

GENETIC STRUCTURE AND CONNECTIVITY OF THE ENDANGERED BUTLER'S
GARTERSNAKE (*THAMNOPHIS BUTLERI*) ACROSS THE FRAGMENTED LANDSCAPE
OF SOUTHWESTERN ONTARIO

By

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A thesis submitted to the Graduate Program in Biology
in conformity with the requirements for
the degree of Master of Science

Queen's University
Kingston, Ontario, Canada
(September, 2017)

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Abstract

Genetic patterns in many species are affected by environmental features that may enable or obstruct gene flow. Geographical isolation of conspecific individuals by uninhabitable expanses can lead to genetic separation. For species impacted by human activities, understanding how they interact with their environment can promote re-establishment of population connectivity and conservation. *Thamnophis butleri* (Butler's gartersnake) is an Endangered species in Ontario, its range comprising three disjunct regional populations: Essex County, Lambton County, and Luther Marsh. *Thamnophis butleri* is a specialist of wetland-adjacent grassland-type habitat, thus its distribution is constrained by Southwestern Ontario's largely agricultural landscape. Genetic differentiation among populations inhabiting these isolated regions was shown in a single previous study. I explored genetic structure more comprehensively, augmenting the geographic sampling and deploying landscape genetics approaches.

In my first data chapter, I tested for population differentiation in both *T. butleri* and the generalist *Thamnophis sirtalis sirtalis* (eastern gartersnake) where the congeners occur sympatrically in Ontario. Assignment analyses revealed clear genetic structure within *T. butleri*, with clusters corresponding to geographical regions: one each in Lambton and Luther Marsh, and two in Essex, one along the shoreline and another in LaSalle, ON and the Ojibway Prairie Complex. Dispersal by river, either aquatically or along riparian corridors, may facilitate connection in the Lambton and Essex shoreline clusters, while the Ojibway/LaSalle cluster may have no need to disperse (occurring in good-quality habitat) or be impeded from dispersing by intervening urban areas. In contrast, I found that *T. s. sirtalis* comprises a single genetic

population, implying that its generalist ecology allows for dispersal through this heavily altered landscape.

In my second data chapter, I explored the relationship between patterns of *T. butleri* genetic differentiation and landscape composition across its Canadian range, using Mantel-based methods to test the correlation along either uniform or habitat-delineated distance matrices. The relationship between landscape and genetics varied among regional populations, from resistance-based in Lambton to distance-based in Essex. Treating open water as a potential impediment or facilitator of dispersal did not alter my conclusions, raising the possibility of aquatic dispersal that may occur at a different spatial scale than terrestrial dispersal.

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Acknowledgements

Project design

- Dr. Stephen Lougheed has been my supervisor through the long haul, and I must thank him for taking me on and giving me this opportunity to delve into this fantastic field of research.
- Thanks to Jeff Row and Megan Hazell, my co-authors, for working with me on making this thesis what it is today.
- I'd also like to thank the members of my committee, Dr. Vicki Friesen and Dr. Shelley Arnott, for making it to all my committee meetings, and for the suggestions that helped develop my project into a model piece of research.

Field sampling

- First and foremost, I must thank Aamjiwnaang First Nation for the continued collaboration on this project: Dennis Plain and Justin Waters for running most of the 2014 field season and for helping us collect samples in 2015, and Sharilyn Johnston for the coordination that made all this possible.
- Thank you to my field assistants Tori Brown (who kept me sane), Mary Alice Snetsinger (best mom ever), Henry Wang, Louisa Kennett, and Mark Szenteczki for being fast on your feet and subjecting your pants to grass stains in the pursuit of the snakes.
- Thank you to everyone who provided me with supplementary DNA samples: Erin Carroll from St. Clair Region Conservation Authority (SCRCA) for sharing a small piece of the demo Butler's gartersnake, Dan Noble and Jon Choquette for the leftover samples from their study, Megan Hazell and her team at Amec Foster Wheeler for the many Windsor samples, Steve Lougheed and Jeff Row for doing quick field runs, and the anonymous researchers who collected the samples in the Lougheed Lab tissue database.
- Thanks also go to Dan Choquette, Steve Marks, Nick Scobel, and the folks who put together the NHIC database for the help figuring out where to look for snakes.
- Thank you to Sarah Fraser, Kathy Richardson, Anne Marie Laurence, Erin Hellinga, Emilee Hines, and Catherine Jong at the Ontario Ministry of Natural Resources and Forestry (OMNRF) for helping me acquire permission to catch and sample my snakes. Thank to Thomas Goniea at the Michigan Department of Natural Resources (MDNR) for the same.
- Thank you to everyone who gave me permission to access public and private lands to look for snakes: Laura McLean (OMNRF), Crystal Allen, Tony Zammit, and Derek Strub (Grand River Conservation Authority), Erin Carroll, Kelli Smith, and Shane White (SCRCA), Sharilyn Johnston and Dennis Plain (Aamjiwnaang First Nation), Dan Lebedyk and Kathryn Arthur (Essex Region Conservation Authority), Greg Norwood and Jody DeMeyere (Detroit River International Wildlife Refuge), Paul Muelle and Paul Cypher (Huron-Clinton Metroparks),

Alicia Ihnken (MDNR), and Jim Kroetsch, Jeff Currier, and Adam Armaly (CH2M), Jim Gallaway, Jon Choquette, and Eric Jolin (private property access).

- I'd also like to thank all the people who helped out here and there with the field work: Kelli Smith and the rest of the field team at SCRCA, Jared Fedora, and Christopher Moser-Purdy for joining in on one-off field visits; and Dr. Oliver Love from the University of Windsor, for giving me extra collection tubes when I ran out in the field.

Lab work and analysis

- I would like to thank everyone in the Lougheed lab who gave me advice and guidance in the lab, particularly Mark Szenteczki who taught me the essentials from the ground up when I began my project.

- Thank you to all the assistants who helped me streamline the lab process, mainly Erin Suenaga and Tori Brown.

- A big thanks to Zhengxin Sun for genotyping my thousands of PCR runs, and for troubleshooting the problem areas.

- And thank you to those who gave me advice on what analyses to do and how to do them, particularly to Mark Szenteczki for showing me how to read my microsatellite results, Becky Taylor for her advice and help with Arlequin, and Jeff Row for getting me through the landscape analysis R code.

Funding

- Thank you to the folks at the Species at Risk Research Fund for Ontario for funding the first year of my research.

- And thank you to my supervisor Dr. Lougheed for making up the rest with his grant from the Natural Science and Engineering Research Council of Canada.

Emotional support

- I really appreciate the love and support from all my friends and family – it really helped me drag myself through the three years of this project. Shout-outs go to Jen, Dionne, Austin, Steve M., Jake, and Hayley. And especially to my parents, who (almost) never thought I'd end up a career student.

- I want to give a final recognition to Patt Austin, who just passed away this summer. Despite being deathly afraid of snakes, she supported my passion for them throughout my whole life.

- And at last, thanks to our friend the Butler's gartersnake!

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List of Abbreviations

AMOVA: analysis of molecular variance
B-Y: Benjamini-Yekutieli (adjustment)
BGS: Butler's gartersnake
COSEWIC: Committee on the Status of Endangered Wildlife in Canada
COSSARO: Committee on the Status of Species at Risk in Ontario
DIC: deviance information criterion
(mt)DNA: (mitochondrial) deoxyribonucleic acid
DU: designatable unit
EGS: eastern gartersnake
ESU: evolutionarily significant unit
EWR: experiment-wide error rate
GIS: geographic information system
GPS: Global Positioning System
HWE: Hardy-Weinberg equilibrium
IBD: isolation by distance
IBR: isolation by resistance
K: number of population clusters
LCP: least-cost path
LD: linkage disequilibrium
MCMC: Markov chain Monte Carlo
MU: management unit
OMNRF: Ontario Ministry of Resources and Forestry
PCR: polymerase chain reaction
Q: matrix of membership assignments
SARA: Species at Risk Act
UTM: Universal Transverse Mercator
WMA: Wildlife Management Area

Chapter 1. General Introduction

1.1. Factors shaping population genetic structure

As human presence continues to grow and impact natural spaces, it becomes increasingly important to understand how the organisms displaced by our activities are affected by the alterations we inflict upon the landscape. A major influence of anthropogenic activities in terms of conservation of biodiversity is habitat loss; indeed, even many of the other ways we affect biological systems (e.g. introduction of non-native species, pollution, climate change) can be intertwined in some way with it (Stein et al. 2000). Habitat loss is thus an area of major ecological and conservation concern, and an important focus of applied research.

There are many ways that habitats may be negatively affected by humans, including degradation, fragmentation, and outright destruction. These are often conflated in the literature, but it is helpful to identify them as distinct albeit related types of habitat loss (Fahrig 2003). Habitat degradation (also called disturbance) indicates an environment that is altered in key attributes relevant to a particular species (e.g. Hannah et al. 1995, Marvier et al. 2004, Devictor et al. 2008). Habitat fragmentation occurs when a landscape becomes isolated into small patches of habitat (e.g. Tilman et al. 1994, Marvier et al. 2004, Devictor et al. 2008). Habitat destruction can be used to describe areas where suitable conditions for a species are completely removed by human activities (e.g. Baur and Baur 1993, Tilman et al. 1994, Marvier et al. 2004).

Among the many causes of anthropogenic habitat loss, the most widespread and influential in terms of the diminution of biodiversity are agricultural and urban land conversion (Stein et al. 2000, Brooks et al. 2002, He et al. 2014). The creation of farmland can eliminate considerable swaths of natural habitat simply due to the sheer area that is involved, especially land conversion from industrial-scale agriculture. Many instances of habitat destruction caused

by agricultural land change are vestiges of historical human expansion, before nature conservation was ever a widespread practice (Kelly 1975, Brooks et al. 2002); this is especially true for regions that have been continuously occupied for millennia like the Middle East, China and Europe. Urban expansion on a large scale is obviously more recent, but in a shorter time has had comparable impact (Stein et al. 2000), with urban growth destroying large areas of natural habitat in areas with high human densities (He et al. 2014).

Associated with human population growth and development are their ever-increasing effects on ecosystems and species, the latter of which may face novel selective pressures. For example, some bird species are more strongly depredated in forests adjacent to agricultural land than in intact forests (Schmiegelow and Mönkkönen 2002). This is a consequence of an edge effect, where changing community structure and microenvironments in boundary areas between degraded and intact habitats have negative fitness consequences for some resident species. Large mammals typically avoid such edge habitats – which can increase fatal interactions with humans – and are confined into progressively smaller areas as more habitat is removed (Kinnaird et al. 2003). For some amphibians, the degradation of buffer zones around the wetlands they inhabit puts them in proximity with adjacent developed lands, and they face greater threat of local extinction (Harper et al. 2008).

Habitat loss is not a threat that exclusively progresses inward from the habitat borders. Fragmentation is the result of patchwork landscape changes that divide habitat into isolated parcels, and can lead to population decline of resident species (Bender et al. 1998). Small population sizes can result, increasing the risk of lower genetic diversity through genetic drift and inbreeding, and also increasing the possibility for local extinction (Frankham 2005). Fragmentation impacts plant as well as animal species, causing increased selfing and lower

genetic diversity, which has been shown to affect rare and common species alike (Honnay and Jacquemyn 2007). Genetic consequences of such population isolation occur across taxa. Large mammalian predators like the grizzly bear, *Ursus arctos*, reside in small, genetically-isolated populations as a consequence of habitat fragmentation across their native western North American range (Proctor et al. 2005), as do amphibians like the Endangered dusky gopher frog, *Rana sevosa*, which is found in a single isolated population with reported low genetic variability (Richter et al. 2009). Genetic differentiation among isolated remnant populations can correlate with patterns of landscape features (e.g. eastern foxsnake, *Pantherophis gloydi*, see Row et al. 2010).

Human-created transportation corridors (e.g. roads, railroads, canals) can be major agents of habitat fragmentation by physically blocking animal movement. Roads can affect species diversity by acting as barriers to gene flow, isolating previously contiguous populations, and also decreasing population recruitment and abundance through vehicular mortality (N. D. Jackson and Fahrig 2011). Many animals are affected by road presence, particularly terrestrial animals, although the problem is not limited to specific taxa. For example, reduced genetic diversity as a result of road-caused isolation has been reported for invertebrates like the flightless violet ground beetle, *Carabus violaceus* (I. Keller and Largiadèr 2003), mammals like the desert bighorn sheep, *Ovis canadensis nelsoni* (Epps et al. 2005), amphibians like the eastern red-backed salamander, *Plethodon cinereus* (Noël et al. 2007), and reptiles like the timber rattlesnake, *Crotalus horridus* (R. W. Clark et al. 2010). The subdivided remnant populations have smaller census and effective population sizes, particularly because roads prevent dispersal and gene flow precluding movement of novel alleles across landscapes (N. D. Jackson and Fahrig 2011). Indeed, some species appear to show active road avoidance contributing to this demographic and

genetic isolation: e.g. the massasauga rattlesnake, *Sistrurus catenatus*, and the box turtles *Terrapene carolina* and *T. ornate* (Shepard et al. 2008). For other taxa, road mortality rather than avoidance causes isolation; this is particularly notable in some owls (Bishop and Brogan 2013), mammals like the Iberian lynx, *Lynx pardinus* (Ferrerias et al. 1992), and many herpetofaunal species (Ashley and Robinson 1996, Row et al. 2007). Mitigation measures, such as walls and ecopassages, can be implemented to reduce fragmentation and road mortality (S. D. Jackson et al. 2015b), but those may prove ineffective if animals are unwilling to use them (Baxter-Gilbert et al. 2015).

Habitat destruction and fragmentation are growing and inescapable threats to natural ecosystems and organisms around the world. Conservation biologists and wildlife managers are turning increasingly to genetic and genomics tools and new spatial analytical approaches to understand the consequences of such habitat alteration. Such approaches afford opportunities to quickly and efficiently survey patterns of genetic structure and diversity that reflect the integrated signature of habitat fragmentation, and resulting altered gene flow, genetic drift and inbreeding that has accrued across multiple generations.

1.2. Population genetics research and Bayesian cluster assignment

Since population genetics arose in the early 20th Century, researchers in the field have sought to quantify spatial genetic patterns and understand the processes that underlie them. Some of the earliest and most influential works in the field were published within a few years of each other, each work contributing to our understanding of how natural populations acquire and retain genetic structure; Ronald A. Fisher (1930), Sewall Wright (1931), and John B. S. Haldane (1932) applied mathematics to the theories of Darwin and Mendel. These works collectively set up the

theoretical foundations for modern population genetics that we use as a framework to understand the impacts of contemporary human activities on natural systems (Provine 1971).

Sewall Wright expanded on this earlier work, developing a metric for quantifying population differentiation that remains widely used: the fixation index or F (S. Wright 1949), which apportions the total genetic diversity among and within populations into different components. F_{ST} , the measure of substructure, is the most commonly reported, theoretically varying from zero (no differences in allelic frequencies among surveyed populations – thus panmixia) to one (complete fixation of alternate alleles at each surveyed locus) (S. Wright 1949). Departures from Hardy-Weinberg equilibrium (Hardy 1908, Weinberg 1908) or linkage equilibrium (Lewontin and Kojima 1960) can provide clues as to the processes that are influencing patterns of genetic diversity. For example, the Wahlund effect is caused by undiagnosed population subdivision or population admixture and is evidenced by reduced observed heterozygosity relative to predicted values across loci (Wahlund 1928).

Researchers were more easily able to test these ideas with the advent of polymerase chain reaction (PCR) in the late 20th Century. PCR allowed for easy replication of targeted segments of DNA, like simple sequence repeats (e.g. DNA microsatellites) and small sequence differences (e.g. single-nucleotide polymorphisms) (e.g. Luikart et al. 1998, A. G. Clark 1990).

Contemporary evolutionary ecology uses genetic approaches to address a wide variety of questions, from phylogenetics among focal organisms and speciation, to the structure and dynamics of natural populations within species.

Most modern population genetics research incorporates Bayesian inference. Bayesian statistics use observed variables to calculate probable distributions of some unknown parameters, essentially calculating an informed or conditional probability. This is typically applied to

population or spatial genetics and genetic clustering using Markov Chain Monte Carlo (MCMC) methods to repeatedly sample genotypes of a set of individuals, which builds a probable membership distribution for a number (K) of population clusters designated *a priori* (Beaumont and Rannala 2004). MCMC methods help to surmount the great computational challenges of population structure inference using large genetic datasets that became increasingly available with PCR (Garibyan and Avashia 2013) and next-generation sequencing (Schuster 2008).

Many programs use Bayesian approaches to infer population structure (Excoffier and Heckel 2006). One of the most popular and widely-used is STRUCTURE (Pritchard et al. 2000), which assigns individuals to one of K genetic clusters with the assumption that populations are genetically stable, there is no linkage disequilibrium (LD), and that individuals mate randomly within each cluster (i.e. Hardy-Weinberg equilibrium, HWE). STRUCTURE can be run with one of two ancestry models to produce a matrix of membership assignments (Q). The no-admixture model assumes that each individual, like its genetic progenitors, belongs to a single genetic cluster, assigning membership to the most probable parent population; the admixture model allows individuals to have originated from more than one cluster, with the Q-matrix showing the proportions of an individual's genome that most resemble each cluster (Pritchard et al. 2000, François and Durand 2010). The admixture model may be more widely applicable for natural populations, as dispersal and subsequent interbreeding are common in nature. Moreover, the admixture model is robust to scenarios where little or no ancestral mixing has occurred, while the reverse is not true for the no-admixture model (François and Durand 2010).

Under the program defaults, both models assign cluster membership using only the provided genetic data (e.g. microsatellites or single-nucleotide polymorphisms), not accounting for any variation derived from the organisms' environment. Bayesian priors (LOCPRIOR) can be

incorporated into either of the above models, placing prior weight in favour of clusters that correlate with the locations from which the individuals were sampled (Hubisz et al. 2009); however, it only uses the discrete locations provided, not accounting for geographic distance between individuals or sample locations. The program TESS (Chen et al. 2007) includes geographical coordinates from each individual sampled, assigning higher correlation to individuals from nearby areas than distant ones, all else being equal (François and Durand 2010). This spatial approach is thought to produce more robust structure inferences (Durand et al. 2009b) when dispersal is limited by distance. As with STRUCTURE, TESS can incorporate both no-admixture or admixture models (Chen et al. 2007).

To determine the genetic structure evident for any species, analyses can be run for a range of selected values of K that typically spans from fewer than to greater than the hypothesized number of clusters (e.g. the number of geographically disparate sampling locales or the number of isolated habitat patches). Methods for selecting the most representative cluster number vary depending on the program, as they are based on different algorithms. For STRUCTURE, we can calculate the mean log likelihood of the data given a value of K , $\ln P(D|K)$ or $\ln P(D)$, and determine visually at which K value the curve reaches a plateau (Pritchard et al. 2000, François and Durand 2010). Evanno et al. (2005) built on this method to develop their ΔK approach, which uses the second order rate of change in the log likelihood between K s. In TESS, we estimate and plot the deviance information criterion (DIC) of each K (Spiegelhalter 2002), and again the optimal value of K is chosen based on where the curve plateaus (Durand et al. 2009b). Unlike $\ln P(D)$, the DIC plateaus toward a minimum value rather than a maximum.

Potential weaknesses of Bayesian analysis programs like STRUCTURE and TESS include spurious diagnosis of the number and membership of genetic clusters due to populations that are

not in equilibrium, i.e. not meeting the assumptions of Hardy-Weinberg and linkage disequilibria within genetic populations (Chen et al. 2007). A bias of STRUCTURE is that it typically cannot correctly identify structure where there is a genetic gradient, e.g. isolation by distance (IBD) (Meirmans 2012). There has been a suggestion too that TESS may provide spurious results under some scenarios of IBD (Guillot 2009, Durand et al. 2009a). Because of these caveats, researchers often combine multiple assignment programs to estimate the cluster number and spatial patterns to overcome the weaknesses of any one program (e.g. Row et al. 2010, Cothran et al. 2011). The optimal values of K from different programs sometimes disagree, as their underlying models make different assumptions (François and Durand 2010); but even if there is disagreement among approaches, the distribution of genetic clusters may be informative of the overall structure.

1.3. The interaction of genetics and landscape features

Population genetics, including insights from new Bayesian approaches, tell us how a species is genetically partitioned across its range, but the reasons for this subdivision can usually only be inferred indirectly. In part, this is because conventional approaches do not incorporate data on geography, topography, habitat, or other environmental variables. As I have indicated above, some relatively new Bayesian analyses, like those encoded in STRUCTURE (Pritchard et al. 2000) cannot include prior information on geographic distances between sampling locales (although can include sampling locales themselves as ‘priors’ via LOCPRIOR). Other programs that do include pairwise geographic distances, like TESS (Chen et al. 2007), model genetic variation as a function of straight-line geographic distance only. This method of mapping genetic similarities of individuals across an ‘undifferentiated’ geographic landscape (i.e. assuming homogeneity of landscape features and no impediments to gene flow; see Proctor et al. 2007,

Noël et al. 2007, Honnay and Jacquemyn 2007) does not account for how real landscape heterogeneity and life histories of focal organisms might inform genetic structure. The discipline of landscape genetics arose to integrate population genetics with the spatial variation described by landscape ecology (Manel et al. 2003, Manel and Holderegger 2013). The relationship between landscape features and phenotypic variation within species has long been recognized (e.g. de Candolle 1820, Wallace 1860). However, it was not until the early 2000s that research on the relationship between genetics and geography began to flourish. The seminal 2003 publication by Stéphanie Manel and colleagues – although not the first to advance the idea of landscape genetics (e.g. see Gerlach and Musolf 2000) – is widely credited as being the trigger that launched the field of landscape genetics (Turner and Gardner 2015).

Landscape genetics studies typically analyse the impact of landscape configuration on genetic population structure and gene flow using simple and partial Mantel non-parametric permutation tests (Manel and Holderegger 2013). These tests determine the correlation between two or more matrices (Mantel 1967); in landscape genetics, Mantel tests are often used to investigate the relationships between matrices of geographical distances or matrices that summarize landscape (predictors), and a matrix of genetic distances (dependent). Genetic distance matrices can be built by determining the proportion of shared alleles between individuals (Manel and Holderegger 2013), although there are many different metrics. Geographic distances are typically straight-line distances between individuals or sites, often corrected for the curvature of the earth. Landscape distances can be measured from a geographic information system (GIS) overlay of the study area – where the landscape is divided into a raster grid of land cover types – by identifying passages along areas of habitat (Manel and Holderegger 2013). A simulated homogenous landscape (i.e. where all pairwise distances are based on geographic

distances) can be used as a null model in landscape genetics. Simple Mantel tests compare the genetic distance matrix with a single landscape matrix, while partial Mantel tests incorporate multiple matrices, e.g. to measure a landscape effect while controlling for IBD (Legendre and Fortin 1989).

Mantel tests use causal modeling, quantifying the correlation between the matrices, and quantifying how well each landscape matrix explains the matrix of pairwise genetic distances (Cushman et al. 2006). A basic testing method uses least-cost path (LCP) models, which for each pairwise comparison determines the ‘optimal’ route through a resistance landscape (Adriaensen et al. 2003). LCP tests can be useful for identifying geographic barriers to dispersal and setting conservation management goals (Gerlach and Musolf 2000, Adriaensen et al. 2003; Cushman et al. 2006); however, LCP analysis has been criticized as not being biologically realistic because most landscapes would have multiple pathways of dispersal (McRae 2006). The isolation by resistance (IBR) model was designed to improve on LCP by allowing for multiple dispersal passages between sample points. Based in circuit theory (McRae 2006), IBR methods model gene flow across landscapes using the same algorithms that describe electrical current in a circuit: connectivity increases with the number of pathways between points and passage is obstructed to some degree by resistant elements (McRae et al. 2008). The IBR approach to modeling connectivity offers advantages over other models and can be more intuitively applied to habitat description and management (McRae et al. 2008).

The Mantel test is the dominant statistical tool for landscape genetics, but is not without criticism. Some potentially limiting factors include highly correlated cost distances among resistance models (Cushman et al. 2013, Zeller et al. 2016), low detectability of landscape effects with high habitat connectivity (Cushman et al. 2013, Zeller et al. 2016), and sensitivity to

violations of linearity (Legendre et al. 2015, Zeller et al. 2016). Researchers must control for these limitations to produce robust results from Mantel tests (Zeller et al. 2016).

1.4. Conservation genetics

Conservation biologists connected with the field of genetics as soon as it could be reasonably applied to natural systems, using it to inform their approaches in preserving species and habitats. Early studies in conservation genetics consisted of surveying levels of genetic variation and making inferences about key aspects of population demographics: e.g. determining genetically effective population size (N_e), estimating genetic connectivity, and identifying phenomena like hybridization and population bottlenecks (Lande 1988, Hedrick and Miller 1992, Roy et al. 1994, Luikart et al. 1998). Such efforts continue to be valuable (e.g. Milián-García et al. 2015), but the field has expanded as new tools and analytical approaches became available. For example, more in-depth DNA-based genotyping has permitted easier phylogenetic classification and population diagnoses, and thus allows for identification of animals to facilitate control of illegal wildlife trade (Holland and Hadfield 2002, Fong et al. 2007). And being able to compare how genetically differentiated species interact varyingly with environmental features across their range has led to more targeted conservation efforts and prioritization (R. W. Clark et al. 2010, Row et al. 2010, Manel and Holderegger 2013).

