timing of the population split in southwestern Ontario (35-970 years), combined with the striking correlation found between population boundaries and habitat fragmentation found in the second chapter, provide strong evidence that anthropogenic factors have likely caused the extensive genetic structure observed in this region.

Traditional *post hoc* phylogeographic approaches derive conclusions by testing for a large number of possible causative factors, which can often lead to falsely attributing causation to some historical factor (Panchal & Beaumont 2007). Because of this, there has been a recent move to include a more robust statistical, hypothesis-testing framework into phylogentic analysis (Knowles & Maddison 2002). ABC analysis coupled with coalescent modeling is well suited to this task (Bertorelle *et al*. 2010). Only recently, however, have programs become available (Cornuet *et al*. 2008; Lopes *et al*. 2009; Wegmann *et al*.

2010) for population geneticists and phylogeographers without extensive computer programming skills. Using this analysis we gained insight into both the historical and contemporary processes that have shaped patterns of diversity for eastern foxsnakes. Further exploration of herpetofauna species with similar geographic ranges using ABC approaches would show whether the large population declines seen here are unique to the relatively ecologically specialized foxsnakes, or comprise a more general trend for terrestrial ectotherms, most of which would likely not have benefited from the infilling of deciduous forest and cooler temperatures beginning in the mid-Holocene. Furthermore, the timing and extent of population fragmentation for other species in southwestern Ontario, which houses the highest density of species at risk in Canada (Environment Canada, 2009), might point to other species that have been similarly impacted in this region.

Literature Cited

Beck N, Peakall R, Heinsohn R (2008) Social constraint and an absence of sex-biased dispersal drive fine-scale genetic structure in white-winged choughs. *Molecular Ecology,* **17,** 4346–4358.

Bertorelle G, Benazzo A, Mona S (2010) ABC as a flexible framework to estimate demography over space and time: some cons, many pros. *Molecular Ecology,* **19,** 2609–2625.

Blouin-Demers G, Weatherhead PJ (2002) Habitat-specific behavioural thermoregulation by black rat snakes (*Elaphe obsoleta obsoleta*). *Oikos,* **97,** 59–68.

Clark R, Brown W, Stechert R, Zamudio K (2010) Roads, interrupted dispersal, and denetic diversity in timber rattlesnakes. *Conservation Biology,* **24,** 1–11.

Cornuet J, Santos F, Beaumont M, Robert C, Marin J, Balding D, Guillemaud T, Estoup A (2008) Inferring population history with DIY ABC: a user-friendly approach to approximate Bayesian computation. *Bioinformatics,* **24,** 2713–2719.

DiLeo M, Row JR, Lougheed SC (2010) Discordant patterns of population structure for two co-distributed snake species across a fragmented Ontario landscape. *Diversity and Distributions,* **16,** 571–581.

Dubey S, Brown G, Madsen T, Shine R (2008) Male-biased dispersal in a tropical Australian snake (*Stegonotus cucullatus*, Colubridae). *Molecular Ecology,* **17,** 3506–3514.

Duffy JE (2002) Biodiversity and ecosystem function: the consumer connection. *Oikos,* **99,** 210–219.

Durand E, Jay F, Gaggiotti OE, Francois O (2009) Spatial inference of admixture proportions and secondary contact zones. *Molecular Biology and Evolution,* **26,** 1963–1973.

Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. *Molecular Ecology*, **17**, 1170–1188.

Environment Canada. 2009. Species at risk in Ontario. Environment Canada, Ottawa, Ontario. Available from http://www.on.ec.gc.ca/wildlife/sar/sar-e.html (accessed March 2010)

Gould S, Johnston R (1972) Geographic variation. *Annual Review of Ecology and Systematics,* **3,** 457–498.

Guillot G, Mortier F, Estoup A (2005) Geneland: a computer package for landscape genetics. *Molecular Ecology Notes,* **5,** 712–715.

Hardy OJ, Pearcy M, Aron S (2008) Small-scale spatial genetic structure in an ant species with sex-biased dispersal. *Biological Journal of the Linnean Society,* **93,** 465–473.

Hirzel A, Le Lay G (2008) Habitat suitability modelling and niche theory. *Journal of Applied Ecology,* **45,** 1372–1381.

Jansen KP, Mushinsky HR, Karl SA (2008) Population genetics of the mangrove salt marsh snake, *Nerodia clarkii compressicauda*, in a linear, fragmented habitat. *Conservation Genetics,* **9,** 401–410.

Knowles LL, Alvarado-Serrano D (2010) Exploring the population genetic consequences of the colonization process with spatio-temporally explicit models: insights from coupled ecological, demographic and genetic models in montane grasshoppers. *Molecular Ecology*, 19, 3727–3745.

Knowles LL, Maddison WP (2002) Statistical phylogeography. *Molecular Ecology,* **11,** 2623–2635.

Lopes J, Balding D, Beaumont M (2009) PopABC: a program to infer historical demographic parameters. *Bioinformatics,* **25,** 2747–2749.

Mayr E (1963) *Animal species and evolution*. Harvard University Press Cambridge, MA,

Mcrae BH (2006) Isolation by resistance. *Evolution,* **60,** 1551–1561.

Murphy MA, Evans JS, Cushman SA, Storfer A (2008) Representing genetic variation as continuous surfaces: an approach for identifying spatial dependency in landscape genetic studies. *Ecography,* **31,** 685–697.

Paine RT (1969) A note on trophic complexity and community stability. *The American Naturalist,* **103,** 91–93.

Panchal M, Beaumont M (2007) The automation and evaluation of nested clade phylogeographic analysis. *Evolution,* **61,** 1466–1480.

Pierson J, Allendorf F, Saab V, Drapeau P, Schwartz M (2010) Do male and female black-backed woodpeckers respond differently to gaps in habitat? *Evolutionary Applications,* **3,** 263–278.

Postma E, Van Noordwijk AJ (2005) Gene flow maintains a large genetic difference in clutch size at a small spatial scale. *Nature,* **433,** 65–68.

Row JR, Blouin-Demers G, Lougheed SC (2010) Habitat distribution influences dispersal and fine-scale genetic population structure of eastern foxsnakes (*Mintonius gloydi*) across a fragmented landscape. *Molecular Ecology,* **19**, 5157-5171.

Row JR, Blouin-Demers G (2006) Thermal quality influences habitat selection at multiple spatial scales in milksnakes. *Ecoscience,* **13,** 443–450.

Saccheri I, Kuussaari M, Kankare M, Vikman P (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature,* **392,** 491–494.

Schmidt K (1938) Herpetological evidence for the postglacial eastward extension of the steppe in North America. *Ecology,* **19,** 396–407.

Schwaner TD, Sarre SD (1988) Body size of tiger snakes in southern Australia, with particular reference to *Notechis ater serventyi* (Elapidae) on Chappell Island. *Journal of Herpetology,* **22,** 24–33.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science,* **236,** 787–792.

Tzika AC, Koenig S, Miller R, Garcia G, Remy C, Milinkovitch MC (2008) Population structure of an endemic vulnerable species, the Jamaican boa (*Epicrates subflavus*). *Molecular ecology,* **17,** 533–544.

Wang Y, Yang K, Bridgman C, Lin L (2008) Habitat suitability modelling to correlate gene flow with landscape connectivity. *Landscape Ecology,* **23,** 989–1000.

Wegmann D, Leuenberger C, Neuenschwander S, Excoffier L (2010) ABCtoolbox: a versatile toolkit for approximate Bayesian computations. *BMC bioinformatics,* **11,** 116.