If species across a wide area consist of more than one demographically independent genetic cluster or population, identifying the pressures acting on each in the different locales can help to create more cogent and relevant conservation strategies (D. Keller et al. 2015). When reconciling scientific recommendations with the needs of policy-makers, the selection of units for conservation must be standardized. This led to the conception of evolutionarily significant units (ESUs). First developed for informing captive breeding programs (Ryder 1986), an ESU is

an isolated subset of a species that has become genetically diverged from conspecifics. ESUs typically cover the broadest genetic differences, often used in examining a species across most or all of the range, e.g. it was recommended that the Komodo dragon, *Varanus komodoensis*, be assigned multiple ESUs corresponding to different islands where it occurs (Ciofi et al. 1999). The concept of ESUs has been debated in the literature, and definitions have been modified to include other facets of genetics. For example, Moritz (1994) suggested that ESUs need not be restricted to scenarios where there is both geographic and genetic isolation (Moritz 1994). Not all conservation units are defined by complete monophyly as is typically the case with ESUs; for example, management units (MUs) are characterized by divergence in allele frequency between groups, implying some demographic independence and restriction of gene flow (Moritz 1994, Palsbøll et al. 2007). Often applied in short-term management applications, MUs can be particularly useful for gauging and mitigating the effects of human activity (Palsbøll et al. 2007). Critiques and disputes of what can be considered an ESU or MU (e.g. Paetkau 1999) can distract from conservation planning, which is what led to the proposal of designatable units (DUs) as an alternative for the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and the Canadian government (Green 2005). DUs account for genetic differences, but are not limited by them; designation is based on a set of biological guidelines that include ecological, genetic, and biogeographical differences (Green 2005). This combination of genetics and organismal biology is biologically relevant, as applying conservation plans based solely on genetic structure can result in management failure (Taylor and Dizon 1999). Evaluating each case individually will allow for better decisions for maintaining species at risk.

1.5. Study species

My study focuses on two snake species: the Endangered Butler's gartersnake, *Thamnophis butleri*, and the Least Concern eastern gartersnake, *Thamnophis sirtalis sirtalis* (Figure 1.1D). Members of the genus *Thamnophis*, the gartersnakes are colubrid snakes endemic to North America (Rossman et al. 1996).

1.5.1. Butler's gartersnake

Butler's gartersnakes, *Thamnophis butleri*, are small, striped snakes, typically reaching 38-51 cm in length at sexual maturity (Rowell 2012). *Thamnophis butleri* are distinguished from sympatric congeners (Figure 1.1D) by the lateral stripes centred on the 3rd scale row extending onto scale rows 2 and 4, by their proportionately small heads that are barely wider than the neck, and by their dark, rich brown colour (Rossman et al. 1996). This colouration helps maintain crypsis when *T. butleri* emerge to bask or hunt, though they will often remain hidden under vegetation or loose debris (Rowell 2012).

Gartersnakes tend to be most active when their bodies are at temperatures of 25-35°C (Peterson et al. 1993). Average body temperature for *T. butleri* during their active season has been reported as 26.1°C, varying between 12.4-34.0°C (Carpenter 1956). Gartersnakes can obviously persist outside their optimal temperatures – in particular, they overwinter in environments below 10°C (Peterson et al. 1993) – but both high temperatures (above 40°C) and low temperatures (below 0°C) can be lethal to exposed *Thamnophis* snakes (Lueth 1941). To maintain a temperature equilibrium, gartersnakes engage in behavioural thermal regulation by choosing appropriate microhabitats (Peterson et al. 1993). *Thamnophis butleri* may sometimes be found basking in the open (COSEWIC 2010) or moving on very hot days, although they typically move to shaded areas when temperatures are high (Carpenter 1956). More frequently,

during their active season, they are found thermoregulating under both natural and artificial cover objects, in situations that require either warming and cooling (COSEWIC 2010, Joppa et al. 2009). When a *T. butleri* individual is found, it is often first detected as a noise under layers of grass or only as daylight is fading, particularly during the summer (Logier 1939). That time of day coincides with the habits of their main prey.

The diet of *T. butleri* consists primarily of Palearctic earthworms, supplemented with leeches (which were probably their principal diet before the introduction of the invasive earthworms) (Carpenter 1952, Catling and Freedman 1980). Their feeding strategies are specialized to their preferred prey. For example, *Thamnophis butleri* were observed *in situ* by Catling and Freedman (1980), probing the earth with their snouts when attempting to locate earthworms or flailing their heads underwater when seeking leeches. Catling and Freedman (1980) also observed, in a controlled experiment, that *T. butleri* were faster at detecting and striking earthworms than *T. s. sirtalis*. The foraging microhabitats of *T. butleri* have not been specifically studied, but it is likely that they regularly hunt under cover objects, especially during the day. This behaviour would be consistent with the secretive nature of the species, and would increase their chances of encountering prey. Earthworms are more likely to move to the surface in cool, moist, and dark conditions (Lee 1985).

While rarely seen during the majority of their active season, *T. butleri* will venture into the open to mate. After their emergence from hibernation in late March or early April (Rossman et al. 1996, Rowell 2012), males will follow pheromone trails to seek reproductive females (Ford 1982), sometime forming aggregations ('mating balls') around a single female (Finneran 1949). After spring mating, gravid female snakes move to safe locations while their offspring develop (Ford and Burghardt 1993). Female *T. butleri* bear their young until July or August, when they

produce litters of between 4 and 20 neonates (Rowell 2012). In late October, *T. butleri* will congregate with other snakes to seek refuge for overwintering in old animal burrows or rocky crevices on south-facing slopes (Carpenter 1953, Morgan 1939, Rossman et al. 1996).

Although there is some movement for reproduction and hibernation, few *T. butleri* travel broadly over their active season. Macartney et al. (1988) calculated mean activity ranges from two individual studies: 8,000 m² over one season for a Michigan population (Carpenter 1952) and 200 m² for an Ontario population in the middle of a season (Freedman and Catling 1979). Although they are known to remain in small areas, census population sizes are not widely known for *T. butleri*, particularly in Ontario, because their demographics are not widely studied and because many sites where they once existed have been destroyed (COSEWIC 2010). The few large *T. butleri* populations in Ontario persist in areas with some level of protection, where their preferred habitat is maintained (COSEWIC 2010). These areas mostly belong to local conservation authorities or areas operated by private conservation initiatives, with some parcels owned and managed by the Ontario Ministry of Natural Resources and Forestry (OMNRF). Most of the Canadian *T. butleri* range, however, consists of remnants that are at risk of habitat loss (COSEWIC 2010).

Thamnophis butleri exist sporadically across a range that extends from Western Ohio and northeastern Indiana, up through eastern Michigan, and into Southwestern Ontario, with two disjunct populations in southeastern Wisconsin and at Luther Marsh Wildlife Management Area (WMA) in Ontario (Figure 1.1A). In Southwestern Ontario, the species occurs in non-contiguous population pockets from the Sarnia area in Lambton County southwest to Windsor and Amherstburg in Essex County, and also in a highly isolated population at Luther Marsh WMA, which lies at the intersection of Dufferin and Wellington counties (Figure 1.1B). There are other

isolated locations recognized as part of the Ontario *T. butleri* range – Parkhill, ON and Skunk’s Misery Complex in Bothwell, ON – but no sightings have been reported in these sites for at least 20 years (COSEWIC 2010). *Thamnophis butleri* is likely extirpated from these locations, as it is from Rondeau Provincial Park and localities along much of the southern and eastern shoreline of Lake St. Clair (COSEWIC 2010). Additionally, several known *T. butleri* sites within the Essex-Lambton range have been lost or are threatened due to land development (Rowell 2012). Disappearance of *T. butleri* in these areas has been attributed to loss of viable habitat either through destruction or succession. Habitat loss and diminution of numbers has led to the species being listed as Endangered by COSEWIC, by the federal Species at Risk Act (SARA), by the Committee on the Status of Species at Risk in Ontario (COSSARO), and by the OMNRF (COSEWIC 2010, COSSARO 2011, OMNRF 2016, SARA 2017).

Ideal *T. butleri* habitat is described as comprising wet meadows or prairies near wetlands (Rowell 2012). European colonization of Southwestern Ontario in the 19th Century is coincident with the beginning of the decline of habitats in this region. Open prairies were prime choices for early colonists due to the ease of setting up farms in areas with few trees to clear. In the mid-to-late 1800s, many wetlands were drained as a low-cost means to create additional farmland (Kelly 1975). Today, Southwestern Ontario retains few of its original wetlands. Extreme Southwestern Ontario (referring to Essex, Kent, and Lambton counties) lost over 95% of the wetlands that existed pre-settlement (c. 1800) (Ducks Unlimited Canada 2010).

Of course, all this farmland was and is beneficial for the Canadian populace. Potential arable land makes up only about 5% of the country, and over half of the prime agricultural land is in Ontario – most in Southern Ontario (Environment Canada 1976). Agricultural lands in Ontario are decreasing, however, primarily due to urban expansion (Walton and Hunter Planning

Associates et al. 1999). The southern part of the province is facing human population growth, particularly around large population centres (Ontario Ministry of Finance 2016). This can lead to competition for land use, as farmland owners and advocates fight urban sprawl for use of the landscape (e.g. Watkins et al. 2003). The struggle between housing and feeding the human population has the potential to sideline the habitat needs of *T. butleri*, and other species that share its habitat needs. Henslow's sparrow (*Ammodramus henslowii*), northern bobwhite (*Colinus virginianus*), and eastern prairie fringed orchid (*Platanthera leucophaea*) are only a few Endangered species that depend on grassland habitat (Tallgrass Ontario 2005). Some prairie-associated species (e.g. Karner blue butterfly, *Lycaeides melissa samuelis*, and greater prairie-chicken, *Tympanuchus cupido*) are already extirpated from Ontario's grasslands (Tallgrass Ontario 2005, OMNRF 2016).

While direct human activities have destroyed much *T. butleri* habitat, human presence has also led to less new habitat being created naturally. Prior to the arrival of Europeans, natural fires (i.e. due to lightning) and those set by First Peoples (to better hunt game or to increase living space) were normal, and led to expansion of grassland landscape (Axelrod 1985, Bakowsky and Riley 1994). Settlers feared the destructive nature of fire – having come from lands where forests were scarce and trees were cultivated to maturity as an important resource – and fire suppression became commonplace (Weakley 1999, H. A. Wright and Bailey 1982). Trees began to succeed the grasses (Axelrod 1985), as woody plants will overtake open spaces without fire to prevent them (Bragg and Hulbert 1976). This means that *T. butleri* have less space available in general, and more specifically, that there are fewer fire-formed spaces. Small-bodied snakes will disperse to recently burned new habitat, possibly due to reduced competition and predation (Larson 2014).

Dispersal of *T. butleri* may also be affected by the network of roads cutting across their range. Many small snake species will avoid roads (Shine et al. 2004, Andrew and Gibbons 2005), and some snakes will become immobile in response to traffic increasing probability of mortality (Andrew and Gibbons 2005). There are also records of Endangered gartersnakes being killed in substantial numbers by road strikes (Dalrymple and Reichenbach 1984). *Thamnophis butleri* are thought to avoid roads (Freedman and Catling 1979), but they do not do so completely, as there are records of vehicular mortality (Choquette 2011).

Landscape fragmentation, habitat loss, and isolation because of a network of roads can create a patchwork of genetically isolated subpopulations (e.g. R. W. Clark et al. 2010). *Thamnophis butleri* has already been shown to have some genetic signature of habitat fragmentation and reduced connectivity in Ontario (Noble et al. 2013). However, there are still many questions as to how these snakes have been shaped by a landscape heavily altered by human activities.

1.5.2. Eastern gartersnake

The common gartersnake (*Thamnophis sirtalis*) can be found over much of temperate eastern Canada and United States, and over a large portion of western North America as well (Figure 1.1C). In Ontario specifically, *T. sirtalis* is present across the southern two-thirds of the province. Perhaps because of the species' extensive geographic distribution there are several subspecies, from nine to twelve according to different compilations (Collins and Taggart 2009, Crother 2012); however, the eastern gartersnake (*Thamnophis sirtalis sirtalis*) is the only subspecies co-distributed with *T. butleri* within my study area (Rossman et al. 1996) and is thus the focus of my study.

Thamnophis s. sirtalis resembles its Endangered counterpart phenotypically (Figure 1.1D), though it is larger at sexual maturity, typically achieving lengths of 46-66 cm long (Rowell 2012). Other differences include a proportionately bigger head, lateral stripes falling on scale rows 2 and 3, and highly variable colouration and markings (Harding 1997). Like *T. butleri*, the striped patterns of *T. s. sirtalis* help them blend in when they are hiding under or moving through grass.

Regular sightings of *T. s. sirtalis* activity are because of foraging or thermoregulatory behaviour. Eastern gartersnakes are highly opportunistic predators, consuming a variety of vertebrates and invertebrates, but they primarily eat earthworms and anurans (Catling and Freedman 1980, Dalrymple and Reichenbach 1981). They depend heavily on visual cues to hunt (Heinen 1994), and have been observed lifting their heads to sight prey (Catling and Freedman 1980). This strategy may be why they are successful anuran predators, as such behaviour could allow them to target the quick-moving prey from a distance. When digesting their prey, or often when simply adjusting their internal temperature, *T. s. sirtalis* can be largely immobile. They can be found seeking refuge under natural or artificial cover objects (Cox et al. 2009, Engelstoft and Ovaska 2000, Huey et al. 1989), but are also frequently observed sunning in the open throughout their active season (Burger 2001, Gibson and Falls 1979, Shine et al. 2000).

Thamnophis s. sirtalis have a long active season in Southern Ontario, and are mainly observed between late March and early November (Rowell 2012). They have a similar thermal regime to *T. butleri* (Dalrymple and Reichenbach 1981, Peterson et al. 1993, Shine et al. 2000), with a broader operational temperature (9.0-35.0°C) (Carpenter 1956). Reproductive habits of *T. s. sirtalis* are also comparable to those of *T. butleri* (Clesson et al. 2002, Ford 1982, Gardner 1955), though *T. s. sirtalis* litters are on average bigger, producing from 6 to 78 young in Ontario

(Rowell 2012). At the end of their active season, *T. s. sirtalis* aggregate in groups, often with other snake species, in below-ground cavities (Carpenter 1953, Morgan 1939), but occasional winter sightings (on mild days) indicate that these snakes can choose to be active essentially year-round (Rowell 2012).

In addition to being active longer than *T. butleri*, *Thamnophis s. sirtalis* also travel farther. In the same studies as discussed for *T. butleri*, *T. s. sirtalis* were recorded as having similar or slightly larger mean range sizes: 8,000 m² in Michigan (Carpenter 1952), and 2,400 m² in Ontario (Freedman and Catling 1979). The largest reported range size for *T. sirtalis* was 142,000 m² for males, 92,000 m² for females in a Kansas population of the red-sided gartersnake, *T. sirtalis parietalis* (Fitch 1965). Eastern gartersnakes are able to take full advantage of their wider range because they can use most habitat types, being most often observed in open areas in the proximity of water bodies (Rossman et al. 1996, Rowell 2012), which includes all habitat types used by *T. butleri*.

Due to their success in many different habitats, the size of their home ranges, and their varied diet, *T. sirtalis* are one of the most common snake species. Many other snake species are negatively impacted by anthropogenic encroachment (Row et al. 2007, Row et al. 2010, R. W. Clark et al. 2010). While this also necessarily affects *T. sirtalis* (e.g. they may choose to avoid roads, or if they do cross, males are less able to follow female scent trails, Shine et al. 2004), the species persists and indeed is common in most human-altered landscapes. Population sizes have not been widely recorded, only estimated for specific sites (e.g. Carpenter 1952, Larsen and Gregory 1989), but they are often the most common snake observed across their range (Ontario Nature Reptile and Amphibian Atlas, https://www.ontarionature.org/protect/species/threats_to_reptiles_and_amphibians.php, see also: Rossman et al. 1996). It has been reported that, for the

most part, their populations are stable (Larsen and Gregory 1989, Rossman et al. 1996). Indeed, human presence may even result in population growth for the species, because these habitat generalists can thrive in disturbed habitats and because the human-introduced Palearctic earthworms are a major part of their diet (Rowell 2012).

1.6. Study objectives

Genetic differences among *Thamnophis butleri* populations in Ontario have not been investigated in depth; the single genetic study of the Canadian range of the species (Noble et al. 2013) was not conclusive on the numbers of genetically-distinct populations nor did it include explicit consideration of spatial genetic patterns and their relation to landscape and habitat preferences. COSEWIC (2010) considers the whole Canadian population to be a single DU, due to sparse and conflicting ecological and genetic information.

One of the overarching goals of my study is to diagnose the number of distinct genetic clusters that exist over the Canadian portion of the *Thamnophis butleri* range using augmented geographic sampling, increased number of DNA microsatellites, and deploying microsatellites that were explicitly designed for the species. I predict that the number of distinct genetic clusters will relate positively to the distribution of remnant habitats with breaks corresponding to tracts of urbanized, or heavily altered agricultural lands as *Thamnophis butleri* are habitat specialists (Marvier et al. 2004). Thus there should be at least three genetic clusters, corresponding to Luther Marsh, Lambton County, and Essex County. There may be additional clusters within the latter two areas because of strong local barriers to dispersal, including dense urban habitation and heavily utilized 4-lane roads. I also analyse co-distributed *Thamnophis sirtalis sirtalis* from across the same sampling range for the same suite of DNA microsatellite markers. Because *T. s. sirtalis* are habitat generalists and should use all habitats including urban gardens and margins of

agricultural lands, I predict that they should be less impacted by landscape fragmentation than the habitat specialist *T. butleri* (see Dileo et al. 2010).

I also use landscape genetics methods to determine whether *T. butleri* dispersal and gene flow are driven by the habitat suitability of landscape features (i.e. IBR) rather than IBD. I will compare pairwise genetics distances with pairwise resistance distances for varying resistance levels of non-habitat landscapes, using both simple and partial Mantel tests (Legendre and Fortin 1989), from which I will infer how much pressure is imposed by resistant landscape types. This will enhance knowledge of the species' distribution in Ontario, its genetic connectivity, and its relation to variation in habitat.

Ultimately, I will combine insights on spatial genetic patterns with inferred landscape associations of *Thamnophis butleri* to make recommendations for conservation planning that will hopefully improve habitat connectivity and increase the likelihood of the species' persistence in Southwestern Ontario.

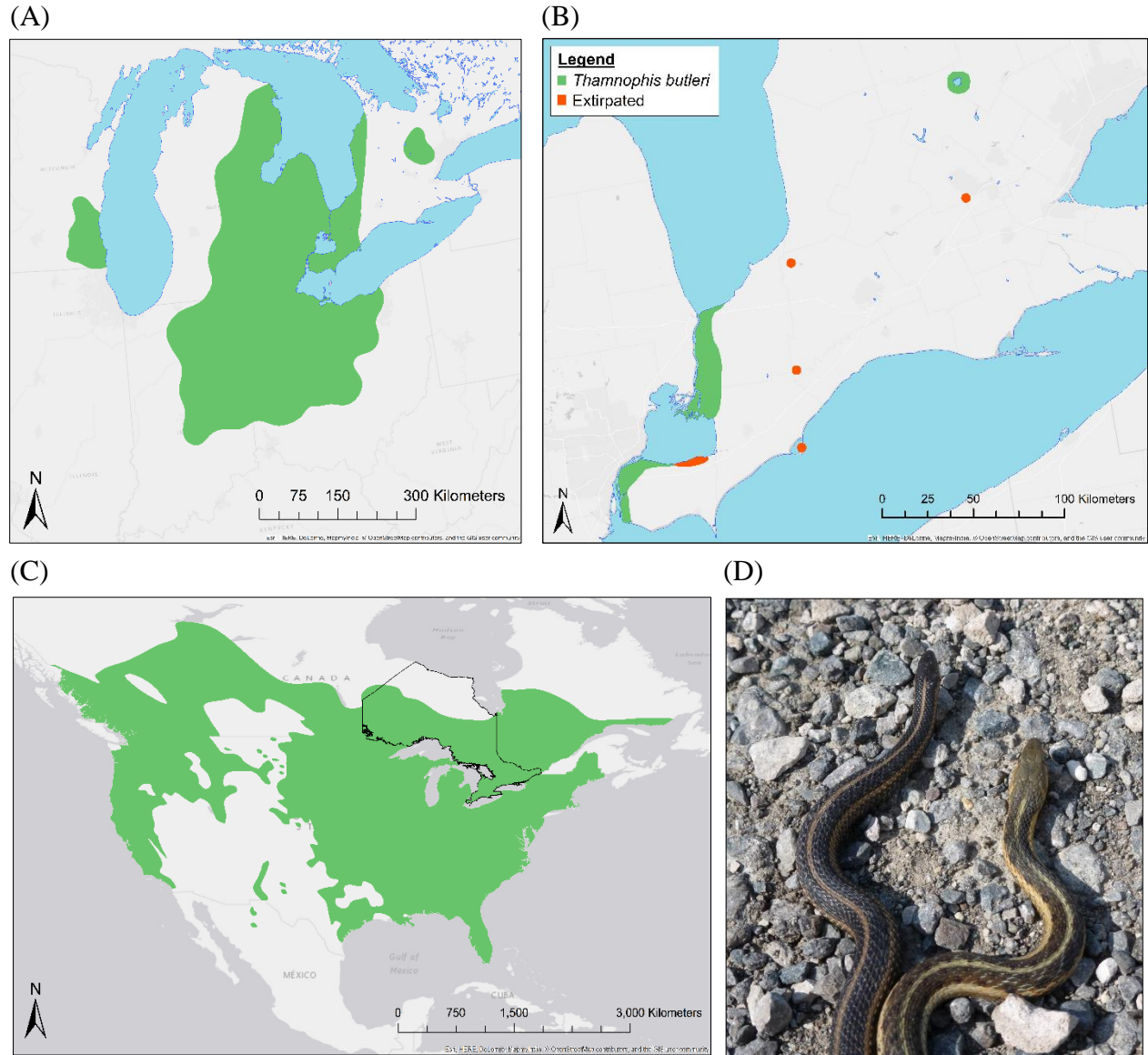


Figure 1.1. (A) Generalized global range of *Thamnophis butleri* (IUCN 2016). (B) Approximated Ontario range of *T. butleri*, based on species sightings (COSEWIC 2010, also: Ontario Nature Reptile and Amphibian Atlas, https://www.ontarionature.org/protect/species/threats_to_reptiles_and_amphibians.php). Populations with no recently confirmed sightings have been labeled as extirpated on this map. (C) Generalized global range of *Thamnophis s. sirtalis* (IUCN 2016), with an outline of Ontario to illustrate the Ontario range of the species. Maps created using ArcMap (ESRI 2015). (D) *T. butleri* (left) and *T. s. sirtalis* (right) from Aamjiwnaang in Lambton County, Ontario.

Chapter 2. Comparing the population structure of the specialist Butler's gartersnake (*Thamnophis butleri*) and the generalist eastern gartersnake (*Thamnophis sirtalis sirtalis*) in Ontario

2.1. Abstract

Variation in life history means that species may respond differently to the same features within a shared landscape, and thus also may show different responses to human-induced habitat fragmentation. We evaluated genetic structure and connectivity of two gartersnake species – a habitat specialist and a generalist – found in primarily in Southwestern Ontario, where much of the land use is agricultural and the road network is dense. We quantified the genetic structure of the specialist Butler's gartersnake (*Thamnophis butleri*) and generalist eastern gartersnake (*Thamnophis sirtalis sirtalis*) across the known Canadian and adjacent Michigan distribution of *T. butleri* using 15 DNA microsatellites, 12 for *T. butleri* and 9 for *T. s. sirtalis*. Bayesian clustering, both spatial and non-spatial, and pairwise comparisons revealed genetic differentiation between the three regional populations of *T. butleri* at Essex County, Lambton County, and Luther Marsh Wildlife Management Area, with further structure within the Essex region between *T. butleri* found in LaSalle and the Ojibway Prairie Complex and those found along the shoreline of the Detroit River. We found a single *T. s. sirtalis* genetic cluster across the entire sampling region, implying that it is not as impacted by habitat loss and isolation. There was no evidence that major rivers prevented gene flow, as individuals from Michigan were grouped with adjacent Ontario clusters. Genetic clustering of *T. butleri* suggests that patches of suitable habitat surrounded by inhospitable habitat preclude dispersal and gene flow, consonant with what has been found for other species within Ontario with similarly disjunct distributions.

Differentiation within the Essex region also probably reflects anthropogenic barriers that impede movement.

2.2. Introduction

Uncovering how genetic structure and diversity vary within a species is a core objective of population genetics, and can increase our understanding of how organisms react to the extrinsic pressures that they encounter. Different environmental features can facilitate or impede dispersal, and consequently, gene flow (McRae et al. 2008). Human population growth and the development that accompanies it can have myriad effects. Organisms may be exposed to novel environments to which they are not adapted, and some may fail to adapt and thus face local extinction (Harper et al. 2008). Large-scale land conversion is common across the globe, particularly for farmland or urban expansion (Stein et al. 2000, He et al. 2014), leaving the remaining habitat divided into fragments, with small populations that have increased probability of lower genetic diversity and genetic isolation (Proctor et al. 2005, Row et al. 2010). Even the barrier of a single road can effect demographic change, by leading to vehicle strikes of animals attempting to cross (Row et al. 2007, Bishop and Brogan 2013) and preventing gene flow (Noël et al. 2007, R. W. Clark et al. 2010). These changes do not impact all species equally. Generalist species may do moderately well in disturbed landscapes, while habitat specialists may react poorly (Devictor et al. 2008). Genetic analyses can uncover in what way and to what degree habitat fragmentation shapes dispersal and connectivity.