Appendix 1

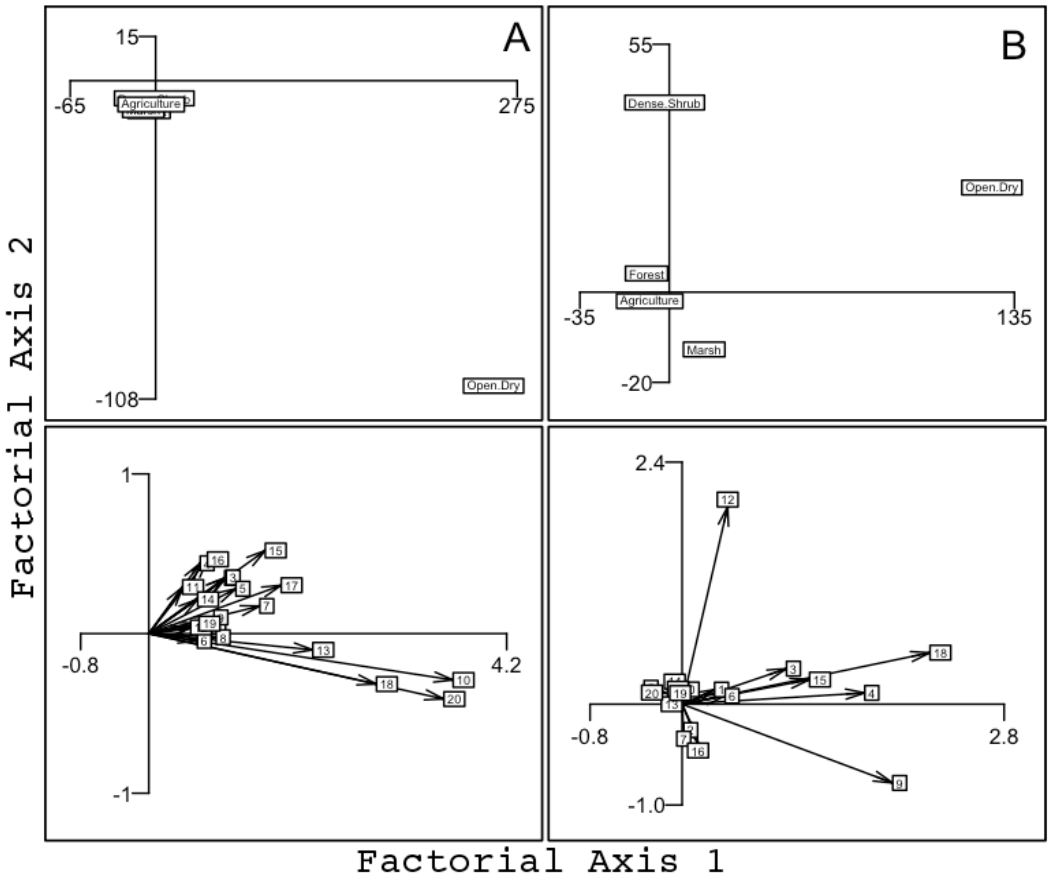

Figure A1.1. Results of eigen analysis (see text for details) with habitat loadings for each habitat type (top) and eigenvectors for each individual foxsnake (bottom) comparing used locations to habitat composition within the home-range of eastern foxsnakes at A) a highly fragmented (HMCA) and B) a low fragmented (PPNP) site in southwestern Ontario.

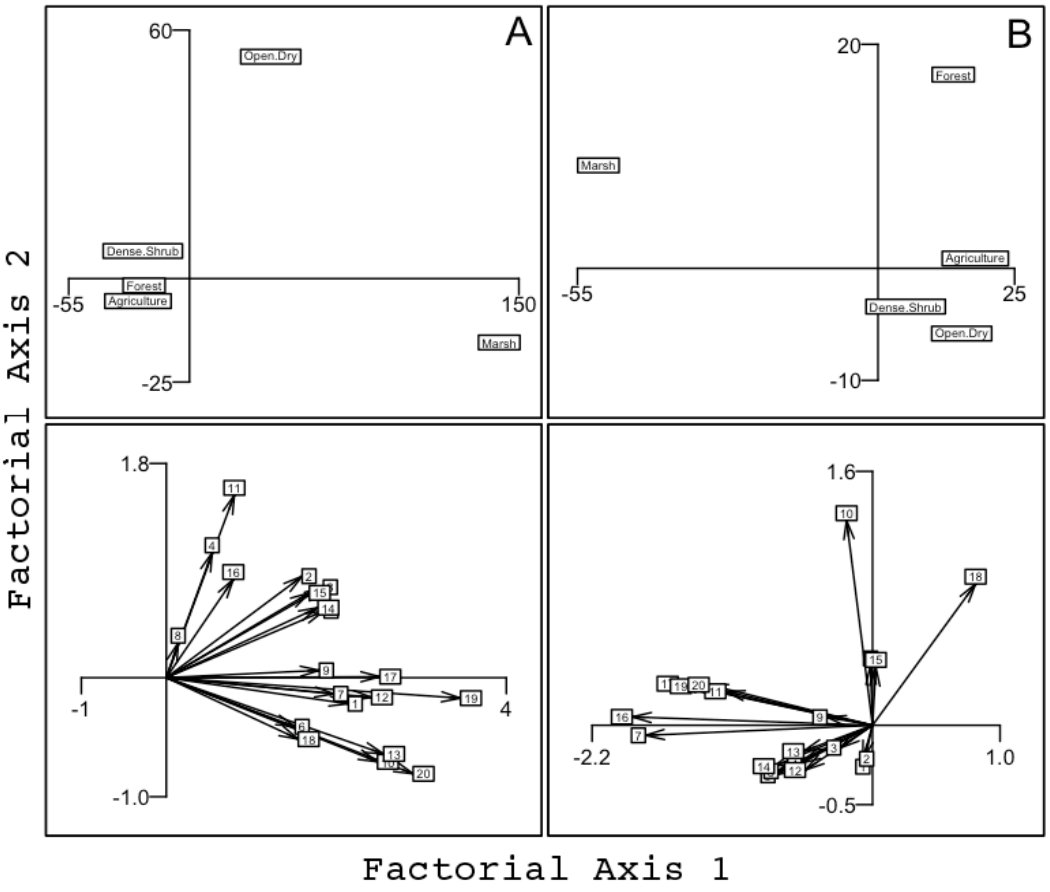

Figure A1.2. Results of eigen analysis (see text for details) with habitat loadings for each habitat type (top) and eigenvectors for each individual foxsnake (bottom), comparing habitat proportions within minimum convex polygon home-ranges of eastern foxsnakes to available habitat composition (circle centered on the hibernation site with a radius equal to the home-range length for each individual) for radio-tracked eastern foxsnakes at A) a highly fragmented (HMCA) and B) a low fragmented (PPNP) site in southwestern Ontario.