Species with specific habitat requirements can have reduced genetic diversity when encountering anthropogenic landscape changes (Fahrig 2003). For example, in contrast to other subspecies the range of the western massasauga rattlesnake (*Sistrurus catenatus tergeminus*) has areas that are only partially unfragmented; in those areas with habitat continuity its genetic

structure is homogeneous, while distinct genetic clusters correspond to populations that are isolated, as occurs range-wide for the other *S. catenatus* subspecies (McCluskey and Bender 2015). For Yunnan snub-nosed monkeys (*Rhinopithecus bieti*) living on the Tibetan Plateau, human-caused habitat gaps correlate strongly with subpopulation genetic structure (Liu et al. 2009). Human activity does not always have a negative effect; indeed, some efforts to repair connectivity can assist species in disturbed areas, e.g. wood frogs (*Lithobates sylvaticus*) that occupy human-made wetlands in Edmonton have maintained one genetic population (Furman et al. 2016).

In this paper we quantify the genetic structure of the Butler's gartersnake (*Thamnophis butleri*), a habitat specialist, and compare it to the sympatric generalist eastern gartersnake (*Thamnophis sirtalis sirtalis*), from the same sites in extreme Southwestern Ontario and nearby Michigan. *Thamnophis sirtalis* is found across an array of habitats spanning forests, wetlands, fallow agricultural fields, open scrub and rocky outcrops throughout North America. Although *T. sirtalis* has many subspecies, only *T. s. sirtalis* is co-distributed with *T. butleri* (Rossman et al. 1996). *Thamnophis butleri* occurs in wetland-adjacent grassland habitat, found intermittently in Michigan and its surrounding states, and Ontario. Its Canadian range – the disjunct regions of Essex County, Lambton County, and Luther Marsh Wildlife Management Area (WMA) – lies entirely within the human-dominated landscape of Southwestern Ontario (Rowell 2012).

In Ontario, the region southwest of Toronto is primarily dedicated to agriculture, as it contains much of Canada's prime farmland (Environment Canada 1976). Agricultural land use in this area became widespread after European settlement, particularly in open grasslands that could be easily tilled (Tallgrass Ontario 2005) and in wetlands that could be drained and filled (Kelly 1975). This land conversion resulted in widespread loss of natural habitats, restricting and

fragmenting the ranges of species that depended on them (Tallgrass Ontario 2005, Ducks Unlimited Canada 2010). The region also has a dense network of roads, with no more than 1.5 km distance between any two (Ontario Nature Reptile and Amphibian Atlas, https://www.ontarionature.org/protect/species/threats_to_reptiles_and_amphibians.php).

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) has classified *T. butleri* as Endangered because it exists in patches of remnant habitat that are isolated across the fragmented landscape, and it is extirpated from some areas where it was previously known to occur (COSEWIC 2010). It seems unlikely that gene flow is occurring among these isolated patches, as *T. butleri* does not travel great distances, and may be somewhat deterred by roads (Freedman and Catling 1979). Genetic division has previously been shown for the species, although the number of genetic clusters was not definitive (Noble et al. 2013). In the present study we quantify genetic structure across the co-distributed ranges of these two congeners. Although they exist in a shared landscape, the habitat specialist *T. butleri* should be more impacted by human-caused habitat loss and fragmentation than its generalist counterpart. With fewer individuals that occur in more isolated populations, we predict that *T. butleri* will exhibit less genetic diversity than *T. s. sirtalis*, and in Ontario will be composed of at least three genetically distinct population clusters: Luther Marsh WMA, Lambton County, and Essex County. In contrast, we predict that *T. s. sirtalis* will consist of either a single population over its unbroken range or at most will show patterns consistent with individual-level isolation by distance (S. Wright 1943). Our findings here for *T. butleri* in Ontario can be used to help delineate designatable units (DUs) and thus set conservation priorities for the species.

2.3. Materials and methods

2.3.1. Sampling locations & methods

We acquired blood and tissue samples from *Thamnophis butleri* (Butler's gartersnakes, BGS) and *Thamnophis s. sirtalis* (eastern gartersnakes, EGS) throughout the known Ontario range of the former and from their adjacent Michigan range. *Thamnophis s. sirtalis* co-occurs with *T. butleri* (Figure 1.1).

We obtained samples (Figure 2.1) over four planned field seasons in 2013 through 2016, and from other researchers who collected samples in 2008, 2009, and 2011. Samples derive from three major regions where these snakes are found: Luther Marsh WMA, in the Grand River watershed ($n_{\text{BGS}} = 10$, $n_{\text{EGS}} = 11$); Lambton County, including the city of Sarnia and localities to the south ($n_{\text{BGS}} = 94$, $n_{\text{EGS}} = 87$); and Essex County, comprising Windsor and sites to its east and south including some islands in the Detroit River ($n_{\text{BGS}} = 114$, $n_{\text{EGS}} = 40$). Potential sampling sites were identified using observations from the Natural Heritage Information Centre (NHIC, <https://www.ontario.ca/page/natural-heritage-information-centre>), supplemented with satellite imagery and knowledge of basic habitat references for *T. butleri* (Harding 1997). We did not obtain samples from Walpole Island, an unceded First Nations reserve known to have both species; we searched but did not find *T. butleri* at Skunk's Misery Complex and in Essex County along the southeast side of Lake St. Clair where the species has been extirpated (COSEWIC 2010). We also obtained samples from Michigan, in St. Clair, Macomb, and Wayne counties along the Great Lakes system, including one island in the Detroit River ($n_{\text{BGS}} = 15$, $n_{\text{EGS}} = 25$).

All field visits occurred during the active season for *Thamnophis* snakes, on clear or partly clear days with temperatures between 15-30°C when snakes are most active (Peterson et al. 1993). We conducted active searches by walking transects through likely habitat, and

surveying visually for gartersnakes. We also checked under natural and human-made cover objects, as a method of more effectively detecting these cryptic animals (Halliday and Blouin-Demers 2015). In addition to live snakes located with these methods, we also sampled road-killed and lawn-mower struck snakes.

Live snakes were captured by hand, and released at the point of capture after processing. We identified the sex of each individual using external tail morphology, and recorded basic morphological measurements (e.g. snout-vent length, vent-tail length, mass) as per our standard snake field protocol. Using either a 29-, 30- or 31-gauge, sterile ½ cc insulin syringe, we extracted a small amount of blood (typically 50-100 µL, always < 250 µL) from the snake's caudal vein 5-10 mm from the tip of the tail, which was cleaned with an alcohol swab prior to sampling. For the few snakes from which blood could not be drawn, typically very small snakes (< 10 g), we clipped < 5 mm from the distal end of the tail. For snakes found dead (road or mower mortality), we clipped a substantial piece of skeletal muscle with skin (2-3 cm). We preserved all tissue samples in 95% ethanol. Sampling site for each snake was recorded using a Garmin Etrex Legend handheld GPS unit in Universal Transverse Mercator (UTM) coordinates.

2.3.2. Laboratory methods

DNA was extracted following the QIAGEN DNeasy Blood & Tissue Quick-start Protocol (QIAGEN 2011), with minor modifications. We measured DNA concentration in extracted samples using a NanoDrop ND-1000 Spectrophotometer (NanoDrop, Wilmington, DE, U.S.A.). Extracted DNA was stored in a -20°C freezer until subsequent analysis.

We chose 22 microsatellite primer sets (forward and reverse) designed for various species (*T. butleri*, *T. sirtalis*, *Thamnophis elegans*, *Nerodia sipedon sipedon*) from various publications (Sloss et al. 2012, Prosser et al. 1999, Garner et al. 2004, Garner et al. 2002, Manier and Arnold

2005, McCracken et al. 1999). With a selection of ~50 extracted *T. butleri* and *T. s. sirtalis* DNA, we ran trial polymerase chain reactions (PCRs) for all primer pairs (see below). Seven of the microsatellite loci were unsuitable (e.g. did not amplify, amplified fragment sizes that are inconsistent with their published repeat motif), but the remaining 15 primer pairs (Table 2.1) worked with both species. Appropriate annealing temperatures were determined using the gradient function of an Eppendorf Mastercycler gradient thermal cycler.

After individual locus testing, each amplification reaction was run as a 2-locus multiplex-PCR containing 10 μ M M13-labeled forward primer and unlabeled reverse primer for both loci (Eurofins Genomics, Toronto, ON, Canada for *3Ts* primers; Invitrogen, Burlington, ON, Canada for all other primers); we used 0.2 μ L of each for the locus with a shorter size range, and 0.4 μ L of each for the locus with a longer size range. The 10 μ L reaction also contained 2 μ L of 10-100 ng/ μ L genomic DNA, 5.5 μ L *Taq* PCR MasterMix (QIAGEN Inc., Toronto, ON, Canada), 1 μ L of 1 μ M labeled Well-Red M13 (Sigma-Aldrich Canada Co., Oakville, ON, Canada), and 0.3 μ L RNase-free double-distilled H₂O. For each plate (~60 reactions), DNA samples were chosen blindly with respect to species and location of origin to minimize biases.

The reactions with primers *Nsm2*, *Nsm3*, *TbuA09*, and *TbuA74* were amplified with an initial denaturing step at 95° C for 5 m, 40 cycles with 95° C for 30 s, annealing at 50° C for 30 s, and extension at 72° C for 45 s, and a final extension at 72° C for 5 m. Thermal cycling conditions for the reactions with other primers were identical but with varying annealing temperatures. *Te1Ca3*, *Te1Ca29*, *TbuA49*, and *TbuA64* had an annealing temperature of 51° C; *Ts010* and *TbuA27* had an annealing temperature of 52° C; *Ts2* and *3Ts* had an annealing temperature of 54° C; *TbuB12* had an annealing temperature of 56° C; *TbuA04a* and *TbuA70* had an annealing temperature of 58° C. Samples were amplified in one of a GeneAmp PCR System

2700 thermal cycler and a GeneAmp PCR System 9700 thermal cycler. Microsatellite genotyping was done with a Beckman Coulter CEQ 8000 Genetic Analysis System (Beckman Coulter, Mississauga, ON, Canada).

For samples where individual microsatellites could not be scored from duplex reactions, we amplified them singly. The protocol was the same as above, except with only one set of primers, and 0.9 μ L RNase-free double-distilled H₂O to keep the volume at 10 μ L. For individuals with lower DNA concentrations (~ 10 ng/ μ L) that failed to amplify, we ran a nested PCR using amplified product from the first reaction (for a few individuals at various primers).

2.3.3. Population structure analysis

We scored microsatellite genotypes with GeneMarker 2.6.3 (SoftGenetics, State College, PA, U.S.A). We assembled the data into separate Microsoft Excel (Microsoft, Mississauga, ON, Canada) files for each species, and binned the fragment sizes using the sort and plot tools.

For all loci – for each species separately – we tested for null alleles, and scoring and typographic errors using MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004). These tests were done for 12 distinct *T. butleri* samples locations, nine of which also had *T. s. sirtalis* samples. Sampling locations were defined as an area \leq 10 km in diameter containing at least five *T. butleri* samples (Table 2.2, Figure 2.2; note exceptions for sparsely-sampled locations, for isolated individuals, and between Aamjiwnaang and North Lambton specifically). *Thamnophis s. sirtalis* samples were grouped into the same locations; if they did not fall within a *T. butleri* location, they were assigned to the closest location.

After formatting the microsatellite data for Arlequin using GenAlEx 6.503 (Peakall and Smouse 2006, 2012), we used Arlequin 3.5.2.2 (Excoffier and Lischer 2010) to test for linkage

disequilibrium between each all pairs of loci and for deviations from Hardy-Weinberg Equilibrium (HWE) for all loci, for all previously determined locations.

We estimated pairwise genetic differentiation between all population pairs using Sewall Wright's F_{ST} (1949) and Slatkin's R_{ST} (1995). The R_{ST} statistic assumes a stepwise mutation model (it sums squared size differences) that is considered to be more appropriate for microsatellites (Valdès et al. 1993, Slatkin 1995). However, at sample sizes below $n = 50$, R_{ST} can have a large sampling variance and F_{ST} is potentially more accurate (Ruzzante 1998). However, F_{ST} can underestimate differentiation if the microsatellites have a high mutation rate (Balloux and Lugon-Moulin 2002). We used both statistics to accommodate these differences in accuracy and interpretation. To control for Type I errors that can occur with multiple tests, we used both a Bonferroni correction (Rice 1989) and a Benjamini-Yekutieli (B-Y) adjustment (Benjamini and Yekutieli 2001), as recommended by Narum (2006).

We also evaluated genetic structure of *T. butleri* and *T. s. sirtalis* using Bayesian clustering analysis in the programs STRUCTURE 2.3.4 (Pritchard et al. 2000) and TESS 2.3.1 (Chen et al. 2007, Durand et al. 2009b). Both programs use multilocus genotype data to assign individuals into one of K genetically distinct populations (Chen et al. 2007). STRUCTURE uses non-spatial clustering methods (Pritchard et al. 2000), while TESS incorporates geographic coordinates from individual sampling locations (Chen et al. 2007). For both species in STRUCTURE we used the admixture model, with a burn-in of 50,000 MCMC steps followed by 100,000 steps. We repeated this 20 times for each value of K value from $K = 1$ to $K = 9$, beyond the number of clusters that we predicted for either species. We also re-ran these analyses in STRUCTURE using the LOCPRIOR model, which weights clustering assignment *a priori* using discrete sampling locations (Hubisz et al. 2009). We assessed the number of discrete genetic

clusters in TESS with the same parameters as for STRUCTURE, using the BYM admixture model (Durand et al. 2009b) for K values from K = 2 to K = 9.

Outputs from STRUCTURE and TESS were summarized using CLUMP 1.1.2 (Jakobsson and Rosenberg 2007) to average the 20 repeats of each K value. Barplots of Q-coefficients were constructed using POPHELPER 2.2.0 (Francis 2017) in R 3.3.2 (R Core Team 2016), with each vertical bar representing a single snake and showing the proportion of its genetic assignment attributable to each cluster. We chose the most representative number of clusters for each STRUCTURE and TESS analysis by interpreting the barplots, accounting for the biological context of the different samples. For the STRUCTURE runs, we supported interpretations using the Evanno et al. (2005) method of determining ΔK , and the mean log likelihood for each value of K, $\ln P(D)$. For the TESS runs, we used the average deviance information criterion (DIC) of each K value, and also looked at the overall representation of membership in each cluster from the Q-matrix. We calculated and plotted the results of all the K-value inference tests in R (R Core Team 2016).

We also estimated pairwise F_{ST} and R_{ST} between all pairs of genetic clusters diagnosed by STRUCTURE and TESS, and ran an analysis of molecular variance (AMOVA; Excoffier et al. 1992) to estimate the total genetic variance apportioned among hierarchical sampling levels.

2.4. Results

Of the 15 microsatellite loci surveyed, 14 were polymorphic for *Thamnophis butleri* (TbuA09 was monomorphic) and all 15 were polymorphic for *Thamnophis s. sirtalis*. The average number of alleles per locus varied among sampled regions: 3.7 ± 1.7 alleles (BGS) and 7.3 ± 3.4 alleles (EGS) in Luther Marsh; 8.3 ± 4.9 alleles (BGS) and 17.2 ± 9.6 alleles (EGS) in

Lambton County; 8.7 ± 4.6 alleles (BGS) and 13.7 ± 7.8 alleles (EGS) in Essex County; 7.3 ± 3.2 alleles (BGS) and 12.2 ± 6.0 alleles (EGS) in Michigan.

Results from MICRO-CHECKER suggested no scoring or mistyping errors. However, at three loci for *T. butleri* (Ns μ 2, Ts010, Te1Ca3) homozygote excess implied the possibility of null alleles. These were discarded for subsequent *T. butleri* analyses. At six surveyed loci for *T. s. sirtalis* (Ns μ 3, Ts2, 3Ts, TbuA27, TbuA74, TbuB12) there was a possibility of null alleles. These loci were discarded for all subsequent analyses of *T. s. sirtalis* samples. Because the discarded loci were different for the two species, we re-ran additional STRUCTURE tests using the six loci (Te1Ca29, TbuA04a, TbuA09, TbuA49, TbuA64, TbuA70) that *T. butleri* and *T. s. sirtalis* still have in common.

We found instances of linkage disequilibrium (LD) for both *T. butleri* and *T. s. sirtalis* between loci at most of the defined locations, although fewer if we correct the p values for multiple testing (Bonferroni correction, Rice 1989; B-Y adjustment; Benjamini and Yekutieli 2001) (see Appendix A – Tables A3, A4). Linkage disequilibrium can pose problems for Bayesian analysis programs like STRUCTURE, which assume linkage equilibrium and independence among loci (Pritchard et al. 2000). Strong linkage between loci can result in spurious conclusions regarding genetic structure (Kaeuffer et al. 2007), although weak linkage is unlikely to change conclusions (Falush et al. 2003). With multiple testing, the experiment-wide error rate (EWR; the probability of at least one test being significant by chance) increases (Waples 2015) and some significant linkage disequilibrium may occur by chance. We found the EWR to be as follows: $EWR_{BGS} = 0.97$ and $EWR_{EGS} = 0.84$ at each location. As no pair of loci exhibited disequilibrium at more than three of the 12 locations tested for *T. butleri* and at no more than two of the 9 locations tested for *T. s. sirtalis*, we retained all for our analyses.

We found departures from HWE for both *T. butleri* and *T. s. sirtalis* at most locations (Tables A1, A2). As with LD, Bayesian analyses assume that populations are in Hardy-Weinberg Equilibrium, and strong deviations may cause misinterpretation (Falush et al. 2003, Kaeuffer et al. 2007). For HWE tests at each location, $EWR_{BGS} = 0.46$ and $EWR_{EGS} = 0.37$. For all *T. butleri* loci and eight of nine *T. s. sirtalis* loci, significant HWE departures occurred at no more than three locations; thus we retained these loci for Bayesian analyses. The remaining locus in the *T. s. sirtalis* samples, TbuA70 did not conform to HWE expectations at over half of our sampling locations. To account for the possibility that this could affect the results, we performed STRUCTURE tests both with and without TbuA70. HWE-departures occurred most frequently at the Essex locations for *T. butleri*.

Pairwise population differentiation between *T. butleri* was evident between many sampled locations indicated (Table A5). Luther Marsh, Aamjiwnaang, and Crystal Bay were distinct from most other locations both for F_{ST} and R_{ST} , with other Essex and Lambton locations showing significant differentiation for pairwise F_{ST} only. Most locations that were sparsely sampled – all Michigan locations and Belle Isle – were not significantly different from other populations for either metric. Pairwise comparison between *T. s. sirtalis* (Table A6) did not reveal many significant differences, aside from pairwise F_{ST} results for Luther Marsh compared to the other locales.

Assignment tests analysis of *T. butleri* revealed strong genetic structure, with distinct genetic clusters (Figures 2.3, 2.4). Similar patterns were evident from both TESS and STRUCTURE, indicating four genetic clusters: Luther Marsh, Lambton County, northwestern Essex County, and the remainder of Essex County plus the Detroit River islands (Michigan samples clustered with adjacent Ontario samples) (Figure 2.5). The barplots of cluster membership from

STRUCTURE's non-spatial assignment showed that clusters aligned with sampling locations (Figure 2.3A). At $K = 2$ northern Essex appeared as a cluster, as was the combination of Lambton and Luther Marsh, while south Essex and the islands appeared to be a mixture of these two. At $K = 3$ Luther Marsh, south Essex, and the islands fell out into their own cluster, along with some of the northern-most samples from Essex. At $K = 4$ Luther Marsh occurs as its own cluster. For $K \geq 5$ the additional clusters revealed genetic differences between the Detroit River islands and evenly split larger populations. The spatial assignment barplots from TESS showed similar patterns, though the clusters tended to be more distinct and Luther Marsh appears as a separate cluster at $K = 3$ (Figure 2.4A).

The assessments for number of clusters in *T. butleri* showed some variation around a single value. From the STRUCTURE results, Evanno's ΔK (Evanno et al. 2005) implies $K = 2$ with some support for $K = 4$ (Figure 2.3B). The mean log likelihood plateaued at $K = 4$ (Figure 2.3C). For TESS, the deviance information criterion (DIC) began to plateau at $K = 4$ or $K = 5$ (Figure 2.4B), with overall membership proportions from the Q-matrix showed strong representation for clusters 1 to 4 (Figure 2.4C).

Barplots for *T. butleri* obtained using LOCPRIOR for all 12 loci were well-resolved and showed a clear geographic pattern (Figure A1). Barplots using only the six loci that were in common with *T. s. sirtalis* were less well-resolved, but suggestive of the same genetic pattern (Figure A2). Overall these analyses supported the structure revealed by the preceding analyses.

Some apparent admixture is evident in both STRUCTURE and TESS (Figures 2.3A, 2.4A) for the *Thamnophis butleri* from Essex County. This conforms to patterns of departures from HWE expectations and LD evident in samples from northern Essex (Tables A1, A3). Because strong levels of structure between populations can mask substructure within a population, a

multi-step approach can be useful (Pisa et al. 2015). We re-ran STRUCTURE using only samples from Essex County, including geographically proximate samples from Michigan and the Detroit River islands (Figure 2.6). At $K = 2$, the cluster distribution matches the Essex portion of the larger barplots (Figures 2.3A, 2.4A); snakes from Michigan (Wayne County), the Detroit River islands, and localities in southern Essex are part of one cluster, along with individuals from within the city of Windsor in northern Essex, while Lasalle and the Ojibway Prairie Complex are part of a different cluster (Figure 2.6A,B). Increasing the number of clusters (K) retains the same basic pattern, but also suggests differences between the islands. At $K = 4$ (Figure 2.6A,B), the island populations from Belle Isle and Crystal Bay appear to be closely related, but Fighting Island snakes are more closely allied with those from Windsor. The Amherstburg population appears more genetically similar to some of the Michigan snakes.

Although neither STRUCTURE nor TESS implied admixture at $K = 4$ within the Lambton County population, STRUCTURE revealed some evidence of substructure at higher K values (Figure 2.3A). I thus ran a STRUCTURE analysis on that subset of my samples as well, including the adjacent Michigan samples (Figure 2.7). The snakes from this county exhibited only weak structure; no snake was mapped as strongly belonging to either of two clusters when $K = 2$. The structure that was present suggested that individuals from Aamjiwnaang might be somewhat genetically distinct from the rest of the snakes in the region (Figure 2.7A,B).

Bayesian analyses of our *T. s. sirtalis* individuals implied no genetic structure across their sampled range (Figures 2.8, 2.9). The barplots from the STRUCTURE runs showed approximately equal contribution from each cluster at all sampling locations for all values of K (Figure 2.8A). At $K = 2$ only a third of individuals had greater than 60% of their genetic composition (Q) belonging to any one cluster, and those individuals were randomly distributed across the

landscape. At $K = 4$ all but five individuals were assigned by less than 60% to any one cluster. At all other values of K , no individual had values of Q greater than 60%. While there was some slight suggestion of genetic similarity among a handful of individuals (e.g. four samples from southern Essex County and five samples from Aamjiwnaang), they were not strongly distinct. In TESS, individuals were assigned almost exclusively to the same cluster (Figure 2.9A), aside from one individual from southern Lambton County that was consistently suggested to be admixed and a few others exhibiting a similar pattern at higher values of K (two from south Lambton, one from Aamjiwnaang, one from Luther Marsh).

Assessment of cluster number supported a single cluster in *T. s. sirtalis*. While the Evanno et al. (2005) ΔK method is not designed to identify $K = 1$ in STRUCTURE, the fact that all predicted values of ΔK were small indicates no strong support for any other value (Figure 2.8B). The mean log likelihood of the STRUCTURE results did not rise to a plateau, and its values were highly variable (Figure 2.8C). In TESS, the DIC is at a plateau throughout (Figure 2.9B), and the overall membership proportions overwhelmingly support the conclusion of a single cluster (Figure 2.9C).

Additional STRUCTURE analyses for *T. s. sirtalis* yielded comparable results. When using the restricted subset of six loci, Q values were evenly apportioned among K clusters (Figure A4). The analysis that removed locus TbuA70 because of possible HWE-departure produced similar barplots (Figure A5). Barplots from the LOCPRIOR model implied some genetic structure, suggesting differentiation between Essex County and Luther Marsh and individuals from Lambton County and Michigan (Figure A3). Regardless, in all additional analyses, ΔK was consistently small and the mean log likelihood plateaued at $K = 1$.

Pairwise values of F_{ST} and R_{ST} identified significant differences between the four *T. butleri* genetic clusters (Table 2.3). Pairwise F_{ST} values ranged between 0.06 and 0.23, pairwise R_{ST} values ranged between 0.06 and 0.52. Essex and Ojibway were the most similar, while Luther was highly distinct from all other locations. There are no comparable values for *T. s. sirtalis*, as there was only one inferred genetic cluster.

The AMOVAs were highly significant ($p < 0.00001$). Using the F_{ST} model for *T. butleri*, 10.3% of the total genetic variation is attributable to differentiation among genetic clusters, 13.4% is explained by among-individual variation within clusters, and 76.3% by within-individual variation. Using the R_{ST} model, 30.2% of the total variation comprises differences among clusters, 9.8% is among-individual variation, and 60.0% is within-individual variation. Again there are no comparable results for *T. s. sirtalis*.

2.5. Discussion

We found a striking difference in genetic structure between two congeners co-distributed in the heavily fragmented landscape of Southwestern Ontario, Canada. For the habitat specialist *Thamnophis butleri*, we found four genetic clusters corresponding to (1) Luther Marsh Wildlife Management Area, (2) Lambton County, (3) Ojibway and LaSalle in Essex County, and (4) Essex sites adjacent to the Detroit River, including islands. In contrast, the habitat generalist *T. s. sirtalis* showed no strong evidence of genetic population structure despite the relatively large spatial scale of our sampling.

While the most coherent interpretation of our TESS and STRUCTURE Bayesian clustering analyses is that there are four genetic clusters for *T. butleri*, the ΔK method (Evanno et al. 2005) did suggest $K = 2$ over $K = 4$, with one cluster comprising Luther Marsh and Lambton County and a second cluster of Essex County, with some admixture south of Windsor and in the Detroit

River islands. However, Luther Marsh is both distant and disjunct from the remaining Ontario range of the species with virtually no likelihood of dispersal; Pritchard et al. (2000) cautioned that interpretations of K should always account for the biology of the focal species. Evanno et al. (2005) note that small sample sizes can produce spurious results when identifying the true number of genetic clusters; we sampled only 10 individuals from Luther Marsh. In sum, we take four clusters as being most consistent with our data and results.

The genetic structure that we delineated for *T. butleri* agrees with findings of Noble et al. (2013), who also found well-supported, geographically delineated genetic clusters. However, this new study provides a more comprehensive description of genetic connectivity in Southwestern Ontario, and particularly a clearer picture of genetic patterns within Essex County by virtue of larger sample sizes from the region (Noble et al., n = 60; present study, n = 114) and a greater number of markers (seven vs. twelve). Other snake species with disjunct distributions in Ontario have genetic clusters that coincide with known range gaps – some of which are caused by local habitat destruction and others which predate European colonization – e.g. eastern foxsnakes (*Pantherophis gloydi*) (DiLeo et al. 2010), massasauga rattlesnakes (Chiucchi and Gibbs 2010), and eastern hog-nose snakes (*Heterodon platirhinos*) (Xuereb et al. 2015).

Evidence that we have provided here strongly supports the conclusion that *T. s. sirtalis* consists of a single genetic population across the range that we sampled. Another Ontario-based study, covering some of the same geographic range, compared populations of *T. s. sirtalis* with the Endangered eastern foxsnake, and found a similar lack of genetic structure for the former (DiLeo et al. 2010).

We found that the habitat specialist *T. butleri* contained lower genetic diversity than did its generalist congener, *T. s. sirtalis*. At every region surveyed, *T. butleri* had an equal number or

fewer alleles than *T. s. sirtalis* for every microsatellite locus. This is often the case among specialist versus generalist species (Habel and Schmitt 2012, Janecka et al. 2016), although not always (Brouat et al. 2004). Similarly, it is common to see higher levels of genetic differentiation in specialist species (DiLeo et al. 2010, Habel and Schmitt 2012), which is the pattern in the *Thamnophis* that we investigated. Lower genetic diversity is not inherently detrimental; being confined to a specific fraction of the landscape, specialist species are often adapted to such scenarios (Habel and Schmitt 2012). It is when conditions change, such as with habitat destruction, that specialists are more susceptible to extinction, particularly when their population sizes are small (Dennis et al. 2011).

We found no compelling evidence that gene flow is impeded by the major rivers that lie along the international border between Canada and the U.S.A. Population assignments grouped *T. butleri* individuals from Michigan and islands with the nearest Ontario cluster, both in analyses examining the whole range of sampling (Figures 2.3, 2.4) and in the supplementary STRUCTURE runs more closely examining Essex and Lambton counties (Figures 2.6, 2.7). *Thamnophis s. sirtalis* comprised a uniform cluster across the Detroit and St. Clair rivers, although the population size of this species is so large that it is unlikely to be an effective test of riverine barriers as impediments to dispersal. Although both species are terrestrial and more often seen on land, both have been observed swimming (COSEWIC 2010, personal observation). Indeed *T. butleri* must have colonized the islands in the Detroit River via aquatic dispersal. This also implies that *T. butleri* from the (unsampled) location of Walpole Island will align genetically with the geographically proximate Michigan and Ontario samples, putting it within the Lambton County cluster.

Essex County is the one region of *T. butleri* occurrence that contains two genetic clusters. One cluster ranges the length of the county along the Detroit River, including individuals from the islands, and the second lies slightly inland, consisting of snakes from LaSalle and the Ojibway Prairie Complex (Figure 2.6). Prior to European settlement, 83% of the county was covered in wetland (Ducks Unlimited Canada 2010), which would have allowed ready access to leeches, an important prey item for *T. butleri* prior to the introduction of Palearctic earthworms (Catling and Freedman 1980); there would have been suitable habitat and food available inland away from large rivers. The more elongated range of the shoreline population could be due to passive dispersal by water, as the river's current could have facilitated more substantial downstream movement than the somewhat limited movement patterns normally evident in *T. butleri* (Macartney et al. 1988). When assigning higher K values in STRUCTURE (Figure 2.6) to Essex County individuals, we found that the more southern Amherstburg individuals fell out as a cluster distinct from the shoreline cluster, which could mean less gene flow is occurring up and down the length of the county. Physical separation can lead to genetic differentiation (Allendorf and Luikart 2007), the source of which could be isolation by distance, with the species not exhibiting sufficient dispersal to allow continued gene flow across the region. Alternatively, the Ojibway/LaSalle population could have become isolated by anthropogenic fragmentation. Human settlement tends to be associated with water edges, so habitat loss along the river could have split the interior and border populations.

Although the regional populations of Lambton County and Luther Marsh apparently each comprise a single genetic cluster, this does not necessarily mean that they are unaffected by changes in the landscape. Pairwise F_{ST} and R_{ST} comparisons showed significant differences between *T. butleri* from Aamjiwnaang and those from locations in both northern and southern

Lambton (Figure A5). The results from STRUCTURE looking solely at Lambton County (Figure 2.7) suggest that individuals from Aamjiwnaang are slightly genetically different from other samples. In time, snakes from that area may comprise a distinct cluster, as population differentiation studies can suffer from a time lag between the action of microevolutionary forces and their effects (Bossart and Prowell 1998). At Luther Marsh, snakes are unlikely to be threatened by landscape conversion, as the Wildlife Management Area is managed by both the local conservation authority and MNRF. However, for *T. butleri*, small population size and the small area occupied (COSEWIC 2010) will likely lead to lower genetic diversity and increased probability of local extinction.

A fundamental question that follows from the genetic clustering of *T. butleri* according to its disjunct distribution in Ontario regards the origin of this pattern. The structure may reflect post-glacial recolonization and historical dispersal into naturally-distributed habitat patches; alternatively, habitat loss caused by European settlement of the area could have destroyed intermediate habitat and impeded gene flow across a now-fragmented range. It is possible that patchy geographical and genetic distribution is the norm for *T. butleri*, as it is for many specialist species (Habel and Schmitt 2012). Although the generalized global range (Figure 1.1A) indicates a mostly continuous distribution with only two disjunctions, we know that this is not the case in Ontario (Figure 1.1B). Aside from the three regional populations, there are also several locations where *T. butleri* has been extirpated. Even in Michigan, the apparent stronghold for the species, sightings of *T. butleri* are sporadic, according to the Michigan Herp Atlas (<https://www.miherpatlas.org/taxon.php?taxon=84>). *Thamnophis butleri* is a relatively sedentary species (Macartney et al. 1988), and this facet of its biology coupled with relative specialization in habitat preference should result in greater differentiation across fine spatial scales.

Mitochondrial DNA sequencing studies have revealed that *T. butleri* in Ontario and Michigan share a single mitochondrial haplotype (Placyk et al. 2012, Noble et al. 2012), with no distinction among the groups that colonized this region of post-glacial North America. The same is true for *T. s. sirtalis* in Ontario and eastern Michigan (Placyk et al. 2007). This is likely due to the founder effect that often follows the retreat of glaciers (Hewitt 2000), coupled with insufficient time for mutation to have added mitochondrial diversity. While mtDNA mutates quickly relative to comparable coding regions in nuclear DNA (Galtier et al. 2009), it still takes millennia for appreciable mtDNA mutations in snakes to accumulate (Eo and DeWoody 2010). Recent isolation – particularly as recent as post-European colonization – would not be reflected in mitochondrial patterns. Ultimately it will be worthwhile to assess *T. butleri* using genomics and the large panel of markers that could be thus employed; this would provide more power to quantify population structure, and may allow us to detect differences in loci of fitness import.

Regardless of whether the genetic structure that we see in *T. butleri* has some origins in natural historical events, human presence undoubtedly has exacerbated the situation. Land conversion of natural habitats, such as grassland and wetland, has been an issue ever since European settlement (Tallgrass Ontario 2005, Ducks Unlimited Canada 2010), as has been the suppression of wildfires that could potentially create new habitat to compensate for loss via natural succession (Weakley 1999, Larson 2014). Known locations of recent *T. butleri* populations have been destroyed for development (COSEWIC 2010). Without conservation efforts targeting habitat preservation and restoration, the species could be eliminated entirely from Ontario. We recommend an approach of improving habitat connectivity, with an eye toward restoration of gene flow – at least within if not among – the three regions. Essex County in particular may benefit from reconnecting populations, particularly if the genetic differentiation

there occurred after European colonization. For areas of low *T. butleri* density, like Luther Marsh, implementing artificial dispersal and gene flow into the region by headstarting juveniles for release may be more beneficial (Conner et al. 2003, King and Stanford 2006).

Our research quantifies the genetic populations of *T. butleri* in Ontario, and highlights the importance of using genetics to help in conservation strategies. Because there is apparently little gene flow occurring among genetic populations, the continued destruction (COSEWIC 2010) of *T. butleri* habitat without any active habitat restoration efforts could result in local extinction of the species or even its disappearance entirely from Canada.

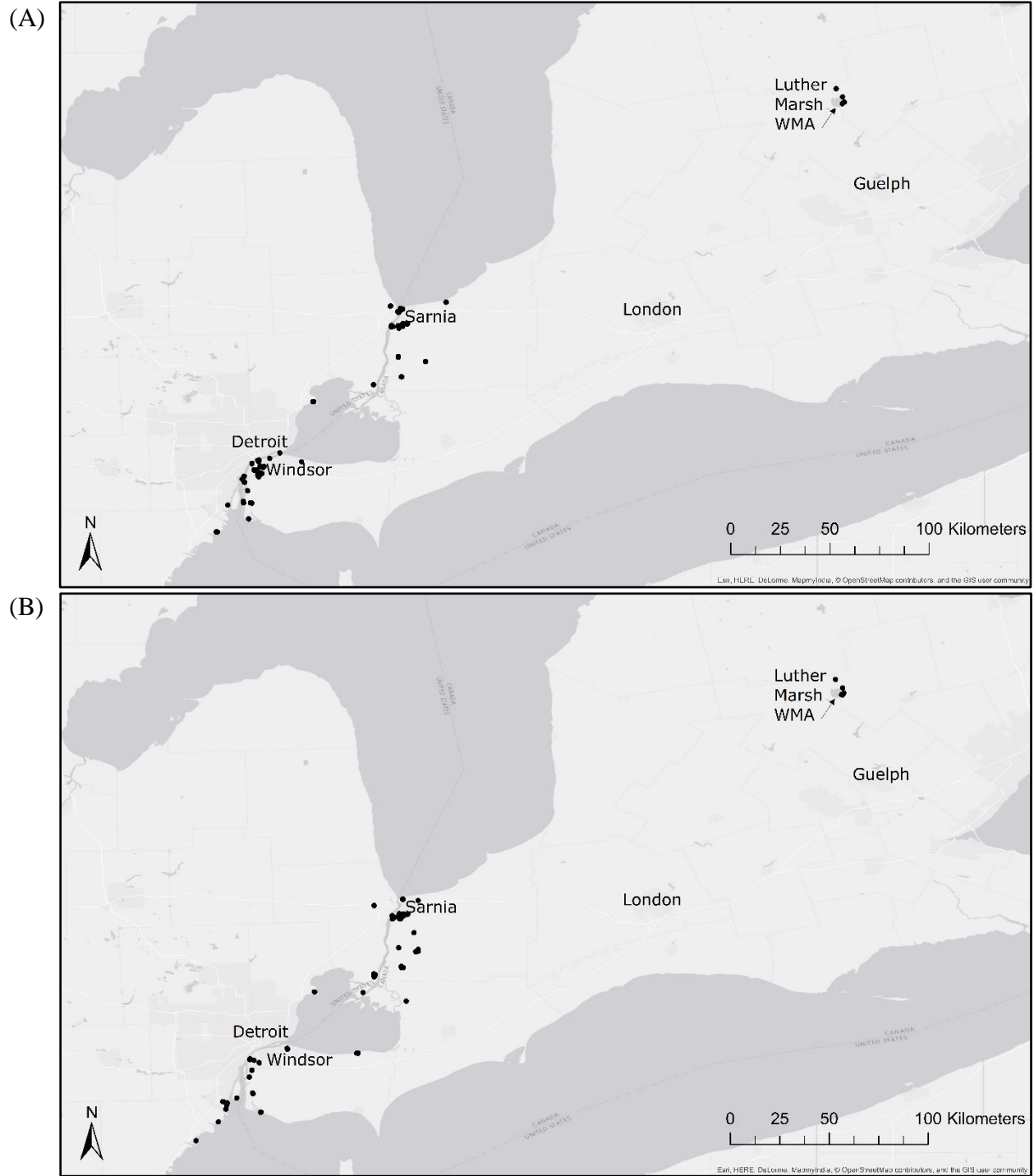


Figure 2.1. (A) Sample locations for all *Thamnophis butleri* used in this study (n = 233). (B) Sample locations for all *Thamnophis s. sirtalis* used in this study (n = 163). Maps created using ArcMap (ESRI 2015).

Table 2.1. Microsatellite primer pairs screened for genotypic surveys of the Butler’s gartersnake (BGS; *Thamnophis butleri*) and the eastern gartersnake (EGS; *T. s. sirtalis*). Shown is primer name, motif, DNA sequence, annealing temperature (°C) used for PCR, allele size range and number of alleles found in this study’s samples (greyed-out values indicate loci dropped for one species), and primer design source reference.

Primer	Repeat Motif	Sequence (5'–3')	T _a (°C)	Size range (bp)		No. alleles		Source
				BGS	EGS	BGS	EGS	
N _{su} 2	(AC) ₁₈	F: TCCTCTTTGGCAGAGTAATAGT R: AGCCGAGAACACACTAGTAAGT	50	159-179	156-202	8	24	Prosser et al. 1999
N _{su} 3	(ATCT) ₁₄ AT(CA) ₂₀	F: CTGACTCACTTCTGACCCTAAT R: AATATTTGCTTGGCTCAAAC	50	151-203	134-212	21	39	Prosser et al. 1999
Ts2	(AAT) ₁₀ (AAT) ₃	F: GGCTAGCCCCTGTGTCCTT R: CACAACCTCCAAATATTGAAGATTA	54	128-200	129-225	13	29	McCracken et al. 1999
3Ts	unknown	F: GGTCACTTAAATACAACGAAATTG GTTAGCT R: CGGACAGCTCTGGCTCCCTTG	54	272-422	254-482	21	36	Garner et al. 2002
Ts010	(ATGG) ₃ (ATGA) ₆	F: TGACTCAGATGCCCTCAGTCTA R: CGGACCAACCAGGAACAGAAAT	52	136-164	132-196	8	14	Manier and Arnold 2005
Te1Ca3	(CA) ₁₃	F: CCCCCACCTACCTACCTG R: TGGGTAGGGCAAAAACCAG	51	103-131	95-123	13	15	Garner et al. 2004
Te1Ca29	(CA) ₅ (N) ₄ (CA) ₄ (CG) ₁ (CA) ₁	F: TGCCTTATTTGCTTGGGTTG R: TCTTTCAACCTGCTTTGTAGACAC	51	151-207	157-231	18	35	Garner et al. 2004
TbuA04a	(AC) ₈	F: AAGGAGCTTGGGGAATCTTG R: CTGGGAATCTTAGCATTCTGC	58	216-224	212-224	5	5	Sloss et al. 2012

Table 2.1. Continued

Primer	Repeat Motif	Sequence (5'–3')	T _a (°C)	Size range (bp)		No. alleles		Source
				BGS	EGS	BGS	EGS	
TbuA09	(AC) ₇	F: CATCTCAACCAAAGTCGCTTC R: GGATGTTGTGGGGTGTTTTC	50	121	119-129	1	6	Sloss et al. 2012
TbuA27	(TG) ₉	F: AA ACTCCAGGGATTTCCAAG R: TGTGTTGCGTGAATACATCC	52	237-245	231-243	4	7	Sloss et al. 2012
TbuA49	(AC) ₂₃	F: CTTGTAGTTTGGGGGAAAAG R: TTTTCAGAGCTGGATGAAGG	51	218-248	216-244	13	15	Sloss et al. 2012
TbuA64	(AC) ₁₇	F: ACATAGAATGCATCTGGTTGG R: GCCATGCAATCATGTATAAGC	51	237-277	237-275	19	20	Sloss et al. 2012
TbuA70	(AC) ₂₁	F: GCCACTTCCACCTAACACAG R: CACTGGTGAGTTGCTCTG	58	164-174	158-196	3	15	Sloss et al. 2012
TbuA74	(AC) ₁₁	F: CTTGGAAATGTCCTGCAATC R: CCCATGCAAGCAATATAACC	50	299-323	228-316	13	13	Sloss et al. 2012
TbuB12	(TCTT) ₁₇	F: CTGCTTTTAATCCCATCACC R: AACTGAAAGCCATTCCTG	56	278-318	340-448	11	27	Sloss et al. 2012

Table 2.2. Locations designated *a priori* as *T. butleri* (BGS) ‘populations’ for genetic analyses, based on groupings of *T. butleri* samples (≤ 10 km diameter sampling area, ≥ 5 individuals sampled). Single samples were grouped with the closest location. Exceptions to these criteria (e.g. sparsely-sampled locations, barriers determining populations) described below. *T. s. sirtalis* (EGS) samples were assigned to the closest *T. butleri* location.

Location	Cause for designation	n _{BGS}	n _{EGS}
Wayne, MI	<ul style="list-style-type: none"> • Population diameter: < 1 km (except for one individual, ~ 15 km N, to which Wayne is the closest location) • Due to the small number of samples, Wayne individuals were grouped by their county • Separated from nearest external sample (~ 6 km E) by the Detroit River 	7	11
Macomb, MI	<ul style="list-style-type: none"> • Population diameter: < 1 km • Due to the small number of samples, Macomb individuals were grouped by their county • ~ 30 km NE to nearest external sample 	2	4
St. Clair, MI	<ul style="list-style-type: none"> • Population diameter: < 1 km (except for one individual, ~ 40 km S, to which St. Clair is the closest location) • Due to the small number of samples, St. Clair individuals were grouped by their county • Separated from nearest external sample (~ 4 km E) by the St. Clair River 	3	10
Crystal Bay (island)	<ul style="list-style-type: none"> • Population diameter: < 1 km • Separated from nearest external sample (~ 3 km E) by the Detroit River 	6	n/a
Fighting Island	<ul style="list-style-type: none"> • Population diameter: ~ 3 km • Separated from nearest external sample (~ 5 km E) by the Detroit River 	14	n/a
Belle Isle	<ul style="list-style-type: none"> • Population diameter: < 1 km • Designated as a distinct location due to being an island • Separated from nearest external sample (~ 4 km SW) by the Detroit River 	3	n/a
South Essex	<ul style="list-style-type: none"> • Population diameter: < 1 km (except for one individual, ~ 8 km S, to which South Essex is the closest location) • Separated from nearest external sample (~ 3 km E) by the Detroit River 	9	17
North Essex	<ul style="list-style-type: none"> • Population diameter: ~ 10 km (except for two individuals, ~ 8 km S & ~ 12 km E respectively, to which North Essex is the closest location without barrier – the Canard River runs across Essex between the locations) • Separated from nearest external sample (~ 4 km NE) by the Detroit River 	85	23
South Lambton	<ul style="list-style-type: none"> • Population diameter: ~ 10 km • Separated from nearest external sample (~ 11 km W) by the Detroit River 	10	42
Aamjiwnaang (Lambton)	<ul style="list-style-type: none"> • Population diameter: ~ 6 km • ~ 6 km N to nearest external samples (designated as a distinct sample due to the barrier of an industrial plant to the north, and based on anecdotal reports by the Aamjiwnaang First Nation Environmental Department that snakes at this location have distinct ecological patterns) 	42	41
North Lambton	<ul style="list-style-type: none"> • Population diameter: ~ 2.5 km (except for one individual, ~ 16 km E, to which North Lambton is the closest location) • Separated from nearest external sample (~ 4 km) by the St. Clair River • ~ 6 km SW to next nearest external location (see Aamjiwnaang) 	42	4
Luther Marsh	<ul style="list-style-type: none"> • Population diameter: ~ 8 km • ~ 175 km W to nearest external sample 	10	11

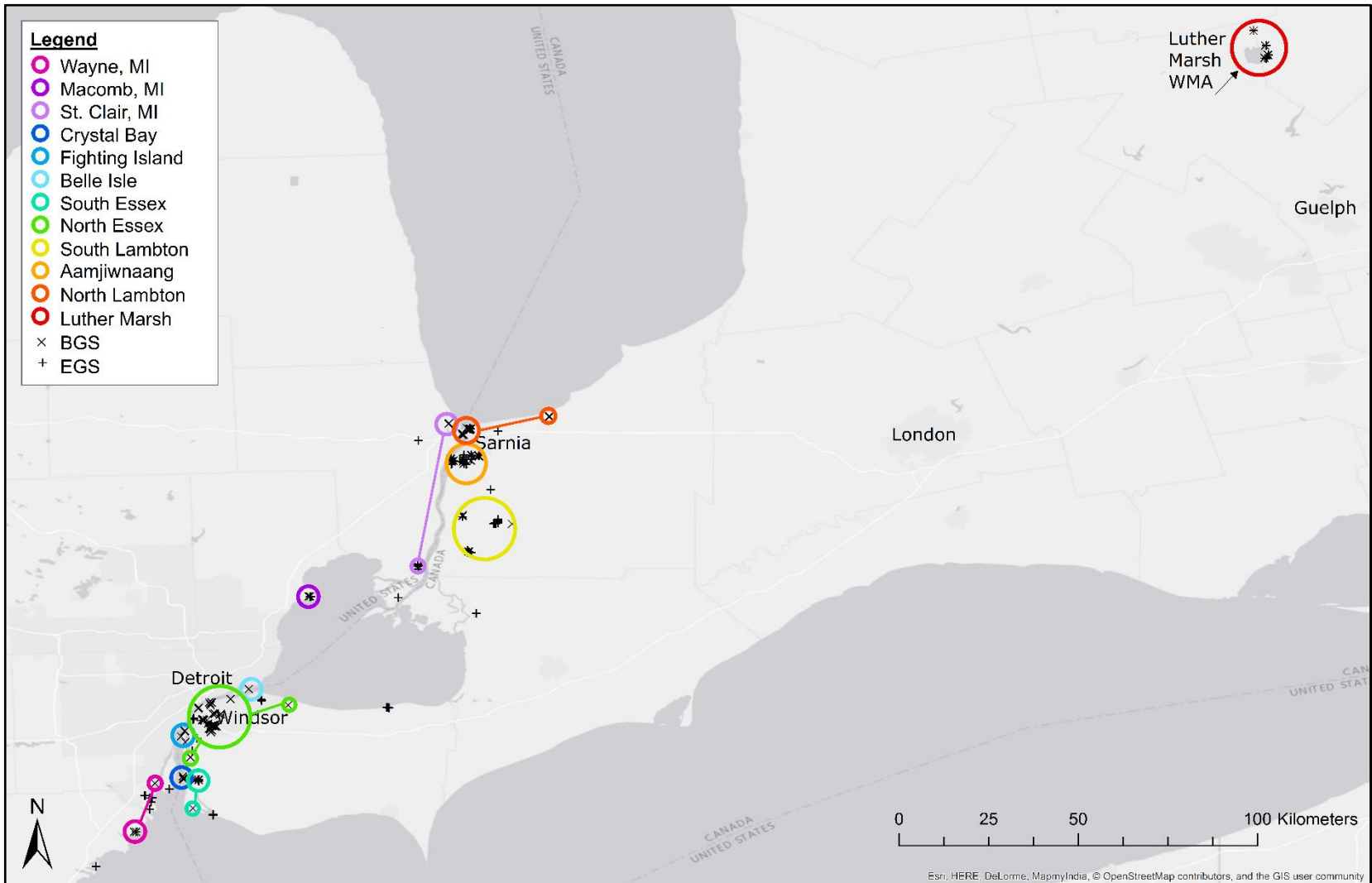


Figure 2.2. Locations designated *a priori* as *T. butleri* (BGS) populations, based on groupings of *T. butleri* samples (≤ 10 km diameter sampling area, ≥ 5 individuals sampled). Single samples were grouped with the closest location. *T. s. sirtalis* (EGS) samples were assigned to the closest *T. butleri* location. Map created using ArcMap (ESRI 2015).