Appendix 2

Ecological Niche Factor Analysis methods and results for eastern foxsnakes across southwestern Ontario.

Ecological Niche Factor Analysis compares the landscape characteristics (derived from environmental and topographical maps) at locations used by individuals of a species to characteristics observed across the entire study region (Hirzel *et al*. 2002) to determine landscape scale habitat use patterns and derive habitat suitability maps. ENFA does not require absence data, making it particularly suited to secretive species, such as snakes, where absence is difficult to determine. Here we use ENFA to quantify habitat suitability patterns for eastern foxsnakes across southwestern Ontario.

Methods

Ecological Niche Factor Analysis

We conducted ENFA in Biomapper 3.2 (Hirzel *et al*. 2002) with 10 environmental descriptors at 40 m^2 resolution (Table A2.1). We used 722 occurrence records, resulting in 510 presence cells (cells with more than one occurrence were not weighted higher). Most records (~72%) were collected by our research team and consisted of live captures and road kills, while the remaining records were obtained from government employees conducting survey work. Agriculture was not included in the analysis because it comprised the majority of the region and, therefore, we considered positive selection for the other habitat types as avoidance of agricultural fields. We first standardized (transformed to standard deviations from the mean) and then Box-Cox transformed (as suggested by the manual) all variables to normalize the distribution of values in each map. Using ENFA, we determined the overall marginality (difference from mean

availability) and specialization (ratio of global variability to species variability) for foxsnakes in this region (Hirzel *et al*. 2002). ENFA also condenses the original variation into a reduced number of factors with the contribution for each variable on each factor measured by the magnitude and direction (in the case of marginality) of its score (Hirzel *et al*. 2002).

We used the factors of the ENFA to derive a habitat suitability map with the geometric mean method, which calculates the geometric mean from each cell to all presence points and assigns a suitability score between 0 and 100% (Hirzel & Arlettaz 2003). We determined the number of factors to retain for the habitat suitability calculation by comparing the eigenvalues with MacArthur's broken-stick distribution (Jackson 1993; Hirzel *et al*. 2002).

Table A2.1. Ecological variables used in ENFA to quantify landscape scale habitat use patterns for eastern foxsnakes (*Pantherophis gloydi*) across southwestern Ontario.

Variable	Description
	Distance
D.Marsh	Distance to nearest marsh (m)
D.Open	Distance to nearest unmaintained open habitat (m)
D.Water	Distance to nearest open water (m)
D.Drain	Distance to neatest drain or small creek (m)
D.Resid	Distance to residential or urban area (m)
	Density
Marsh.Den	Density of marsh habitat
Open.Den	Density of unmaintained open habitat
Drain.Den	Density of drain or small creeks
Resid.Den	Density of residential or urban areas
Road.Den	Density of roads

To evaluate the predictive power of our habitat model, we used a Boyce Index (Boyce *et al*. 2002) with a moving window (20 classes) (Hirzel *et al*. 2006) and our presence only data in a 10-fold cross-validation approach (Fielding & Bell 1997; Hirzel *et al*. 2006). This approach divides the data into 10 partitions (using 9 to build the model and 1 for evaluation) and evaluates the predictability by comparing the ratio of the predicted frequency of evaluation points based on the model (P) in each habitat class to the expected frequency (E) based on a random model (Hirzel *et al*. 2006). Boyce's Index varies between -1 and 1, with zero representing a random model. Subsequently, we divided the suitability scores into habitat classes by examining a plot of P/E ratio for each habitat class. Classes where the ratio was < 1 were classified as unsuitable because there are less evaluation point presences within that habitat class than expected by chance. Areas with constant values within the plot cannot be distinguished from one another and therefore were grouped in successively higher classes (Hirzel *et al*. 2006).

Results

Ecological Niche Factor Analysis

The global marginality (1.5) and specialization (1.2) demonstrated that individuals selected cells far from the global mean and with a narrower distribution of values than are present within the study area. The strongest variables in the marginality factor showed that individuals were much more likely to be found in cells with a low distance to, and with a high density of, surrounding marshes and semi-natural open habitat (Table A2.2). No single specialization factor explained a significant amount of the specialization making interpretation difficult. On the first and second axes, however, open and marsh

variables had the strongest values, showing that foxsnakes were specializing on these habitat types.