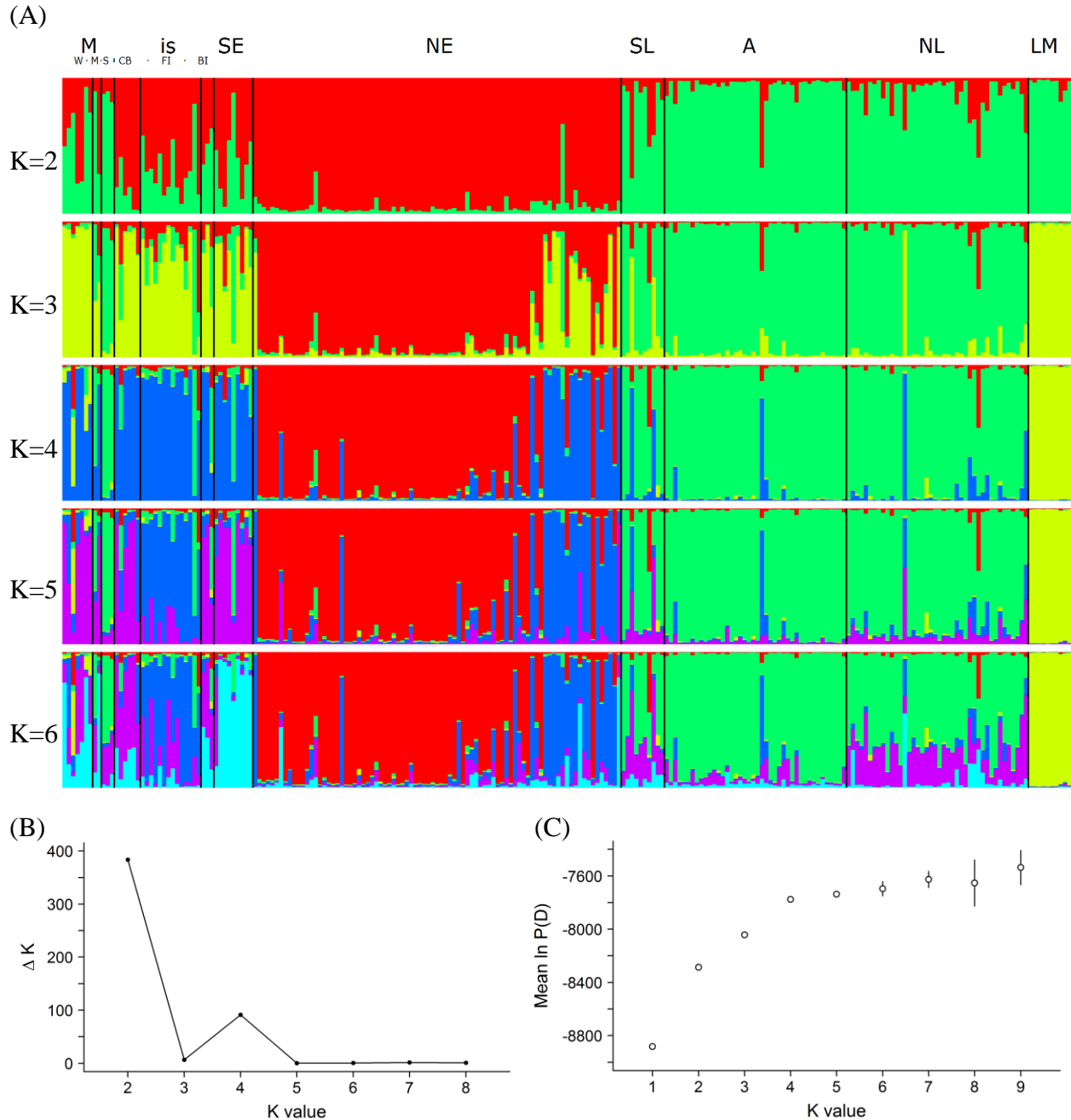


Figure 2.3. (A) Barplots of *T. butleri* population membership proportions assigned by STRUCTURE for $K = 2$ to $K = 6$. The barplots are organized southwest to northeast (with Michigan and the islands grouped on the left), and divided by the designated sampling locations (M_W = Wayne, Michigan; M_M = Macomb County, Michigan; M_S = St. Clair County, Michigan; is_{CB} = Crystal Bay; is_{FI} = Fighting Island; is_{BI} = Belle Isle; SE = South Essex County; NE = North Essex County; SL = South Lambton County; A = Aamjiwnaang; NL = North Lambton County; LM = Luther Marsh WMA). Each vertical bar represents a single snake. Genetic structure in Essex County, particularly that apparent with the NE location, is explored in greater depth in Figure 2.6. For STRUCTURE runs of $K = 2$ to $K = 9$, (B) Evanno's ΔK indicates $K = 2$, with a smaller peak at $K = 4$, and (C) the mean \ln probability of the data plateaus at $K = 4$. Overall, this suggests that $K = 4$ may be the most representative number of clusters.

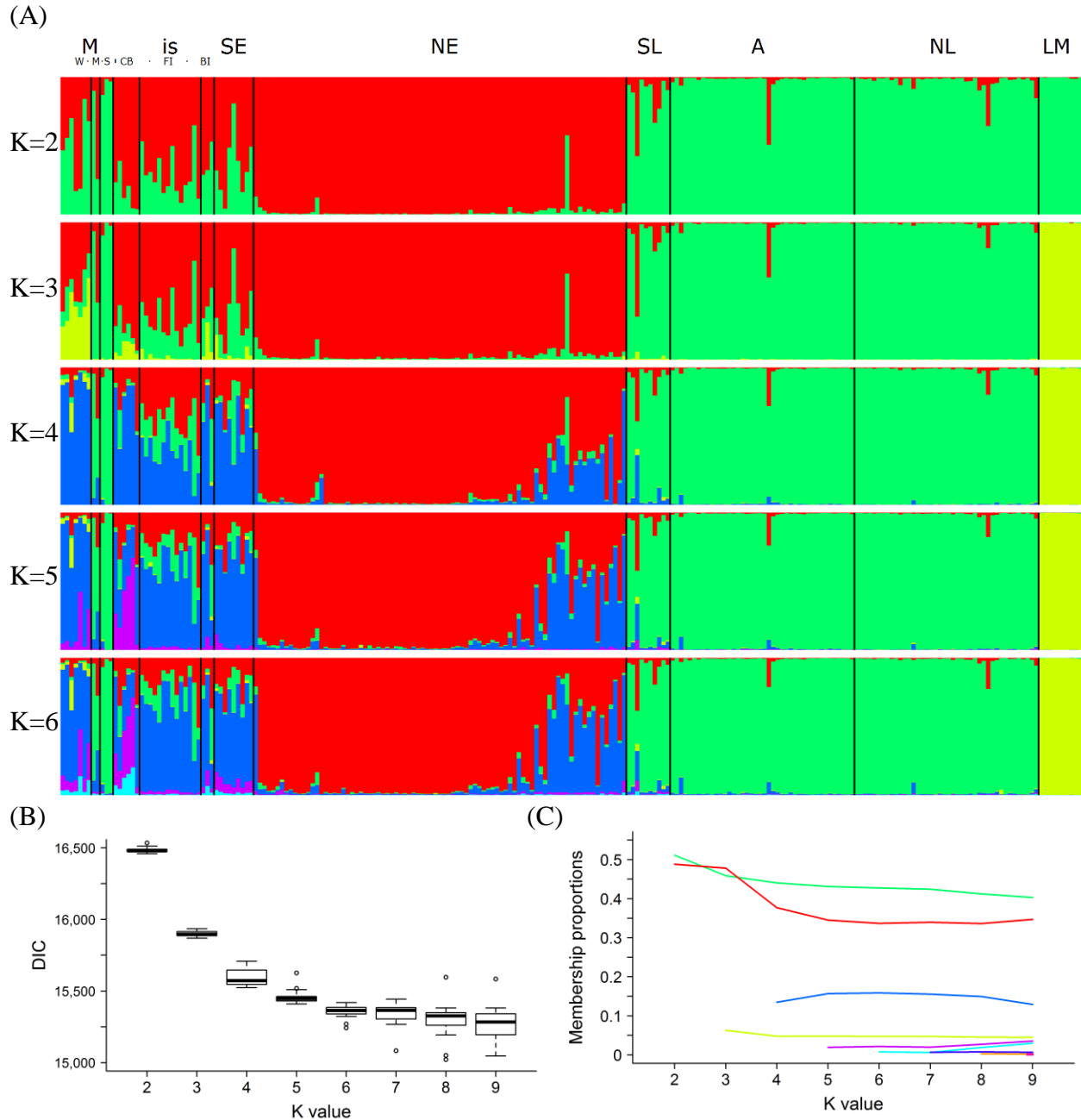


Figure 2.4. (A) Barplots of *T. butleri* population membership proportions assigned by TESS for $K = 2$ to $K = 6$. The barplots are organized southwest to northeast (with Michigan and the islands grouped on the left), and divided by the designated sampling locations (M_W = Wayne, Michigan; M_M = Macomb County, Michigan; M_S = St. Clair County, Michigan; is_{CB} = Crystal Bay; is_{FI} = Fighting Island; is_{BI} = Belle Isle; SE = South Essex County; NE = North Essex County; SL = South Lambton County; A = Aamjiwnaang; NL = North Lambton County; LM = Luther Marsh WMA). Each vertical bar represents a single snake. Genetic structure in Essex County, particularly that apparent with the NE location, is explored in greater depth in Figure 2.6. For TESS runs of $K = 2$ to $K = 9$, (B) the DIC values plateau at $K = 4$, and (C) the overall membership proportions show that the first four (red, green, yellow, then blue) clusters are strongly represented, implying $K = 4$. Overall, this suggests that $K = 4$ may be the most representative number of clusters.

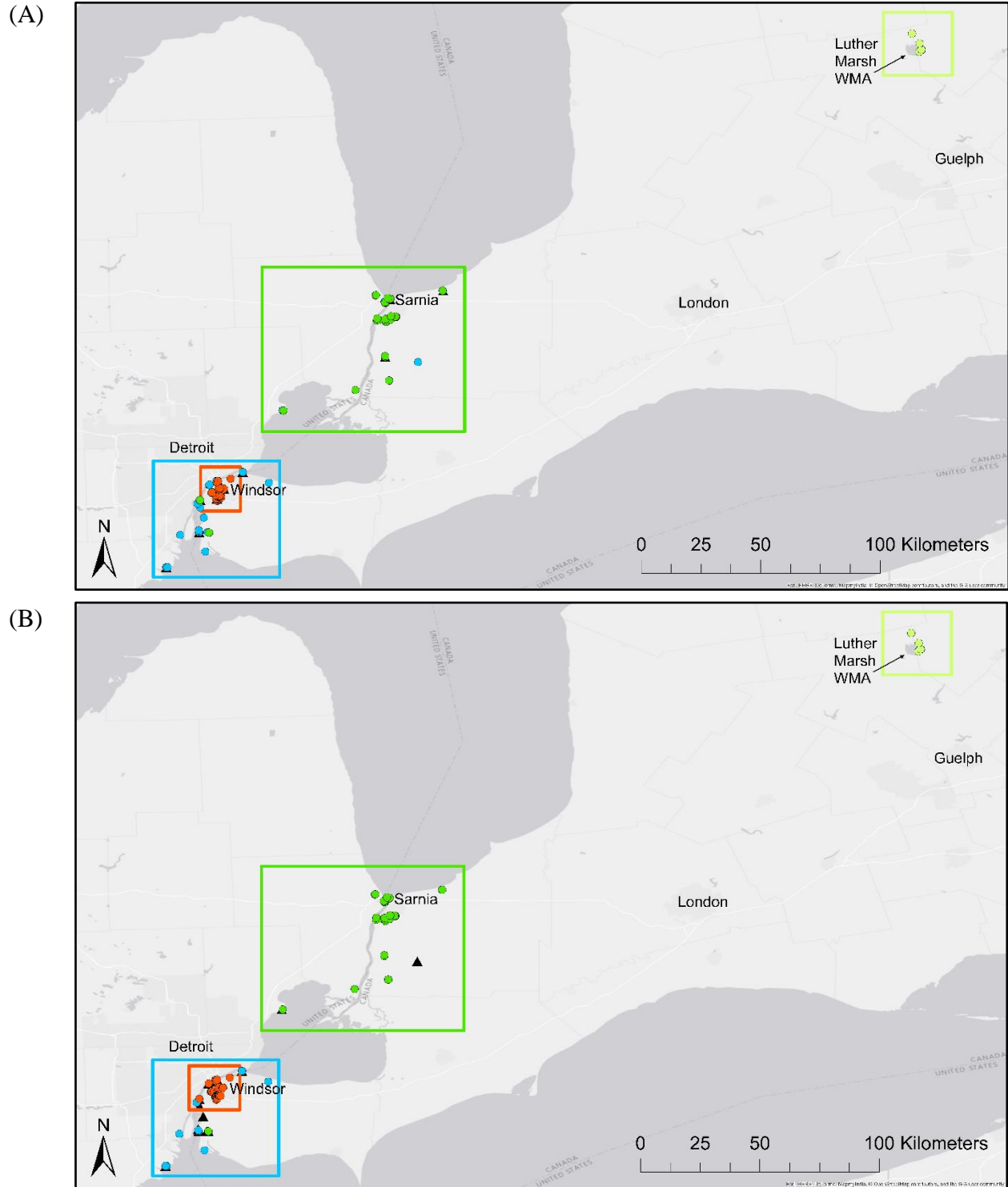


Figure 2.5. Geographic distribution of *T. butleri* genetic clusters based on (A) STRUCTURE and (B) TESS results when $K = 4$, with colours corresponding to that analysis (Figures 2.3, 2.4). Individuals with at least 60% of their Q-matrix assigned to a single cluster are shown with that cluster's colour. Individuals with less than 60% of their genotype belonging to a single cluster (possibly signalling admixture) are represented by black triangles. Map created using ArcMap (ESRI 2015).

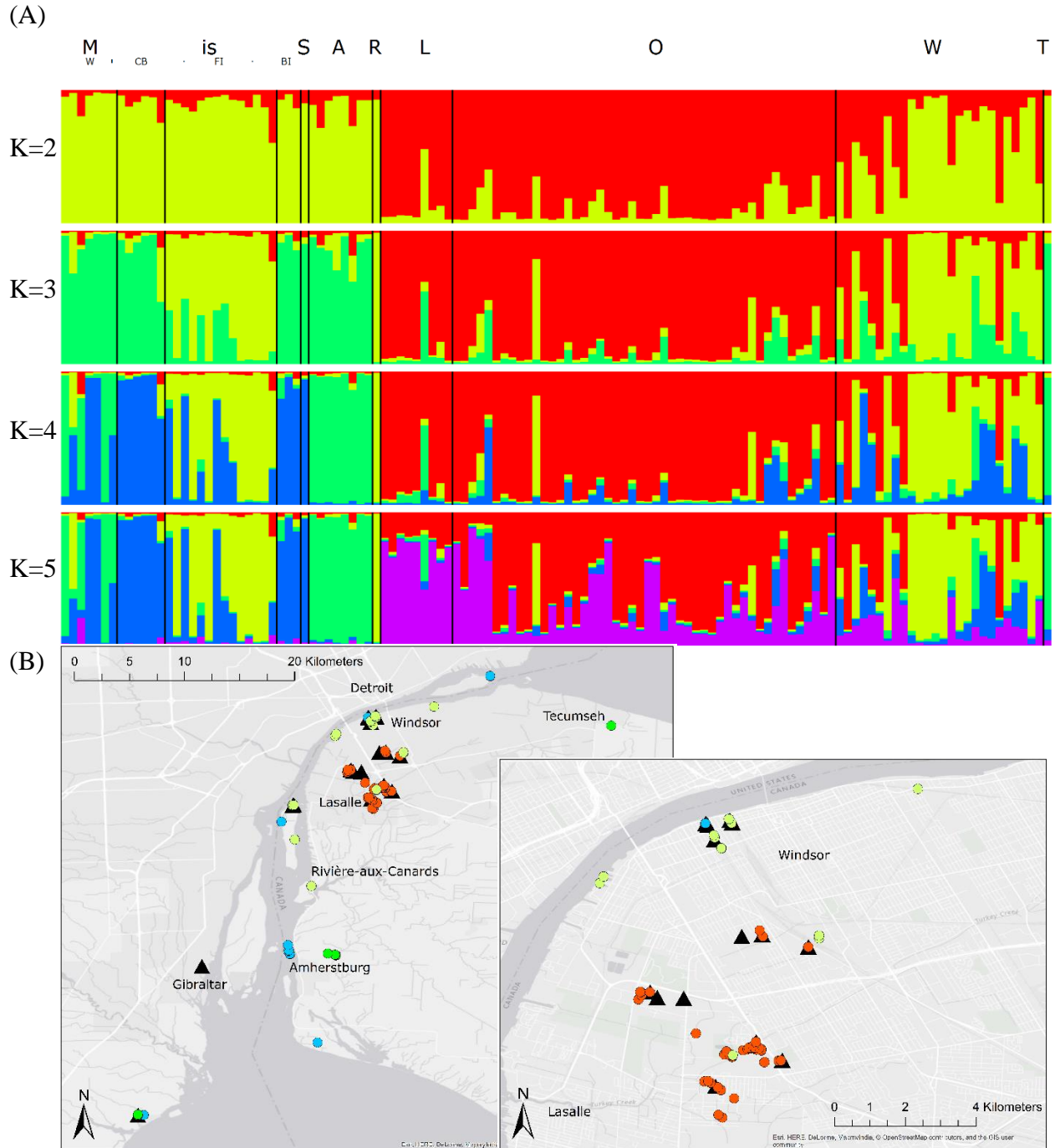


Figure 2.6. (A) Barplots of population membership proportions assigned by STRUCTURE for $K = 2$ to $K = 5$, for *T. butleri* in Essex County and adjacent Michigan locales. The barplots are organized southwest to northeast (with Michigan and the islands grouped on the left), and divided by local regions (M_W = Wayne, Michigan; is_{CB} = Crystal Bay; is_{FI} = Fighting Island; is_{BI} = Belle Isle; S = Sunset Beach; A = Amherstburg; R = Rivière-aux-Canards; L = LaSalle; O = Ojibway Prairie Complex; W = Windsor; T = Tecumseh). Each vertical bar represents a single snake. (B) Geographic distribution of clusters when $K = 4$. Individuals belong to the genetic cluster with the corresponding colour from $K = 4$ in (A); individuals with less than 60% of their genotype belonging to a single cluster are represented by black triangles. Inset image is a closer view of the Windsor-area samples. Maps created using ArcMap (ESRI 2015).

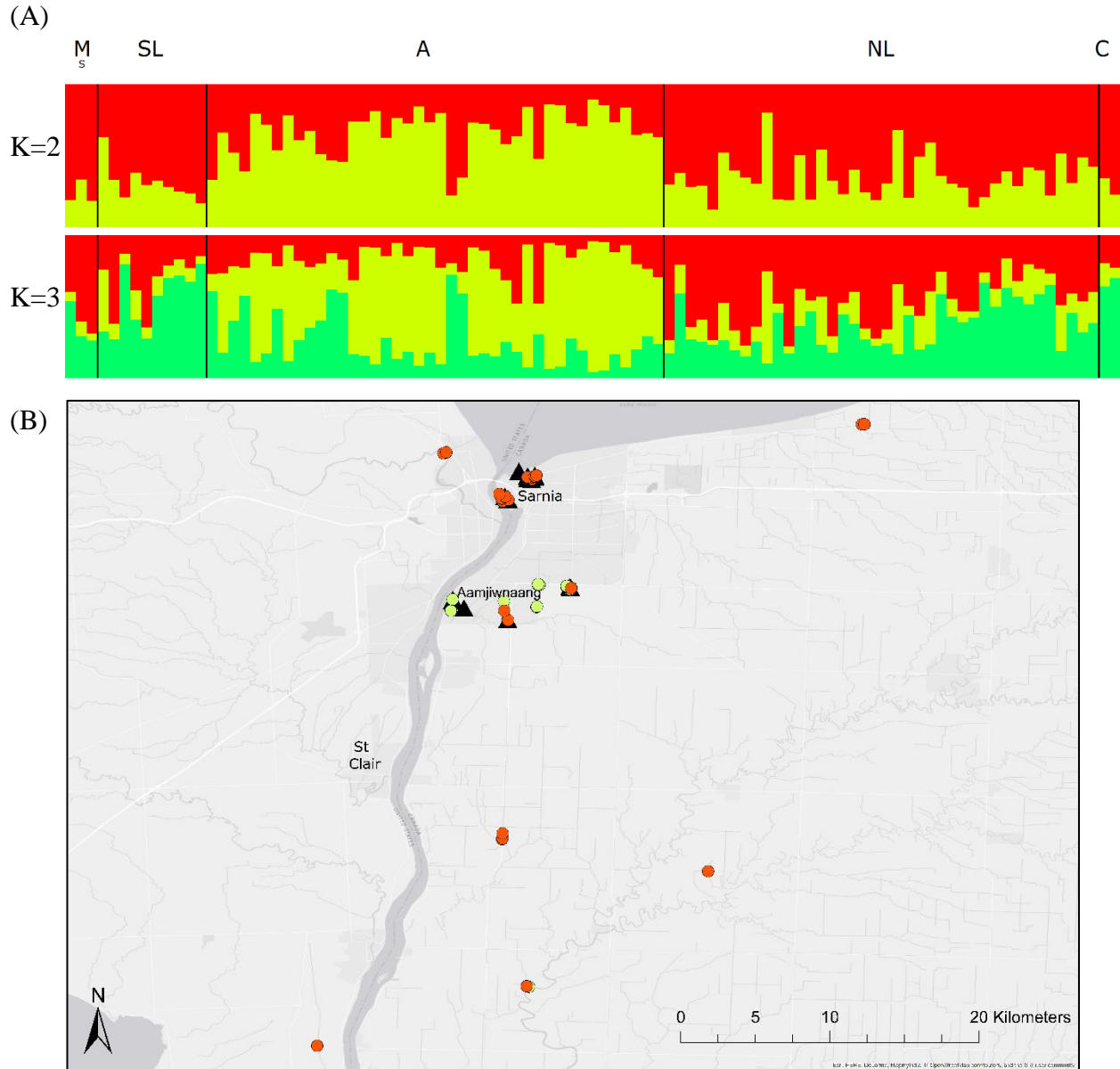


Figure 2.7. (A) Barplots of population membership proportions assigned by STRUCTURE for $K = 2$ to $K = 3$, for *T. butleri* in Lambton County and adjacent Michigan locales. The barplots are organized south to north, and divided by the designated sampling locations, with isolated samples shown separately (M_s = St. Clair County, Michigan; SL = South Lambton County; A = Aamjiwnaang; NL = North Lambton County; C = Camlachie). Each vertical bar represents a single snake. (B) Geographic distribution of clusters when $K = 2$. Individuals belong to the genetic cluster with the corresponding colour from $K=2$ in (A); individuals with less than 60% of their genotype belonging to a single cluster are represented by black triangles. Map created using ArcMap (ESRI 2015).

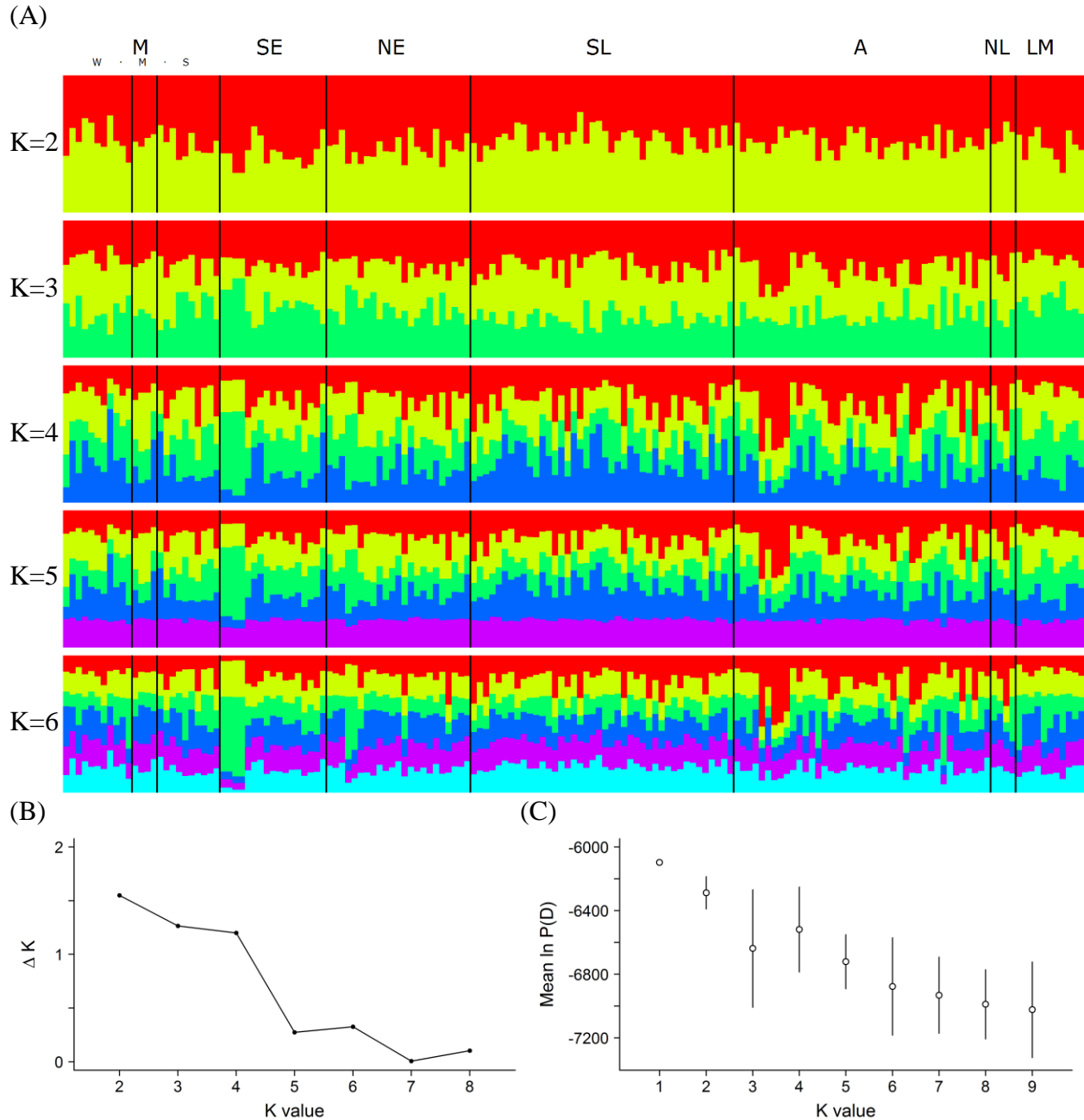


Figure 2.8. (A) Barplots of *T. s. sirtalis* population membership proportions assigned by STRUCTURE for $K = 2$ to $K = 6$. The barplots are organized southwest to northeast (with Michigan areas grouped on the left), and divided by the designated sampling locations (M_w = Wayne, Michigan; M_m = Macomb County, Michigan; M_s = St. Clair County, Michigan; SE = South Essex County; NE = North Essex County; SL = South Lambton County; A = Aamjiwnaang; NL = North Lambton County; LM = Luther Marsh WMA). Each vertical bar represents a single snake. For STRUCTURE runs of $K = 2$ to $K = 9$, (B) Evanno's ΔK does not strongly indicate any K value, and (C) the mean \ln probability of the data plateaus at $K = 1$, with high variability between runs at larger values of K . Overall, this suggests that $K = 1$ may be the most representative number of clusters.

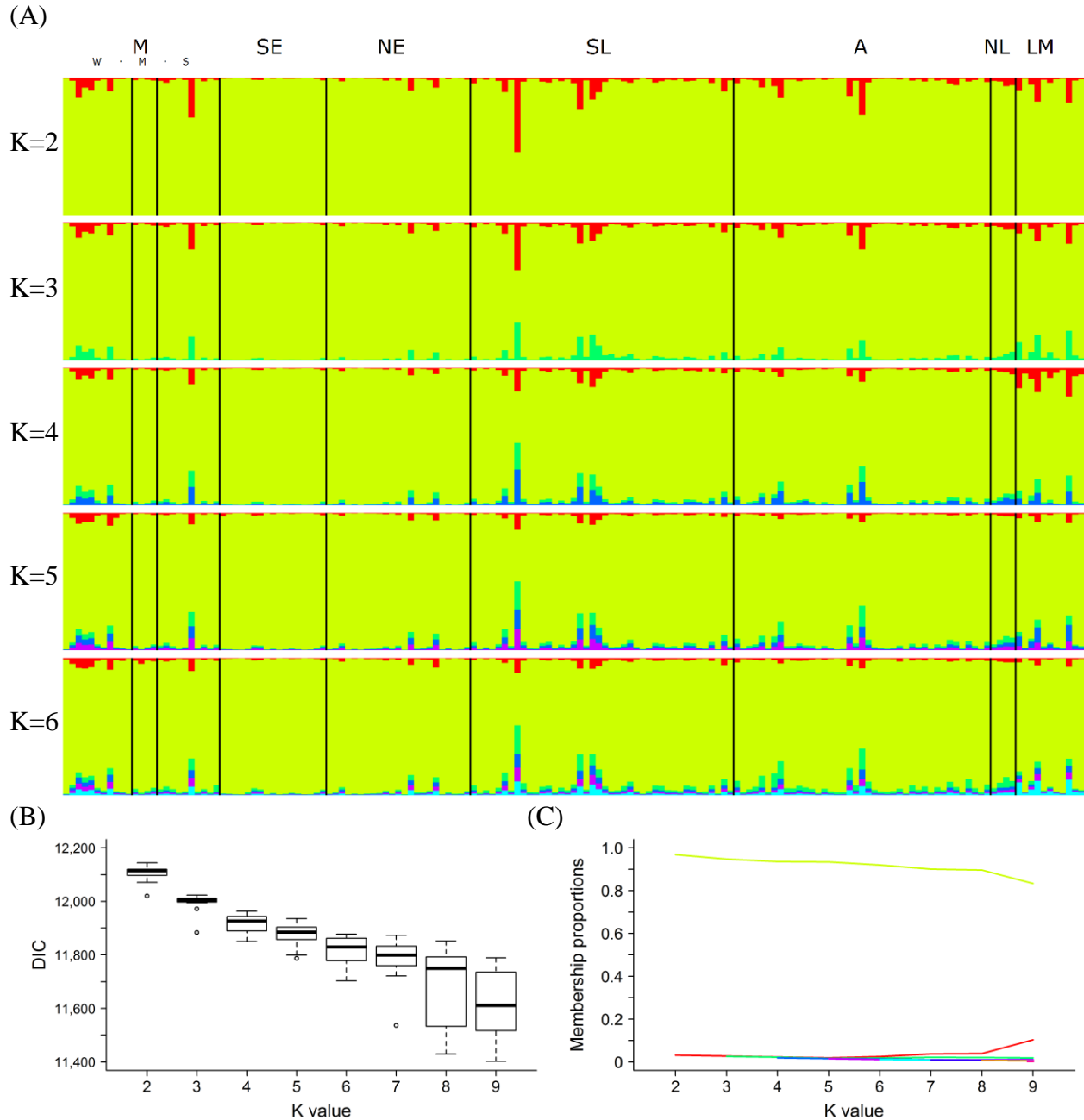


Figure 2.9. (A) Barplots of *T. s. sirtalis* population membership proportions assigned by TESS for $K = 2$ to $K = 6$. The barplots are organized southwest to northeast (with Michigan areas grouped on the left), and divided by the designated sampling locations (M_W = Wayne, Michigan; M_M = Macomb County, Michigan; M_S = St. Clair County, Michigan; SE = South Essex County; NE = North Essex County; SL = South Lambton County; A = Aamjiwnaang; NL = North Lambton County; LM = Luther Marsh WMA). Each vertical bar represents a single snake. For TESS runs of $K = 2$ to $K = 9$, (B) the DIC trend is a slowly decreasing plateau across all K values, and (C) the overall membership proportions show that only the first (yellow) cluster is strongly represented. Overall, this suggests that $K = 1$ may be the most representative number of clusters.

Table 2.3. Estimates of pairwise F_{ST} (above diagonal) and R_{ST} (below diagonal) between each of the four genetic populations determined for *Thamnophis butleri*. All pairwise comparisons were significant ($\alpha = 0.05$ without correction for multiple testing (indicated in bold), $\alpha = 0.020$ with a Benjamini-Yekutieli (B-Y) adjustment, $\alpha = 0.0083$ with a Bonferroni correction (\ddagger indicates significance for both B-Y and Bonferroni corrections)).

	LaSalle & Ojibway	Essex & islands (+ adj. MI)	Lambton (+adj. MI)	Luther Marsh
LaSalle & Ojibway	—	0.056\ddagger	0.13\ddagger	0.24\ddagger
Essex & islands (+ adj. MI)	0.060\ddagger	—	0.064\ddagger	0.17\ddagger
Lambton (+ adj. MI)	0.45\ddagger	0.26\ddagger	—	0.23\ddagger
Luther Marsh	0.52\ddagger	0.32\ddagger	0.17\ddagger	—

Chapter 3. Landscape genetics of Butler's gartersnake (*Thamnophis butleri*) in Ontario

3.1. Abstract

Species movement can be facilitated or impeded by different features of a landscape. Quantifying this relationship can improve our understanding of how genetic structure arises within natural landscapes, and how human-altered landscapes impact genetic diversity and population persistence. We investigated the landscape genetics of Butler's gartersnake (*Thamnophis butleri*) at its northern range periphery and across its entire known Canadian distribution, an area heavily impacted by agriculture and urbanization. Using landcover data from a federal government crop inventory of Ontario and DNA microsatellite markers, we ran simple and partial Mantel tests within all previously diagnosed regional Canadian populations of the species: Essex County, Lambton County, and Luther Marsh Wildlife Management Area. We classified grassland- and wetland-type layers as suitable habitat, and the remaining land cover – mostly agricultural and urban – as resistant to movement. Genetic distances correlated with all landscape-driven (isolation by resistance, IBR) and distance-driven (isolation by distance, IBD) Mantel models for all three regions. Controlling each IBR model with the respective IBD model, and *vice versa*, revealed that genetic structure of *T. butleri* in Lambton is landscape-driven, with strong anthropogenic barriers to dispersal. Genetic structure within the Essex population is better explained by IBD, although some landscape features may act at a finer scale than what we considered here. We found that water did not impede gene flow in these regions, raising the possibility of dispersal along major rivers that may enhance connectivity among individuals along waterways. Our sample sizes from Luther Marsh were small and thus our conclusions provisional, but the genetic patterns may be landscape-driven as the confidence interval of

Mantel matrix correlations, controlling IBR for IBD, did not overlap zero. This is the first study to assess landscape genetic patterns in *T. butleri* and provides a framework for conservation and land stewardship.

3.2. Introduction

The field of landscape genetics is an emerging discipline (Manel and Holderegger 2013) that quantifies the effects of landscape composition on the genetic structure of populations. This field is particularly relevant in areas where natural landscapes are being fragmented through the rapid encroachment of humans. For example, animals are often deterred by anthropogenic features, choosing not to cross roads (Shepard et al. 2008) or to avoid urban areas (Whitcomb et al. 1981), which can result in a reduction of gene flow (e.g. R. W. Clark et al. 2010). Similarly, increased mortality associated with human activities – such as road kills (Ashley and Robinson 1996), pesticides (Grabuski et al. 2004), and increased incidences of predation by both human persecution and generalist predators (Burger and Zappalorti 2016) – can reduce genetically effective population sizes and consequently genetic diversity (e.g. N. D. Jackson and Fahrig 2011).

To discover the degree to which wildlife populations can be shaped by the surrounding environment, researchers examine the association between genetic and landscape structure (Manel and Holderegger 2013). Analytical methods to examine the relationship between genetically clustered populations and landscape configuration saw wide application beginning in the early 21st Century (Manel et al. 2003). A common approach is the use of a Mantel non-parametric permutation test to assess the correlation between a pair of matrices (Mantel 1967), which is adapted for landscape genetics by building matrices of genetic, geographic and environmental distances. Individuals from a focal species or population are sampled and used as

the unit of study, which reduces the bias of pre-defining populations (Manel et al. 2003). Environmental variation is modelled with a variety of ‘cost’ distances that describe its suitability for animal movement between pairs of locations (Manel and Holderegger 2013). Additional matrices can be incorporated into the analyses using partial Mantel tests (Smouse et al. 1986). By evaluating how well competing models explain genetic patterns, researchers can interpret how wildlife interacts with its habitat and test whether particular anthropogenic or natural features impede or facilitate dispersal (e.g. Row et al. 2010, Savage et al. 2010).

This study investigates how the Butler’s gartersnake (*Thamnophis butleri*) has been affected by the profound habitat fragmentation in its Canadian range. *Thamnophis butleri* specializes in grassland and wetland habitat (Rowell 2012), and all Canadian incidences of the species occur in the heavily developed region of Southwestern Ontario, in Essex and Lambton counties, as well as Luther Marsh Wildlife Management Area (WMA), which is located north of the city of Guelph (COSEWIC 2010). Southern Ontario has the highest human population density in Canada (Statistics Canada 2008), and the intensity of urban development, supporting transportation corridors, and industrial agriculture has fragmented the landscape and relegated *T. butleri* to remnant patches of suitable habitat (COSEWIC 2010). Because of the ease of land conversion for agriculture and rich soils, European settlers in the region chose natural grasslands and wetlands to establish farmland (Tallgrass Ontario 2005). In extreme Southwestern Ontario, where most Canadian *T. butleri* occur, over 95% of the wetlands have been lost, largely converted for agriculture (Kelly 1975, Ducks Unlimited Canada 2010).

Given the severity of the habitat loss and fragmentation, we hypothesize that *T. butleri* dispersal is limited to its remaining suitable habitat and constrained by the intervening unsuitable landcover matrix. We evaluated whether the relatedness among genotypes of *T. butleri* sampled

across the northern periphery of their distribution are better explained by isolation by distance (IBD) models that attribute genetic differences to physical distance only, or by isolation by resistance (IBR) models that correlate genetic divergences between individuals with specific environmental features. If the distribution of these habitat specialists is impeded by unsuitable land cover that has been shaped by anthropogenic development, then we predict genetic structure will be better explained by IBR models.

3.3. Materials and methods

3.3.1. Genetic differentiation

Sample collection, DNA extraction, and genotyping of *T. butleri* DNA samples is described in detail in Chapter 2. In brief, we obtained blood or tissue samples from 233 *T. butleri* individuals from Ontario and adjacent Michigan locations. After extracting DNA using established protocols, we genotyped each individual for 12 DNA microsatellite loci. For our landscape genetics analysis, we first created a matrix of Bray-Curtis dissimilarities (Bray and Curtis 1957) between individuals to determine the proportion of shared alleles and pairwise genetic differentiation using the package *adegenet* 2.0.1 (Jombart 2008) in R 3.4.0 (R Core Team 2017).

3.3.2. Regions analysed

Because of the distance between regional areas of *T. butleri* populations and evidence that these comprise demographically distinct populations (see Chapter 2), we performed separate landscape analyses on Lambton County (n = 94), Essex County (n = 114), and Luther Marsh WMA (n = 10), recognizing that small sample size in the latter compromises our power to evaluate the landscape associations. We omitted Michigan samples because our land cover

imagery did not cover the United States. We created a shapefile in ArcMap (ESRI 2015) for each area (Figure 3.1), leaving a buffer of at least 5 km beyond every sampled individual, a value that is about ten times larger than the longest dispersal distance measured for a *T. butleri* individual over a season (Freedman and Catling 1979). This buffer only applies to areas of connected land, however, because most populations are near the international border with the U.S.A., and the Ontario land inventory does not extend far enough across the border for sufficient buffer. Regardless, continuous open water, like the St. Clair and Detroit rivers and Lake St. Clair, creates a natural barrier along the international border.

3.3.3. Resistance modeling

We obtained land cover data from the Government of Canada's annual crop inventory (Agriculture and Agri-Food Canada 2016), which uses satellite imagery to assign land cover types in a raster file, based in the World Geodetic System 84 coordinate system. We clipped the raster map to each shapefile (Figure 3.1) using the R packages `rgdal` 1.2-7 (Bivand et al. 2017) and `raster` 2.5-8 (Hijmans 2016).

We selected the landcover layers of wetland, grassland, fallow areas, pasture/forages, and shrubland as suitable habitat for *T. butleri*. Wetland and grassland make up ideal *T. butleri* habitat (Rowell 2012); fallow areas and pasture are agricultural fields that are considered 'cultural meadows' that can and do function as open grassland habitat for many species including *T. butleri*; shrubland has low-height woody vegetation, often with a ground layer of grass or wetland (Agriculture and Agri-Food Canada 2016). We classified these as non-resistant landcover that would allow snakes free movement. We hypothesized that the remaining land classes (i.e. all regularly-harvested agricultural covers, forests, barren land, urban land, and open water) would impede gene flow, and classified these features as resistant.

Spurious conclusions might arise from our simple binary classification of urban land and open water as being exclusively resistant. While most urban features are barriers to gene flow in snakes (e.g. Shepard et al. 2008, R. W. Clark et al. 2010) and most urban land is undoubtedly not conducive to *T. butleri* population persistence or movement, several sampled individuals from northern Essex County were captured on railway-adjacent land, which is included in the urban land layer (Agriculture and Agri-Food Canada 2016). To address this uncertainty at least in part, we ran an additional analysis for the Essex county area excluding all samples collected from rail lines to test whether their inclusion significantly influenced the results. Open water can similarly act as a barrier to gene flow in terrestrial snakes (King and Lawson 2001), and would not be considered preferred *T. butleri* habitat. However, they have occasionally been observed swimming (COSEWIC 2010), and are often found near watercourses, implying that water may not be a strong barrier to movement. Previous analysis revealed that samples from Michigan were most genetically similar to samples from the most geographically proximate Ontario populations (Figures 2.3, 2.4). We estimated pairwise F_{ST} (S. Wright 1949) and R_{ST} (Slatkin 1995) using with Arlequin 3.5.2.2 (Excoffier and Lischer 2010) to test formally whether *T. butleri* from Ontario are more strongly differentiated from Michigan snakes across the river system than from Ontario snakes equally distant across land. We also estimated pairwise differentiation between locations described in Table 2.2, further separating genetically distinct populations (e.g. Ojibway and LaSalle from North Essex), and distant Michigan samples. We also ran a set of parallel landscape analyses in which we included water as a non-resistant land type.

We constructed multiple IBR models to test whether patterns of genetic distinctiveness of *T. butleri* is shaped by landscape features. We varied three factors that explore how *T. butleri*

might disperse across a potentially resistant landscape: the spatial scale, the resistance threshold, and the level of resistance.

The spatial scale is a summary of the landscape variables, based on landscape distance that a snake might regularly cover, allowing us to ignore the effects of very small habitat or barrier features. Activity ranges of *T. butleri* have been observed to span as small an area as 200 m² over a few months (Freedman and Catling 1979) up to 8000 m² over an entire season (Carpenter 1952); the latter study found that ranges were more likely to be long and narrow rather than squares, likely due to habitat limitations as they seldom strayed far from wetland edges. For this reason, we focused on straight-line geographic distances over which individual snakes might travel. Carpenter (1952) recorded 305 m as the furthest straight-line distance covered by a single *T. butleri* individual, and Freedman and Catling (1979) observed a single female that moved 517 m in a season of observation. These distances are extremes, and while some individuals might undertake substantial movements (COSEWIC 2010), most individuals do not travel extensively. The same studies found average minimum movement distances to be 115 m (Carpenter 1952) and 70 m (Freedman and Catling 1979).

The Agriculture and Agri-Food Canada (2016) imagery has a cell resolution of 30 m. We considered spatial scales of 10 cells (300 m) as a representation of the maximum *T. butleri* seasonal movement, 5 cells (150 m) as an upper limit of the average seasonal movement, and 3 cells (90 m) as the lower limit of average seasonal movement. As the averages determined by previous studies were for minimum movement distances, we chose an upper limit that was larger than distances travelled by over 85% of snakes and a lower limit that was around the mean value (Freedman and Catling 1979).

The resistance threshold is the lowest proportion of habitat-type land cover that still accommodates snake movement through a given region. We chose 5% and 20% thresholds where percentages of habitat below these values within the moving window are barriers to movement. The assigned resistance value describes the degree to which these barriers are deterrents to movement. We tested four levels of resistance, 5 (very low), 10, 50, and 100 (virtually no movement through cell). As a null model, we also tested gene flow across an undifferentiated landscape for each spatial scale, evaluating whether isolation by distance (IBD) is a better explanation for patterns of genetic distances compared to the IBR hypotheses represented by our resistance models (Table 3.1).

To evaluate the IBR and IBD models, we used Mantel tests (Mantel 1967) to determine the correlation between the matrix of genetic differences between individuals and matrices of either uniform or resistance distances. Then we controlled for straight-line distance and landscape resistance in turn with partial Mantel tests (Smouse et al. 1986). All simple and partial Mantel tests were performed with the *ecodist* 1.2.9 package (Goslee and Urban 2007).

We tested for the linearity of our cost distance models by plotting cost distances against the genetic distances and inspecting the shape. We also log-transformed the cost distances of the models and compared the Mantel *r*-values and R^2 measures of transformed and untransformed distance matrices, as recommended by the Zeller et al. (2016).

3.4. Results

3.4.1. Water as an impediment to gene flow

Levels of pairwise genetic differentiation between *T. butleri* populations in Ontario and Michigan do not support the contention that major rivers between Lake Huron and Lake Erie

impede gene flow. Along the St. Clair River, Michigan samples were not genetically distinct from any Ontario samples. The only significant F_{ST}/R_{ST} values were between Ontario locations lying on the same side of the river (Table 3.2). Across the Detroit River, the Michigan samples to the south were not strongly distinct from any other location. There were genetic dissimilarities between most of the other locations, both in Ontario and Michigan, as displayed by significant F_{ST}/R_{ST} values (Table 3.3).

3.4.2. Landscape resistance

In Lambton County, the Mantel tests for all IBD and IBR models indicated significant ($p = 0.001$) correlation with the genetic distance matrix (Table 3.4). All IBR models except for the 300 m spatial scale, 20% threshold models were significant ($p < 0.02$) when controlling for straight-line distance with a partial Mantel test. Conversely, partial Mantel tests that controlled for resistance showed no correlation between genetic and geographic distances, thus not supporting an IBD hypothesis. The models with the highest Mantel correlations were those at a 90 m spatial scale and 20% threshold, and at a 150 m spatial scale and 5% threshold, with the topmost being LS3_20C and LS3_20D at Mantel $r = 0.26$, $p = 0.001$ (Table 3.4).

All IBD and IBR models were significantly correlated ($p = 0.001$) with genetic distance in Essex County as well, but in contrast, the partial Mantel tests controlling for geographic distance showed no correlation with resistance, while those controlling for resistance still showed a significant relationship ($p \leq 0.003$) between physical distance and genetic differences (Table 3.5). The IBD models were the most strongly correlated with genetic distance (e.g. UD5: Mantel $r = 0.43$, $p = 0.001$). Analysing the Essex region without the railway-associated samples revealed the same trend (Table 3.6), with Mantel matrix correlations being slightly higher (e.g. UD5: Mantel $r = 0.47$, $p = 0.001$).

Landscape distance at Luther Marsh also correlated significantly with genetic distance between individuals ($p < 0.035$) for all IBD and IBR models (Table 3.7). None of the partial Mantel tests controlling for distance or for resistance were significant. However, only 10 *T. butleri* were sampled from the Luther Marsh region, so the Mantel analyses in this region had less statistical power. A spatial scale of 90 m was most strongly supported by the Mantel tests, with the highest being for models LS3_20B, LS3_20C, and LS3_20D at Mantel $r = 0.47$, $p = 0.012$, $p = 0.013$, $p = 0.008$, respectively.

Mantel tests including open water as a non-resistant landscape feature showed significant correlation between genetic distances and the IBD and IBR models in all regions. However, the Mantel r -values were mostly less than (and occasionally the same as) those in the equivalent tests where water was classified as resistant (Tables B1, B2, B3), indicating lower support for its inclusion as a non-resistant layer. In Lambton, the tests with the highest Mantel r -values followed the same trends as described above. Some of the less correlated models exhibited support for IBD rather than IBR (Table B1). In Essex, the Mantel tests followed the same trend with and without classifying water as resistant (Table B2). At Luther Marsh, the significant trends were as described previously, but with the inclusion of water all partial Mantel tests had confidence intervals that overlapped zero (Table B3).

The cost/genetic distance plots all appeared linear. Mantel tests of log-transformed cost distances showed significant relationships between the genetic distance matrix and the IBD and IBR models (Tables B4, B5, B6), similar to those found with untransformed cost distances. Most Mantel r -values were smaller after log transformation, except a few in the Essex region (Table B5); these were some of the lower Mantel r -values among the models and those models did not behave differently. A comparison of R^2 values told a similar story (Tables B7, B8, B9).

3.5. Discussion

Our results showed significant correlation for all IBD and IBR models with the genetic distances of *T. butleri* in Ontario, but correlations varied among the regional populations when controlling each of the IBD and IBR models by limiting the influence of the opposite model, suggesting that different factors may be influencing genetic structure in these different regional populations.

Despite the apparent homogeneity of the population in Lambton County (Figure 2.5), we found that complete landscape resistance at a 90 m spatial scale and 20% threshold (LS3_20D) best described patterns of genetic structure across the region (Table 3.4); all Gen~Res+Dist partial Mantel tests were significant, unlike the Gen~Dist+Res partial Mantel tests. Sites containing *T. butleri* were clustered in three areas strongly separated by two swaths with unsuitable landcover: an area of dense urban development between northern Sarnia and Aamjiwnaang First Nation land and an area with farmland and roads separating Aamjiwnaang from sites further south in the county. Samples from locations in Sarnia and in southern Lambton were genetically similar; samples from Aamjiwnaang showed a suggestion of genetic distinction from the others (Figure 2.7). This structure likely explains the stronger fit of matrices of resistance distances with genetic distances than with simple geographic distance, as IBD typically presents a gradient of allele frequencies across a range (Meirmans 2012). The isolation of *T. butleri* in Aamjiwnaang, if borne out by further analyses, could be recent and, while genetic differentiation is not strong enough for it to form a distinct cluster, this separation may eventually result in a genetically unique population.