Analysis using MacArthur's broken-stick distribution suggested that we retain 7 of the 10 axes, which explained 96% of the total explained variation (100% of the marginality and 91% of the speciation). The Boyce's Index (0.836 ± 0.16) from the 10-fold cross validation was far from zero indicating that our model had relatively good predictive power. We used the P/E curve to divide the suitability scores into four habitat classes (Fig A2.2): unsuitable (0-30), marginal (30-41), suitable (41-81), and optimal (81-100). Unsuitable habitat was classed as values with a P/E ratio <1 and the other divisions were made where there were obvious changes in the trends of the graph (Hirzel et al. 2006).

Table A2.2. Correlations between ecological variables (Table 1) and ENFA factors. The first factor explains 100% of the marginality and percentages in brackets indicate the amount specialization explained by each factor. The number of symbols indicates the strength of marginality (factor 1) or degree of specialization (factor 2-7) and zeros indicate no significant difference between factor and global scores.

Variable	Factor 1 (15%)	Factor 2 (21%)	Factor 3 (15%)	Factor 4 (15%)	Factor 5 (13%)	Factor 6 (11%)	Fact 7 (8%)
D.Marsh	----	0	******	*	*****	0	**
D.Open	----	******* *	**	****	**	*	**
D.Water	----	**	*****	*	**	****	**
D.Drain	0	*	***	***	***	*****	*
D.Resid	-	0	**	**	****	****	*****
Marsh.Den	+++++	0	*	0	**	*	**
Open.Den	+++++	*****	**	****	******	*	****
Drain.Den	0	***	****	****** *	*	*****	*
Resid.Den	0	*	*	0	**	****	*****
Road.Den	+	0	*	**	0	***	****

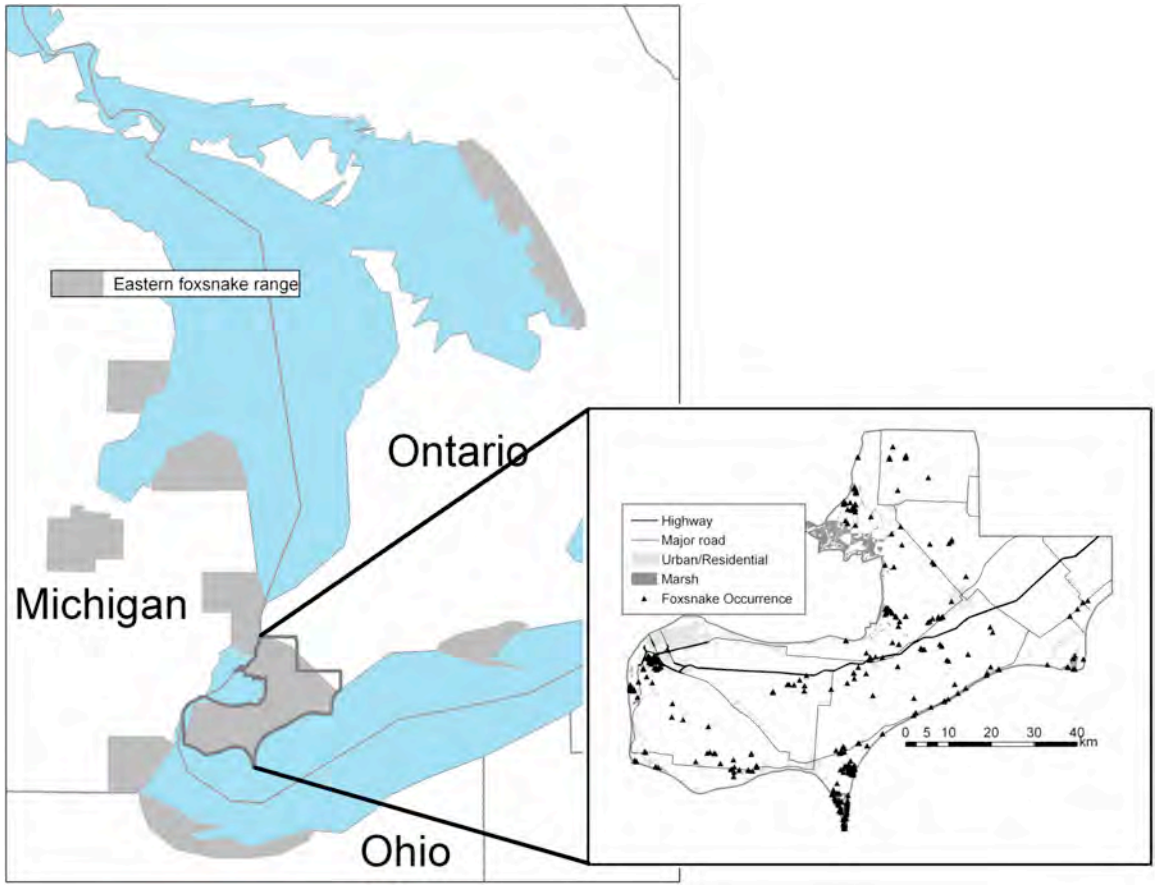

Figure A2.1. Current approximate range of eastern foxsnakes (*Pantherophis gloydi*) in grey with region of ecological niche factor analysis shown in inset. Triangles correspond to occurrence records.

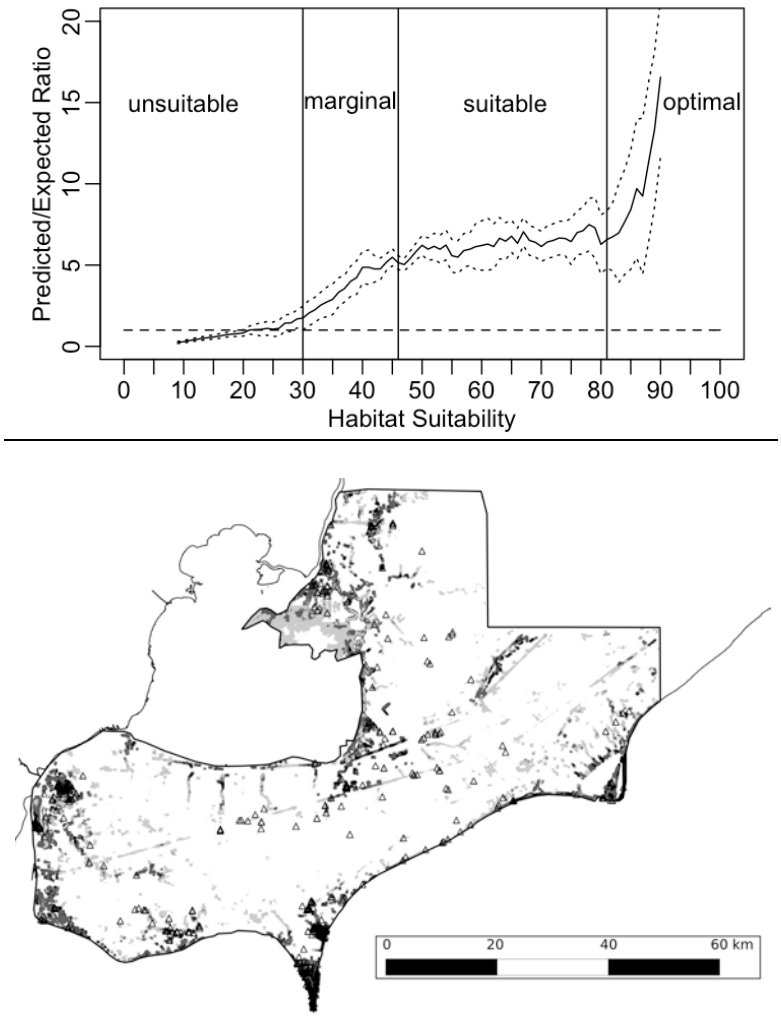

Figure A2.2. a) Habitat suitability classes (derived from Ecological Niche Factor Analysis) based on the predicted/expected ratio of evaluation points within a 20 class moving window and based on a 10 fold cross validation (see text for details). b) Resulting habitat suitability map for eastern foxsnakes outlining unsuitable (white) to optimal (black) habitat. Open triangles are foxsnake occurrence records.

Literature Cited

Boyce MS, Vernier PR, Nielsen SE, Schmiegelow FKA (2002) Evaluating resource selection functions. *Ecological Modelling,* **157,** 281–300.

Fielding AH, Bell JF (1997) A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environmental Conservation,* **24,** 38–49.

Hirzel AH, Arlettaz R (2003) Modeling habitat suitability for complex species distributions by environmental-distance geometric mean. *Environmental Management,* **32,** 614–623.

Hirzel AH, Hausser J, Chessel D, Perrin N (2002) Ecolocial-niche factor analysis: how to compute habitat-suitability maps without absence data? *Ecology,* **83,** 2027–2036.

Hirzel AH, Le Lay G, Helfer V, Randin C (2006) Evaluating the ability of habitat suitability models to predict species presences. *Ecological Modelling,* **199,** 142–152.

Jackson DA (1993) Stopping rules in principal components analysis: a comparison of heuristic and statistical approaches. *Ecology,* **74,** 2204–2214.

Appendix 3

Table A3.1. Number of samples, locations, cluster names (Chapter 4) and population names (Chapter 5) for samples used throughout this thesis.