The retained connectivity between *T. butleri* individuals in northern and southern Lambton County sites may be due to gene flow around Aamjiwnaang, either further inland or

along the margins of or even in the St. Clair River (Figure 3.1A). Inland dispersal is unlikely, as the species has never been recorded in that area and all known locations are closer to water (COSEWIC 2010). Also, the northernmost samples are surrounded by the city of Sarnia, without any apparent habitat corridors to permit movement. However, the St. Clair River may be a corridor to dispersal as there was no evidence of genetic differentiation between Ontario and Michigan populations across that the river (Table 3.2). While resistance models that included open water as suitable habitat were less correlated with genetic matrices, the most strongly correlated models, at a spatial scale of 300 m and a threshold of 5%, still attributed structure to resistance and not geographic distance. If dispersal is facilitated by flowing water, then the spatial scale needed to model movement through water may be larger than that of other habitat variables.

A potential flaw of these analyses is that all Michigan locations were sparsely sampled, with some locations having only one sample; thus, our analyses including these samples might not have a high enough power to detect a significant effect. It is generally agreed that using large sample sizes and/or many loci provide more accurate assessments of genetic differentiation (Ruzzante 1998, Kalinowski 2005). When working with small sample sizes, F_{ST} is preferable statistic to R_{ST} , as it has a lower sampling variance (Ruzzante 1998) particularly for higher values of F_{ST} (greater than 0.05) (Kalinowski 2005). However, R_{ST} assumes a stepwise mutation model that is more appropriate for microsatellites (Slatkin 1995, Ruzzante 1998).

For *T. butleri* in Essex County, IBD models best explained genetic structure while IBR models were not significant when controlling for geographic distance (Table 3.4); i.e. of the partial Mantel tests, only Gen~Dist+Res were significant. This suggests that for the Essex County region our landscape resistance models are no better at describing genetic structure than

simple pairwise distances between individuals. This could be because most *T. butleri* sampling sites in the region, except those in the Ojibway Prairie Complex, were small areas of habitat situated within a highly resistant landscape, such as railway embankments and overgrown parks. These features may have been too small for the scale of the raster imagery (30 m squares) to accurately reflect their presence, and consequently our IBR models were unable to adequately capture all facets of local *T. butleri* habitat. Unlike the situation in Sarnia, in Windsor many of the rail lines run along or link the region's *T. butleri* range rather than bisecting it, and *T. butleri* have been found associated with railway-adjacent land. When we removed samples collected on railway corridors, all IBD and IBR models were more highly correlated with the remaining individuals, although IBD still stood out as the superior model (Table 3.6). Adding open water as a land classification type that might permit dispersal did not appreciably change model trends and, as with the Lambton County samples, individuals on the Michigan side of the Detroit River were not genetically distinct from Ontario individuals (Table 3.3). While a historical association could account for this similarly, we know that some river crossing must be occurring, as the recent appearance of *T. butleri* presence on the Detroit River islands (COSEWIC 2010) must have been due to aquatic dispersal. Landscape analysis that captures this network of small habitat features could potentially provide additional insight into the effect of landcover on *T. butleri* gene flow.

As Luther Marsh consisted of only 10 samples, we had little statistical power for our regional landscape analyses (Landguth et al. 2012). Additionally, as it is located on conservation land, there are fewer land cover elements that might impede movement. Even so, all Mantel tests were significant, and most of the partial Mantel tests controlling IBR models for distance had confidence intervals that did not overlap with zero (Table 3.7). It is possible that, with a greater

sample size, genetic patterns for Luther Marsh *T. butleri* might be shown to relate to landscape patterns. When including open water as a habitat layer, all partial Mantel tests had confidence intervals that included zero, which may mean that *T. butleri* do not swim across Luther Lake. All evidence of gene flow across water that we found were for moving water, not open water bodies.

We used Mantel tests to examine the relationship between the genetic structure of *T. butleri* and habitat configuration within regional populations in its Ontario range. While these tests are the most widely-used method of quantifying the relationship between genetic and landscape structure (Manel and Holderegger 2013), there has been criticism that they have been too broadly applied and their limitations ignored. Zeller et al. (2016) identified several limitations that could impact the results of Mantel tests including deviations from linearity, time lag of genetic equilibrium, insufficient fragmentation of landscapes to impact genetic patterns, and correlation of similar models.

We found no evidence that lack of linearity affected our interpretations as our transformed models did not outperform our original models, and visual inspection of distance relationships implied linearity. It is possible that populations are not in genetic equilibrium and a time lag could be influencing our results, particularly as we identified the possibility of reduced gene flow occurring in Lambton samples. However, Zeller et al. (2016) acknowledged that this challenge does not greatly affect Mantel results. Low levels of fragmentation, which allow relatively uninhibited gene flow, can result in poor performance of Mantel tests (Cushman et al. 2013, Zeller et al. 2016). Among the regions we tested, this could present problems at Luther Marsh, but we do not assert strong meaning to our findings in that area because of small sample size. Our landscape genetics investigation of *T. butleri* is mostly focused on Lambton and Essex

counties, where there is considerable fragmentation. We are thus not concerned that the landscape itself introduces bias in these regions.

The final limitation identified by Zeller et al. (2016) is that similarity between models could mean that they are highly correlated, and that the correct model cannot be confidently selected. The authors suggest that refining resistance values is not an appropriate use of Mantel tests. Our models share parameters, and the Mantel r -values are all similar, particularly when two models have the same spatial scale or threshold. It is likely that identifying the correct model may not be practical. However, we discussed general trends in addition to identifying the top models, and often the top models include the same parameters. As such, we draw similar conclusions from our models, so correlation of the models is not a major issue.

We have shown that how landscape shapes dispersal and gene flow of *T. butleri* varies among regions. To more robustly assess the relationship between genetic structure and environmental heterogeneity, we recommend finer-scale landscape analysis with better GIS data and higher-resolution habitat maps, particularly in the Essex region where genetic differentiation is strong. Genetic matrices based on larger, genome-wide panels of markers may provide better measures of relatedness among individuals. At Luther Marsh WMA, better sampling could provide more confidence in landscape genetic analysis of the region. In the future, real-time monitoring of how the landscape continues to interact with gene flow in *T. butleri* may be beneficial to understanding how changes in the region may continue to affect the persistence of this Endangered species in Southwestern Ontario.

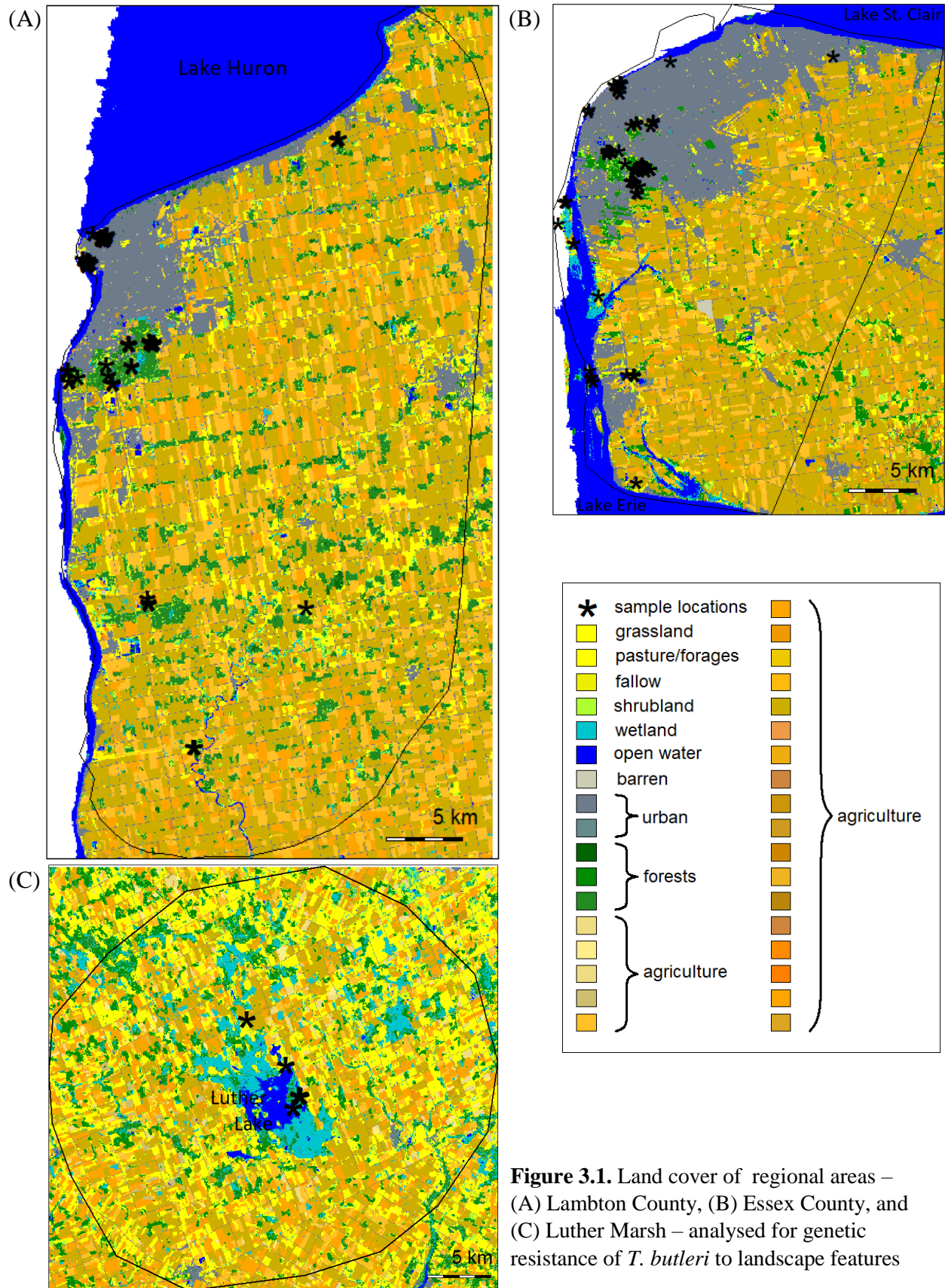


Figure 3.1. Land cover of regional areas – (A) Lambton County, (B) Essex County, and (C) Luther Marsh – analysed for genetic resistance of *T. butleri* to landscape features

Table 3.1. Landscape models for a range of spatial scales of *T. butleri* movement. IBD models demonstrate movement across undifferentiated landscapes, IBR models assign thresholds of resistance that will create a barrier to movement and proportion of resistance conferred by the barriers.

Resistance model	Spatial scale ¹	Threshold	Resistance
UD10	10	undifferentiated landscape	
LS10_5A	10	5	5
LS10_5B	10	5	10
LS10_5C	10	5	50
LS10_5D	10	5	100
LS10_20A	10	20	5
LS10_20B	10	20	10
LS10_20C	10	20	50
LS10_20D	10	20	100
UD5	5	undifferentiated landscape	
LS5_5A	5	5	5
LS5_5B	5	5	10
LS5_5C	5	5	50
LS5_5D	5	5	100
LS5_20A	5	20	5
LS5_20B	5	20	10
LS5_20C	5	20	50
LS5_20D	5	20	100
UD3	3	undifferentiated landscape	
LS3_5A	3	5	5
LS3_5B	3	5	10
LS3_5C	3	5	50
LS3_5D	3	5	100
LS3_20A	3	20	5
LS3_20B	3	20	10
LS3_20C	3	20	50
LS3_20D	3	20	100

1. 10 = 10 cells (300 m). 5 = 5 cells (150 m) 3 = 3 cells (90 m). Cells are 30 m from the Agriculture and Agri-Food Canada (2016) imagery used to quantify habitat.

Table 3.2. Estimates of pairwise F_{ST} (above diagonal) and R_{ST} (below diagonal) between each of 8 *Thamnophis butleri* locations in Lambton County and Michigan, along the St. Clair River. Significant p values (accounting for multiple testing) are bolded: † indicates significant p values for a Benjamini-Yekutieli (B-Y) adjustment ($\alpha = 0.015$); ‡ indicates significance for both a B-Y adjustment and a Bonferroni correction ($\alpha = 0.0033$).

	Macomb, MI (n = 2)	St. Clair, MI South (n = 1)	St. Clair, MI North (n = 2)	Lambton South (n = 10)	Lambton Aamj. (n = 42)	Lambton North (n = 42)
Macomb, MI	—	0.11	0.020	0.086	0.10	0.039
St. Clair, MI South	-0.51	—	0.028	0.14	0.20	0.10
St. Clair, MI North	-0.082	0.13	—	0.060	0.077	0.0017
Lambton South	-0.098	-0.29	-0.096	—	0.064 ‡	0.046 ‡
Lambton Aamj.	0.19	0.11	0.046	0.13 †	—	0.047 ‡
Lambton North	-0.011	-0.17	-0.058	0.0044	0.051 †	—

Table 3.3. Estimates of pairwise F_{ST} (above diagonal) and R_{ST} (below diagonal) between each of 8 *Thamnophis butleri* locations in Essex County and Michigan, along the Detroit River. Significant p values (accounting for multiple testing) are bolded: † indicates significant p values for a Benjamini-Yekutieli (B-Y) adjustment ($\alpha = 0.013$); ‡ indicates significance for both a B-Y adjustment and a Bonferroni correction ($\alpha = 0.0018$).

	Wayne, MI South (n = 6)	Wayne, MI North (n = 1)	Crystal Bay Is. (n = 6)	Fighting Is. (n = 14)	Belle Isle (n = 3)	Essex South (n = 9)	Essex Ojibway (n = 58)	Essex North (n = 27)
Wayne, MI South	—	0.034	0.093	0.16	0.12	0.15	0.19	0.14
Wayne, MI North	-0.30	—	0.11 †	0.076 ‡	0.074	0.091 ‡	0.13 ‡	0.073 ‡
Crystal Bay Is.	0.85	0.67 †	—	0.11 ‡	0.13 †	0.15 ‡	0.12 ‡	0.099 ‡
Fighting Is.	0.11	0.13	0.33 ‡	—	0.078 †	0.11 ‡	0.095 ‡	0.045 ‡
Belle Isle	-0.071	0.052	0.38	-0.098	—	0.12	0.11 ‡	0.072 †
Essex South	-0.11	0.0048	0.48 †	0.0093	-0.062	—	0.13 ‡	0.079 ‡
Essex Ojibway	0.34	0.30 ‡	0.18 †	0.042	0.0058	0.15	—	0.044 ‡
Essex North	0.082	0.11	0.37 ‡	0.0061	-0.054	-0.0015	0.067 †	—

Table 3.4. Mantel tests for correlations between matrices of genetic distance and cost distances of each IBD and IBR model (Gen~Res), partial Mantel tests controlling for the effect of geographical distance from the IBD model (Gen~Res+Dist), and partial Mantel tests controlling for the effect of resistance from each IBR model (Gen~Dist+Res) for the Lambton County region. Significant p-values are bolded.

Model	Gen~Res				Gen~Res+Dist				Gen~Dist+Res			
	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI
UD10	0.21	0.0010	0.17	0.26								
LS10_5A	0.24	0.0010	0.21	0.28	0.14	0.0010	0.095	0.17	-0.056	0.87	-0.095	-0.014
LS10_5B	0.24	0.0010	0.21	0.28	0.13	0.0030	0.094	0.16	-0.017	0.61	-0.057	0.027
LS10_5C	0.23	0.0010	0.21	0.25	0.11	0.0030	0.077	0.15	0.056	0.15	0.015	0.10
LS10_5D	0.22	0.0010	0.20	0.25	0.11	0.0050	0.074	0.15	0.076	0.078	0.032	0.13
LS10_20A	0.22	0.0010	0.19	0.25	0.066	0.093	0.031	0.096	-0.0090	0.56	-0.044	0.031
LS10_20B	0.22	0.0010	0.19	0.25	0.065	0.137	0.032	0.10	0.012	0.40	-0.027	0.054
LS10_20C	0.21	0.0010	0.18	0.24	0.066	0.116	0.029	0.10	0.045	0.22	0.0020	0.091
LS10_20D	0.21	0.0010	0.18	0.24	0.066	0.121	0.027	0.10	0.052	0.18	0.0020	0.097
UD5	0.22	0.0010	0.17	0.25								
LS5_5A	0.24	0.0010	0.21	0.28	0.16	0.0020	0.12	0.18	-0.089	0.96	-0.122	-0.055
LS5_5B	0.25	0.0010	0.22	0.29	0.15	0.0020	0.12	0.18	-0.057	0.86	-0.089	-0.015
LS5_5C	0.25	0.0010	0.23	0.28	0.15	0.0020	0.12	0.18	0.0080	0.43	-0.034	0.055
LS5_5D	0.25	0.0010	0.23	0.28	0.15	0.0020	0.11	0.18	0.029	0.30	-0.015	0.076
LS5_20A	0.23	0.0010	0.20	0.27	0.12	0.017	0.072	0.15	-0.056	0.82	-0.096	-0.0040
LS5_20B	0.24	0.0010	0.21	0.27	0.12	0.012	0.076	0.16	-0.040	0.76	-0.085	0.0050
LS5_20C	0.24	0.0010	0.21	0.28	0.13	0.012	0.080	0.17	-0.0080	0.57	-0.051	0.046
LS5_20D	0.24	0.0010	0.21	0.28	0.13	0.0060	0.085	0.17	0.0000	0.49	-0.052	0.048
UD3	0.21	0.0010	0.17	0.25								
LS3_5A	0.23	0.0010	0.20	0.27	0.12	0.018	0.069	0.14	-0.047	0.79	-0.081	-0.011
LS3_5B	0.24	0.0010	0.21	0.27	0.12	0.014	0.083	0.15	-0.027	0.69	-0.061	0.019
LS3_5C	0.24	0.0010	0.22	0.27	0.13	0.0090	0.092	0.17	0.018	0.37	-0.026	0.066
LS3_5D	0.24	0.0010	0.22	0.27	0.13	0.0080	0.087	0.17	0.035	0.26	-0.0090	0.076
LS3_20A	0.24	0.0010	0.21	0.27	0.14	0.016	0.10	0.17	-0.069	0.88	-0.104	-0.029
LS3_20B	0.25	0.0010	0.22	0.28	0.15	0.0060	0.11	0.18	-0.052	0.81	-0.092	-0.010
LS3_20C	0.26	0.0010	0.23	0.29	0.16	0.0030	0.12	0.20	-0.011	0.58	-0.053	0.038
LS3_20D	0.26	0.0010	0.23	0.29	0.16	0.0030	0.12	0.19	0.0020	0.47	-0.043	0.053

Table 3.5. Mantel tests for correlations between matrices of genetic distance and cost distances of each IBD and IBR model (Gen~Res), partial Mantel tests controlling for the effect of geographical distance from the IBD model (Gen~Res+Dist), and partial Mantel tests controlling for the effect of resistance from each IBR model (Gen~Dist+Res) for the Essex County region. Significant p-values are bolded.

Model	Gen~Res				Gen~Res+Dist				Gen~Dist+Res			
	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI
UD10	0.43	0.0010	0.40	0.45								
LS10_5A	0.38	0.0010	0.35	0.41	0.028	0.31	-0.0060	0.061	0.22	0.0010	0.19	0.25
LS10_5B	0.33	0.0010	0.30	0.36	0.027	0.28	-0.0090	0.061	0.29	0.0010	0.26	0.32
LS10_5C	0.24	0.0010	0.21	0.28	0.026	0.30	-0.0070	0.062	0.37	0.0010	0.34	0.39
LS10_5D	0.21	0.0010	0.18	0.25	0.025	0.32	-0.0070	0.059	0.38	0.0010	0.36	0.41
LS10_20A	0.41	0.0010	0.38	0.44	0.024	0.31	-0.010	0.061	0.15	0.0030	0.10	0.18
LS10_20B	0.39	0.0010	0.36	0.41	0.026	0.29	-0.010	0.065	0.20	0.0010	0.16	0.23
LS10_20C	0.35	0.0010	0.32	0.38	0.028	0.30	-0.0020	0.062	0.26	0.0010	0.22	0.29
LS10_20D	0.34	0.0010	0.32	0.37	0.028	0.29	-0.0030	0.063	0.27	0.0010	0.24	0.31
UD5	0.43	0.0010	0.40	0.45								
LS5_5A	0.38	0.0010	0.36	0.41	0.036	0.23	0.0010	0.072	0.21	0.0010	0.18	0.24
LS5_5B	0.34	0.0010	0.31	0.38	0.035	0.24	0.0010	0.067	0.28	0.0010	0.24	0.30
LS5_5C	0.26	0.0010	0.23	0.30	0.031	0.27	-0.0040	0.063	0.35	0.0010	0.32	0.38
LS5_5D	0.24	0.0010	0.21	0.28	0.030	0.27	-0.0080	0.067	0.37	0.0010	0.34	0.39
LS5_20A	0.38	0.0010	0.35	0.41	0.036	0.24	0.0030	0.070	0.21	0.0010	0.18	0.25
LS5_20B	0.34	0.0010	0.31	0.38	0.035	0.24	0.0010	0.066	0.28	0.0010	0.25	0.31
LS5_20C	0.26	0.0010	0.23	0.30	0.031	0.27	-0.0090	0.066	0.35	0.0010	0.33	0.38
LS5_20D	0.24	0.0010	0.21	0.28	0.030	0.28	-0.0060	0.067	0.37	0.0010	0.33	0.39
UD3	0.43	0.0010	0.40	0.46								
LS3_5A	0.40	0.0010	0.37	0.42	0.042	0.21	0.0080	0.076	0.18	0.0010	0.15	0.21
LS3_5B	0.37	0.0010	0.34	0.40	0.047	0.19	0.011	0.083	0.24	0.0010	0.20	0.27
LS3_5C	0.31	0.0010	0.28	0.35	0.053	0.16	0.015	0.085	0.31	0.0010	0.28	0.34
LS3_5D	0.30	0.0010	0.27	0.33	0.054	0.16	0.018	0.087	0.33	0.0010	0.30	0.35
LS3_20A	0.40	0.0010	0.37	0.43	0.042	0.22	0.0050	0.078	0.16	0.0010	0.12	0.19
LS3_20B	0.38	0.0010	0.35	0.42	0.046	0.18	0.011	0.083	0.21	0.0010	0.18	0.24
LS3_20C	0.34	0.0010	0.31	0.37	0.051	0.16	0.016	0.088	0.29	0.0010	0.25	0.31
LS3_20D	0.32	0.0010	0.29	0.35	0.051	0.17	0.017	0.084	0.30	0.0010	0.27	0.33

Table 3.6. Mantel tests for correlations between matrices of genetic distance and cost distances of each IBD and IBR model (Gen~Res), partial Mantel tests controlling for the effect of geographical distance from the IBD model (Gen~Res+Dist), and partial Mantel tests controlling for the effect of resistance from each IBR model (Gen~Dist+Res) for the Essex County region with railway-adjacent samples removed. Significant p-values are bolded.

Model	Gen~Res				Gen~Res+Dist				Gen~Dist+Res			
	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI
UD10	0.47	0.0010	0.45	0.50								
LS10_5A	0.45	0.0010	0.42	0.47	0.0090	0.45	-0.020	0.039	0.18	0.0010	0.14	0.20
LS10_5B	0.42	0.0010	0.39	0.45	0.0060	0.45	-0.016	0.039	0.24	0.0010	0.20	0.27
LS10_5C	0.32	0.0010	0.29	0.36	>0.0000	0.47	-0.022	0.024	0.36	0.0010	0.33	0.39
LS10_5D	0.27	0.0010	0.24	0.31	-0.0050	0.50	-0.024	0.018	0.40	0.0010	0.38	0.43
LS10_20A	0.45	0.0010	0.42	0.47	-0.0040	0.53	-0.039	0.035	0.17	0.0010	0.12	0.20
LS10_20B	0.43	0.0010	0.40	0.46	-0.0020	0.51	-0.039	0.034	0.22	0.0010	0.18	0.25
LS10_20C	0.39	0.0010	0.36	0.42	0.0010	0.48	-0.034	0.034	0.29	0.0010	0.25	0.32
LS10_20D	0.38	0.0010	0.35	0.41	0.0020	0.47	-0.032	0.041	0.30	0.0010	0.26	0.33
UD5	0.47	0.0010	0.44	0.50								
LS5_5A	0.45	0.0010	0.42	0.48	0.0070	0.44	-0.026	0.043	0.16	0.0030	0.12	0.20
LS5_5B	0.43	0.0010	0.40	0.46	0.0040	0.46	-0.033	0.043	0.22	0.0010	0.18	0.25
LS5_5C	0.37	0.0010	0.34	0.41	-0.0030	0.51	-0.035	0.033	0.31	0.0010	0.27	0.34
LS5_5D	0.35	0.0010	0.32	0.39	-0.0070	0.54	-0.036	0.034	0.34	0.0010	0.30	0.36
LS5_20A	0.45	0.0010	0.42	0.48	0.025	0.37	-0.010	0.057	0.15	0.0030	0.12	0.18
LS5_20B	0.44	0.0010	0.40	0.46	0.023	0.35	-0.014	0.058	0.21	0.0010	0.17	0.24
LS5_20C	0.40	0.0010	0.37	0.43	0.022	0.35	-0.014	0.051	0.27	0.0010	0.24	0.30
LS5_20D	0.37	0.0010	0.33	0.41	0.0002	0.49	-0.033	0.058	0.32	0.0010	0.27	0.35
UD3	0.47	0.0010	0.44	0.50								
LS3_5A	0.45	0.0010	0.42	0.48	0.027	0.33	-0.015	0.069	0.15	0.0020	0.10	0.19
LS3_5B	0.44	0.0010	0.41	0.47	0.035	0.27	-0.0040	0.078	0.20	0.0010	0.16	0.23
LS3_5C	0.39	0.0010	0.36	0.43	0.048	0.22	0.013	0.084	0.28	0.0010	0.25	0.31
LS3_5D	0.38	0.0010	0.35	0.41	0.051	0.19	0.012	0.084	0.30	0.0010	0.27	0.33
LS3_20A	0.45	0.0010	0.42	0.48	0.031	0.32	-0.0040	0.070	0.15	0.0030	0.11	0.18
LS3_20B	0.44	0.0010	0.41	0.47	0.037	0.25	<0.000	0.075	0.20	0.0010	0.15	0.23
LS3_20C	0.40	0.0010	0.36	0.43	0.044	0.24	0.010	0.081	0.28	0.0010	0.24	0.31
LS3_20D	0.38	0.0010	0.35	0.41	0.045	0.21	0.0090	0.075	0.30	0.0010	0.26	0.32

Table 3.7. Mantel tests for correlations between matrices of genetic distance and cost distances of each IBD and IBR model (Gen~Res), partial Mantel tests controlling for the effect of geographical distance from the IBD model (Gen~Res+Dist), and partial Mantel tests controlling for the effect of resistance from each IBR model (Gen~Dist+Res) for the Luther Marsh region. Significant p-values are bolded.