Location	Cluster Name	Population Name	N
Ojibway/Lasalle	Group 1	SWont 1	48
Holiday Beach	Group 1	SWont 1	16
Cedar Creek	Cedar	SWont 3	28
Ruscom	Group 2	SWont6	16
Big Creek	Group 2	SWont6	26
Lambton	Group 2	SWont6	27
Chatham	Group 2	SWont6	15
Sheldon Marsh	Group 3	SWont 7	25
Rondeau	Group 3	SWont 7	23
Maumee Bay	Group 4	SWont 2	16
Bass Islands	Group 4	SWont 2	54
Pelee Island	Group 4	SWont 2	34
Kelly's Island	Group 4	SWont 2	13
Point Pelee	Group 5	SWont 4	73
Hillman Marsh	Group 5	SWont 4	68
Talbot	Talbot	SWont 5	28

Norfolk	Norfolk	Norfolk	78
Georgian Bay Islands	NA	GeoBay 1	119
Killbear	NA	GeoBay 2	41
Lower Michigan	NA	L. Mich	33
Illinois	NA	Illinois	27
Wisconscin	NA	Wisc.	12
Upper Michigan	NA	U.Michigan	12

Appendix 4

Non-spatial assignment test results for eastern foxsnakes across southwestern Ontario and northwestern Ohio.

Genetic assignment tests probabilistically assign individuals to populations based on their genotype and allow researchers to identify boundaries between populations and to move away from delineations of populations based on geographic location alone (reviewed in Manel et al. 2005). Because of their superiority at detecting fine scale population structure when genetic clusters are spatially distinct (Chen *et al*. 2007) we primarily used spatial clustering programs throughout this study. However, for comparative purposes we also conducted the analysis with non-spatial assignment tests and present the results below.

Methods

We ran STRUCTURE 2.3.3 (Pritchard *et al*. 2000) for 200,000 (100,000 burn-in) MCMC iterations 20 times from k=1 to k=10 using admixture analysis and default parameters. The ideal cluster number was chosen based on when the values for log probability of data reached a plateau. Following the choice of the number of clusters, we ran an additional 100 replicates for that number of clusters and averaged the top 10 models in CLUMPP 1.2 (Jakobsson & Rosenberg 2007) and displayed clusters using DISTRUCT 1.1 (Rosenberg 2004).

Results

The log probability of data reached a plateau at k = 7 (Fig. A2.1), which was one less cluster identified through the spatial assignment tests. The top 10 runs (based on

highest log probability of data) from 100 replicates were averaged in CLUMPP (Jakobsson & Rosenberg 2007) and produced similar results to the spatial assignment tests (Fig. A2.2), but with more evidence of admixture and some of the fine scale structure absent.

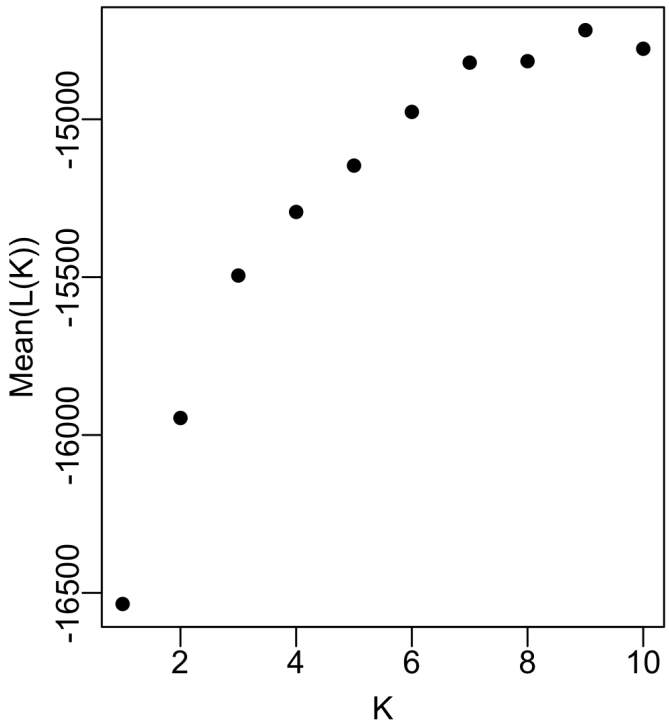

Figure A4.1. Mean log probability of data L(K) as a function of k for 20 replicate STRUCTURE 2.3.3 runs (200 000 MCMC (100 000 burnin) iterations and default admixture parameters) with 585 eastern foxsnakes samples spread across southwestern Ontario and northwestern Ohio.

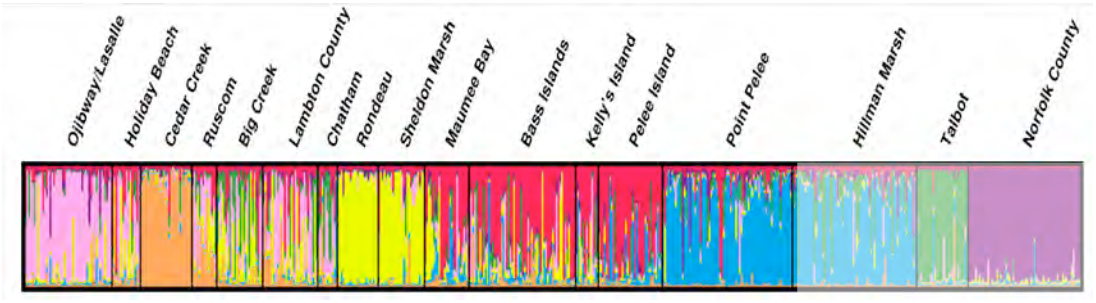

Figure A4.2. Bar plots representing admixture coefficients for eastern foxsnakes from a non-spatial assignment test performed in STRUCTURE 2.3.3. The top 10 runs (highest log probability of data) from 100 replicates were averaged in CLUMPP 1.2 and displayed with DISTRUCT 1.1.

Literature Cited

Chen C, Durand E, Forbes F, François O (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Molecular Ecology Notes,* **7,** 747–756.

Jakobsson M, Rosenberg N (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics,* **23,** 1801–1806.

Pritchard J, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics,* **155,** 945–959.

Rosenberg N (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes,* **4,** 137–138.

Appendix 5

Table A5.1. Prior distribution ranges used for parameters in Approximate Bayesian computation models (Illustrations of models in Fig 4.2a.) designed to estimate the demographic history of foxsnake populations.

Parameters	Model			Values	
Eastern foxsnakes	*Decline*	*Drop*	*Stable*	Min	Max
N.Now	X	X	X	100	3000
N.ancest*	X	X	X	40000	200000
N.bot*			X	20	100
T.drop		X		10	1000
T.decline	X			10	1500
T.stable			X	20	600
T.bot			X	150	1000
Mutation*	X	X	X	1.0×10^{-4}	9.0×10^{-4}
Western foxsnakes					
N.Now	X	X	X	100	20000
T.drop		X		10	3000
T.decline	X			10	3000
T.stable			X	20	1000
T.bot			X	600	4000

*same priors used for eastern and western populations

Table A5.2. Prior distribution ranges used for parameters in Approximate Bayesian computation models (Illustrations of models in Fig 2a.) designed to estimate the demographic history of foxsnake populations in southwestern Ontario.

Parameters	Model			Values	
Eastern foxsnakes	*Bot.Drop*	*Bot.Stable*	*2.Drop*	Min	Max
N.Now	X	X	X	200	2000
N.SWontario	X		X	2000	50000
N.ancest	X	X	X	20000	100000
N.bot	X	X		10	50
T.stable		X		10	500
T.split	X		X	10	500
T.split		X		10	1500
T.Drop			X	1000	2500
T.bot	X	X		500	2500
Mutation	X	X	X	1.0×10^{-4}	9.0×10^{-4}

Table A5.3. Prior distribution ranges used for parameters in Approximate Bayesian computation models (Illustrations of models in Fig 2a.) designed to estimate the demographic history of foxsnake populations across their range.

Parameters	Model			Values	
	Bot.Decline	*Colonize*	*Decline*		
N.nWest	X	X	X	1000	20000
N.nWest.bot		X		20	400
N.sWest	X	X	X	1000	20000
N.swest.bot		X		20	1000
N.swOnt	X	X	X	400	2000
N.bot.swOnt		X		20	400
N.swOntAll	X	X	X	2000	100000
N.Mich	X	X	X	400	2000
N.bot.Mich		X		20	400
N.Norfolk	X	X	X	400	2000
N.bot.Norfolk		X		20	400
N.GeoBay	X	X	X	400	2000
N.bot.GeoBay		X		20	400
N.West	X		X	10000	100000
N.East	X		X	10000	100000
N.Fox	X		X	20000	200000

N.bot.fox	X			100	1000
N.Ancest.fox	X	X		20000	200000
T.split.swOnt	X	X	X	10	80
T.split.EW	X	X	X	200	2000
T.sp.ea	X		X	100	1500
T.sp.Mich		X		200	1500
T.sp.Norfolf		X		200	1500
T.sp.GeoBay		X		200	1500
T.sp.we	X	X	X	200	1500
T.shrink	X	X		1500	3000
T.ancest	X	X		2000	5000
Mutation	X	X	X	1.0×10^{-4}	9.0×10^{-4}