Model	Gen~Res				Gen~Res+Dist				Gen~Dist+Res			
	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI
UD10	0.40	0.021	0.20	0.71								
LS10_5A	0.42	0.020	0.23	0.75	0.18	0.18	0.059	0.29	-0.12	0.74	-0.25	0.030
LS10_5B	0.42	0.030	0.22	0.74	0.18	0.16	0.058	0.30	-0.11	0.74	-0.24	-0.0080
LS10_5C	0.43	0.034	0.24	0.71	0.19	0.16	0.065	0.30	-0.086	0.70	-0.21	0.073
LS10_5D	0.43	0.028	0.24	0.72	0.19	0.16	0.075	0.28	-0.081	0.68	-0.19	0.086
LS10_20A	0.43	0.020	0.23	0.72	0.22	0.10	0.10	0.36	-0.15	0.82	-0.27	-0.028
LS10_20B	0.43	0.024	0.24	0.75	0.21	0.11	0.11	0.36	-0.12	0.77	-0.24	0.010
LS10_20C	0.44	0.031	0.24	0.72	0.21	0.14	0.13	0.35	-0.069	0.67	-0.17	0.11
LS10_20D	0.44	0.034	0.25	0.72	0.21	0.12	0.089	0.32	-0.056	0.63	-0.18	0.12
UD5	0.39	0.028	0.16	0.71								
LS5_5A	0.41	0.024	0.19	0.73	0.20	0.16	0.063	0.29	-0.13	0.77	-0.28	0.036
LS5_5B	0.42	0.018	0.22	0.74	0.21	0.13	0.071	0.33	-0.11	0.72	-0.27	0.064
LS5_5C	0.43	0.024	0.23	0.73	0.21	0.12	0.096	0.32	-0.063	0.67	-0.25	0.13
LS5_5D	0.43	0.027	0.22	0.73	0.21	0.13	0.067	0.33	-0.052	0.63	-0.25	0.16
LS5_20A	0.42	0.029	0.23	0.73	0.21	0.15	0.084	0.36	-0.14	0.79	-0.30	0.076
LS5_20B	0.42	0.032	0.19	0.72	0.22	0.15	0.080	0.35	-0.11	0.77	-0.27	0.10
LS5_20C	0.43	0.023	0.24	0.74	0.23	0.11	0.093	0.36	-0.079	0.72	-0.26	0.21
LS5_20D	0.44	0.021	0.21	0.73	0.23	0.10	0.093	0.34	-0.066	0.64	-0.26	0.15
UD3	0.45	0.014	0.24	0.71								
LS3_5A	0.46	0.011	0.26	0.75	0.13	0.24	-0.029	0.25	-0.036	0.58	-0.13	0.10
LS3_5B	0.46	0.012	0.27	0.74	0.13	0.25	-0.0070	0.25	-0.0080	0.49	-0.12	0.14
LS3_5C	0.46	0.010	0.24	0.74	0.14	0.23	-0.024	0.26	0.036	0.39	-0.10	0.15
LS3_5D	0.46	0.016	0.26	0.73	0.15	0.19	0.011	0.28	0.048	0.36	-0.12	0.16
LS3_20A	0.46	0.013	0.25	0.73	0.14	0.26	0.012	0.26	-0.040	0.58	-0.15	0.08
LS3_20B	0.47	0.012	0.29	0.75	0.15	0.22	0.0000	0.29	-0.020	0.53	-0.15	0.14
LS3_20C	0.47	0.013	0.26	0.75	0.17	0.17	0.056	0.30	0.0080	0.46	-0.12	0.13
LS3_20D	0.47	0.0080	0.27	0.74	0.18	0.17	0.033	0.34	0.017	0.42	-0.11	0.15

Chapter 4. General Discussion

Much of the recent biodiversity loss and species extinction seen worldwide is due to human-caused habitat loss (Brooks et al. 2002, Millennium Ecosystem Assessment 2005), and it is projected that this will continue, with numerous ecosystems to see declines in resident species within the next few decades (Millennium Ecosystem Assessment 2005). Loss of suitable grassland habitat has greatly reduced the range of *Thamnophis butleri* in Ontario (COSEWIC 2010). Today, the species exists in a small number of isolated habitat patches across the southern portion of the province, and growing fragmentation has resulted, and likely will continue to result, in genetic isolation and diminution of sizes of regional populations. My thesis addresses key knowledge gaps for the species, by identifying the current number of genetic clusters, ascertaining how fragmentation of preferred habitat has impacted population connectivity and gene flow, and assessing whether genetic structure relates to the degree of habitat specialization by comparison to a more widespread and common generalist congener. This new information can be applied to conservation prioritization and management of *T. butleri* to ensure its continued persistence in Canada.

Thamnophis butleri from areas of Lambton County, Essex County, and Luther Marsh Wildlife Management Area that are not connected by suitable habitat are genetically distinct. These different genetic populations are apparently differently impacted by their respective landscapes. Genetic distinctiveness among Lambton County *T. butleri* individuals relates to variation in the landscape mosaic, particularly urban development around Aamjiwnaang land. In contrast Essex County appears to have greater connectivity across major developed areas, perhaps due to grassy passages along railway lines or other such corridors. Continued loss of

habitat along river margins likely poses the greatest threat to this regional genetic cluster. Within this region, however, we see some evidence of differentiation between Ojibway Prairie and LaSalle and individuals that lie along the shoreline, which may either reflect the signature of historical colonization into this area, or restricted dispersal out of the Ojibway/LaSalle location because that area contains some of the best and most extensive *T. butleri* habitat in the region (Tallgrass Ontario 2005). A lack of dispersal into the area may be due to a scarcity of available migrants nearby, or because the area is bounded by development. In contrast, Luther Marsh is unlikely to be threatened by anthropogenic habitat loss, as most of the relevant area is conservation land; the small regional distribution of *T. butleri* is a more critical concern. We can draw a lesson from Skunk's Misery where a similarly isolated population of *T. butleri* was extirpated due to the loss of suitable grassland habitat, from natural forest succession rather than human-caused destruction (COSEWIC 2010).

Currently, the species is assigned to a single designatable unit (DU), but it may be advisable to designate unique DUs to each of the three regional populations. One simple reason that treating them as a single group may not apply is that restoring connectivity between these regions is likely impractical. *Thamnophis butleri* only disperse only short distances (Carpenter 1952, Freedman and Catling 1979), and disjunctions of unsuitable habitat between the regions are simply too large to re-establish or maintain connection. Another reason that distinct DUs might be merited is that differing pressures appear to be impacting these different populations. An argument against dividing the present single DU is the observation that only a single mitochondrial haplotype exists, implying some genetic uniformity (COSEWIC 2010). Mutation of mtDNA is relatively fast in snakes, but it still occurs over millennia (Eo and DeWoody 2010). The fact that we see such depauperate mitochondrial diversity simply reflects post-glacial

dispersal from Michigan into Ontario (Placyk et al. 2012). Aforementioned patterns of genetic structure in nuclear markers probably began to arise after European settlement in the 1800s – the major threats facing the species undoubtedly developed conjointly with anthropogenic pressures. Additionally, of all the DNA in eukaryotic cells, the mitochondrial genome is a very small component and may not completely reflect the life history of a species.

While habitat preservation should be considered an overarching goal of conservation for Ontario *T. butleri*, there are management nuances that can be applied in the different regions. For example, areas without direct urban pressures, like Luther Marsh, may benefit from prescribed burning. Open areas preferred by *T. butleri* will succumb to ecological succession, but a balance may be achieved by the wildfires that allow grassland habitats persist in a habitat mosaic (H. A. Wright and Bailey 1982). Loss of habitat will often result in animals seeking more favourable conditions (S. D. Jackson et al. 2015a), and small snakes are among the first species to colonize newly created grasslands after fire has occurred (Larson 2014). Habitat creation is also important in regions where habitat destruction and fragmentation are prevalent, particularly as a mitigation measure. There are several instances of local extirpation in Lambton and Essex counties (COSEWIC 2010), often due to loss of habitat. Creating replacement sites, by burning and/or seeding vacant areas, could allow the snakes to persist. Mitigation of the impacts of road construction is also recommended, like ecopassages under or over roads and wildlife barriers along roads that funnel dispersal toward the crossings, to reduce road deaths and allow dispersal and gene flow (S. D. Jackson et al. 2015b). Monitoring efforts are recommended where *T. butleri* intersect with human presence. Protected areas dedicated to this species should be established and maintained, limiting access and preventing degradation. As well, public areas that are likely to acquire grassland characteristics should either be kept from overgrowing or let go to seed.

Thamnophis butleri may move into urban parks that are mowed infrequently (CBC News 2016), and many can be killed at once by subsequent mowing (personal observation). In Essex County, emphasis should be placed on improving connectivity for snake movement. Areas along existing corridors, like hydro lines, railway land and urban walkways, could be made more habitable, and new habitat created at intermediate locations would increase likelihood of dispersal and connectivity. There is notable genetic isolation between Ojibway/LaSalle and the Essex shoreline; if this differentiation arose only recently (a matter requiring further study), it may be both desirable and possible to reconnect these local populations. This might improve global genetic diversity in the region. In Lambton County, re-connecting the local clusters of individuals may not be practicable, as much of the intervening land is suburban. I would recommend monitoring the Aamjiwnaang location for dispersal from other areas of Lambton County. Assisted gene flow that introduces immigrants from different locations (Aamjiwnaang—Sarnia/southern Lambton) may be feasible (Shine and Bonnet 2009) and worthwhile, if it can be shown that Aamjiwnaang or other local clusters are suffering from lower genetic diversity or there is evidence of inbreeding. I do not recommend translocation of adult snakes, as fidelity to their home ranges may make them unable to settle or successfully overwinter (Reinert and Rupert 1999); however, researchers have found success headstarting neonates (Conner et al. 2003), including the rare plains gartersnake, *Thamnophis radix* (King and Stanford 2006), sister species to *T. butleri* (de Queiroz et al. 2002).

Further research could expand the geographic coverage of sampling and augment the landscape analysis. I sampled across the known Ontario range, only excluding Walpole Island, which likely belongs to the Lambton County cluster. However, genetic sampling across the global range, including the bulk of the range in the U.S.A., would provide a better picture of the

genetic structure of the species, and the impacts of recent human activities. Using genomics would also prove fruitful, both because it would allow us to increase substantially the number of markers for quantification of key population parameters (e.g. migration, gene flow), but also because it might afford insights into diversity at loci of adaptive importance. A more fine-scale assessment of relevant landscape features could allow for more explicit evaluation of elements that facilitate or impede gene flow. The inclusion of railways and roadways would allow us to test whether transportation infrastructure forms barriers to dispersal. The identification of sites where grass is perpetually overgrown (even within urban settings) would provide more direct tests of the utility of such habitat to dispersal or persistence of *T. butleri*. Finally, investigating temporal shifts in the genetic structure of *T. butleri* and other impacted herpetofauna would provide a clearer picture of how the changing landscape in this heavily human-impacted region shapes dispersal and species persistence, and would provide more direct tests of anthropogenic influences on this species.

My thesis provides a robust assessment of *Thamnophis butleri* population genetic structure in Ontario, and to my knowledge, it is the first to apply a landscape genetics approach to this Endangered species. Being able to quantify how land use shapes populations is essential for designing conservation strategies; working to maintain connectivity and genetic diversity may help preserve the Butler's gartersnake in Ontario for future generations.

Chapter 5. Summary of Findings in Data Chapters

Chapter 2: Comparing the population structure of the specialist Butler's gartersnake

(*Thamnophis butleri*) and the generalist eastern gartersnake (*Thamnophis s. sirtalis*) in Ontario

Bayesian assignment methods uncovered the genetic structure of *T. butleri* and *T. s. sirtalis* sampled from the Ontario *T. butleri* range, and from adjacent regions Michigan. I found that:

1. *Thamnophis butleri* in Ontario belong to four distinct genetic clusters: Luther Marsh WMA, Lambton County, and the shoreline and Ojibway/LaSalle in Essex County.
2. *Thamnophis s. sirtalis* in Ontario belong to a single genetic cluster.
3. *Thamnophis* individuals in Michigan cluster with adjacent Ontario populations.
4. *Thamnophis butleri* does not experience gene flow among regional populations.
5. Genetic subdivision in Essex County may be due to different dispersal pressures.

Chapter 3: Landscape genetics of Butler's gartersnake (*Thamnophis butleri*) in Ontario

Landscape genetics analyses using Mantel tests of Ontario *T. butleri* revealed that:

1. Genetic structure is driven by landscape in Lambton County, with the suggestion of further subdivision at Aamjiwnaang.
2. Genetic structure is driven by geographic distance in Essex County.
3. Luther Marsh may be distance driven, but sample size is too small to determine.
4. Water was shown to not be a deterrent to dispersal, explaining connectivity along the river-adjacent populations.

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Appendix A: Supplemental Material for Chapter 2

Table A1. Observed (H_o) and expected (H_e) heterozygosity for each *Thamnophis butleri* locus at 12 sampling locations (Table 2.2) in Michigan and Ontario. Bolded p values indicate a significant departure from Hardy-Weinberg Equilibrium (HWE).

Locus	Wayne, MI			Macomb, MI			St. Clair, MI			Crystal Bay Is.		
	H_o	H_e	P	H_o	H_e	P	H_o	H_e	P	H_o	H_e	P
TbuA04a	0.57	0.66	0.81	0.50	0.50	1.0	1.0	0.73	1.0	0.0000	0.48	0.030
TbuA09	monomorphic			monomorphic			monomorphic			monomorphic		
TbuA49	0.43	0.75	0.027	0.50	0.83	0.34	0.67	0.93	0.19	0.50	0.80	0.22
TbuA64	1.0	0.79	0.24	1.0	0.83	1.0	0.67	0.73	1.0	0.83	0.80	1.0
TbuA70	0.43	0.67	0.096	0.50	0.50	1.0	monomorphic			0.83	0.62	0.39
Te1Ca29	0.57	0.82	0.091	0.50	0.50	1.0	0.67	0.73	1.0	0.17	0.68	0.0040
TbuA27	0.43	0.38	1.0	0.50	0.50	1.0	0.67	0.53	1.0	0.17	0.41	0.27
TbuA74	0.71	0.57	1.0	0.50	0.50	1.0	0.67	0.60	1.0	1.0	0.85	0.88
TbuB12	1.0	0.90	1.0	1.0	0.83	1.0	0.33	0.60	0.20	1.0	0.88	0.77
Nsq3	0.71	0.88	0.40	1.0	1.0	1.0	0.67	0.60	1.0	0.83	0.82	1.0
Ts2	0.43	0.76	0.17	1.0	0.83	1.0	0.33	0.33	1.0	0.83	0.86	0.81
3Ts	0.71	0.90	0.23	0.50	0.83	0.33	0.67	0.80	0.61	0.83	0.62	0.64

Locus	Fighting Is.			Belle Isle			Essex South			Essex North		
	H_o	H_e	P	H_o	H_e	P	H_o	H_e	P	H_o	H_e	P
TbuA04a	0.71	0.65	0.35	0.33	0.33	1.0	0.33	0.69	0.069	0.46	0.69	0.0000
TbuA09	monomorphic			monomorphic			monomorphic			monomorphic		
TbuA49	0.79	0.76	0.11	0.67	0.80	0.60	0.67	0.84	0.26	0.62	0.79	0.0020
TbuA64	0.79	0.80	0.98	0.67	0.87	0.47	0.56	0.82	0.15	0.76	0.84	0.081
TbuA70	0.50	0.42	1.0	0.33	0.60	1.0	0.22	0.21	1.0	0.38	0.37	1.0
Te1Ca29	0.71	0.87	0.053	1.0	0.93	1.0	0.44	0.58	0.084	0.62	0.71	0.0040
TbuA27	0.14	0.14	1.0	monomorphic			0.22	0.21	1.0	0.29	0.28	1.0
TbuA74	0.64	0.52	0.35	0.67	0.80	0.61	0.33	0.31	1.0	0.60	0.70	0.048
TbuB12	0.71	0.81	0.60	1.0	0.93	1.0	0.78	0.80	0.25	0.72	0.78	0.0030
Nsq3	1.0	0.86	0.60	1.0	0.87	1.0	0.67	0.67	0.34	0.69	0.80	0.042
Ts2	0.64	0.65	0.50	0.67	0.53	1.0	0.67	0.67	0.66	0.56	0.71	0.0003
3Ts	0.86	0.85	0.85	0.67	0.73	1.0	0.78	0.88	0.64	0.62	0.79	0.0000

Locus	Lambton South			Lambton Aamj.			Lambton North			Luther Marsh		
	H_o	H_e	P	H_o	H_e	P	H_o	H_e	P	H_o	H_e	P
TbuA04a	0.30	0.36	0.31	0.38	0.66	0.0001	0.62	0.75	0.096	0.40	0.67	0.15
TbuA09	monomorphic			monomorphic			monomorphic			monomorphic		
TbuA49	0.70	0.81	0.18	0.64	0.72	0.59	0.81	0.85	0.26	0.30	0.43	0.045
TbuA64	0.70	0.89	0.018	0.71	0.73	0.98	0.90	0.84	0.45	0.60	0.62	1.0
TbuA70	0.30	0.28	1.0	0.45	0.51	0.56	0.33	0.31	0.57	monomorphic		
Te1Ca29	0.70	0.78	0.62	0.43	0.79	0.0000	0.50	0.75	0.0000	0.60	0.55	0.24
TbuA27	0.10	0.10	1.0	0.050	0.050	1.0	0.10	0.09	1.0	monomorphic		
TbuA74	0.50	0.56	0.58	0.26	0.24	1.0	0.52	0.57	0.28	0.50	0.68	0.42
TbuB12	0.70	0.79	0.93	0.69	0.82	0.14	0.48	0.70	0.0004	1.0	0.77	0.33
Nsq3	0.60	0.66	0.092	0.57	0.60	0.14	0.60	0.71	0.11	0.60	0.80	0.40
Ts2	0.50	0.63	0.18	0.55	0.53	0.76	0.52	0.57	0.61	0.80	0.73	0.71
3Ts	0.60	0.87	0.056	0.69	0.72	0.32	0.86	0.90	0.18	0.60	0.85	0.049

Table A2. Observed (H_o) and expected (H_e) heterozygosity for each *Thamnophis s. sirtalis* locus at 9 sampling locations (Table 2.2) in Michigan and Ontario. Bolded p values indicate a significant departure from Hardy-Weinberg Equilibrium (HWE).

Locus	Wayne, MI			Macomb, MI			St. Clair, MI		
	H_o	H_e	P	H_o	H_e	P	H_o	H_e	P
TbuA04a	0.45	0.52	0.24	0.50	0.43	1.0	0.10	0.48	0.022
TbuA09	0.45	0.59	0.76	0.75	0.75	0.66	0.30	0.65	0.013
TbuA49	1.0	0.91	0.89	1.0	0.93	1.0	0.80	0.90	0.27
TbuA64	1.0	0.93	1.0	0.50	0.89	0.13	0.70	0.86	0.076
TbuA70	0.55	0.78	0.011	0.50	0.82	0.31	0.30	0.71	0.0070
Te1Ca29	0.91	0.94	0.22	1.0	0.96	1.0	0.80	0.90	0.42
Nsq2	0.82	0.94	0.072	0.75	0.86	0.66	1.0	0.87	0.17
Ts010	0.73	0.90	0.27	0.50	0.75	0.085	0.70	0.89	0.089
Te1Ca3	0.45	0.78	0.027	0.50	0.61	0.43	0.40	0.80	0.0030

Locus	Essex South			Essex North			Lambton South		
	H_o	H_e	P	H_o	H_e	P	H_o	H_e	P
TbuA04a	0.24	0.22	1.0	0.18	0.35	0.0020	0.30	0.33	0.69
TbuA09	0.65	0.62	1.0	0.64	0.53	0.39	0.47	0.64	0.038
TbuA49	0.82	0.91	0.025	0.86	0.88	0.21	0.88	0.88	0.90
TbuA64	0.88	0.90	0.13	0.91	0.92	0.57	0.88	0.92	0.012
TbuA70	0.35	0.56	0.049	0.27	0.55	0.0006	0.60	0.74	0.034
Te1Ca29	0.88	0.91	0.053	0.77	0.91	0.037	0.84	0.94	0.30
Nsq2	0.59	0.80	0.0070	0.82	0.91	0.22	0.79	0.93	0.019
Ts010	0.76	0.85	0.38	0.82	0.86	0.68	0.74	0.89	0.019
Te1Ca3	0.53	0.54	0.065	0.64	0.79	0.054	0.77	0.81	0.85

Locus	Lambton Aamjiwnaang			Lambton North			Luther Marsh		
	H_o	H_e	P	H_o	H_e	P	H_o	H_e	P
TbuA04a	0.34	0.36	0.77	0.25	0.61	0.14	0.18	0.17	1.0
TbuA09	0.54	0.68	0.066	0.75	0.61	1.0	0.55	0.52	0.31
TbuA49	0.95	0.88	0.16	0.75	0.93	0.31	0.73	0.90	0.29
TbuA64	0.90	0.91	0.29	0.75	0.93	0.32	0.82	0.81	0.64
TbuA70	0.49	0.64	0.047	0.75	0.79	0.77	0.45	0.66	0.18
Te1Ca29	0.78	0.94	0.0006	0.50	0.86	0.085	1.0	0.95	1.0
Nsq2	0.73	0.86	0.010	1.0	0.93	1.0	0.73	0.90	0.072
Ts010	0.78	0.88	0.018	1.0	0.93	1.0	0.73	0.79	0.47
Te1Ca3	0.71	0.83	0.048	0.75	0.75	0.32	0.64	0.66	1.0

Table A3. Probability (p) values from tests for linkage disequilibrium between all pairs of *Thamnophis butleri* loci at 12 sampling locations (Table 2.2) in Michigan and Ontario. Significant p values are bolded, indicating linkage disequilibrium between those two loci. To account for multiple testing, corrected significance is also shown: † indicates significant p values for a Benjamini-Yekutieli (B-Y) adjustment ($\alpha = 0.010$); ‡ indicates significance for both a B-Y adjustment and a Bonferroni correction ($\alpha = 0.00076$).

		Wayne, MI											
		TbuA04a	TbuA09	TbuA49	TbuA64	TbuA70	Te1Ca29	TbuA27	TbuA74	TbuB12	Nsµ3	Ts2	3Ts
Macomb, MI	TbuA04a	—	1.0	0.51	0.85	0.099	0.89	0.42	0.40	0.91	0.54	0.75	0.052
	TbuA09	1.0	—	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	TbuA49	1.0	1.0	—	0.10	0.50	0.0040 [†]	0.57	0.24	0.62	0.072	0.49	0.37
	TbuA64	0.66	1.0	0.94	—	0.54	0.38	0.28	0.32	0.80	0.99	0.52	0.86
	TbuA70	1.0	1.0	0.65	0.79	—	0.72	0.012	0.66	0.12	0.013	0.14	0.23
	Te1Ca29	1.0	1.0	0.63	0.61	0.34	—	0.80	0.62	0.54	0.32	0.70	0.61
	TbuA27	0.34	1.0	1.0	0.55	1.0	1.0	—	0.67	0.21	0.40	0.98	0.26
	TbuA74	0.35	1.0	1.0	0.66	1.0	1.0	0.34	—	0.33	0.074	0.63	0.47
	TbuB12	0.67	1.0	0.85	0.57	0.67	0.80	0.67	0.67	—	0.061	0.43	0.50
	Nsµ3	0.94	1.0	0.22	0.81	0.64	0.73	0.95	0.63	0.76	—	0.011	0.54
	Ts2	0.67	1.0	0.90	0.40	0.66	0.78	0.97	0.68	0.37	0.36	—	0.018
	3Ts	0.36	1.0	0.35	0.76	1.0	1.0	0.39	0.32	0.77	0.91	0.78	—
		St. Clair, MI											
		TbuA04a	TbuA09	TbuA49	TbuA64	TbuA70	Te1Ca29	TbuA27	TbuA74	TbuB12	Nsµ3	Ts2	3Ts
Crystal Bay	TbuA04a	—	1.0	0.84	0.50	1.0	0.88	0.83	0.37	0.32	0.47	0.67	0.67
	TbuA09	1.0	—	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	TbuA49	0.32	1.0	—	0.68	1.0	0.67	0.69	0.93	0.24	0.51	0.59	0.41
	TbuA64	0.018	1.0	0.55	—	1.0	0.43	0.41	0.95	0.14	0.20	0.60	0.72
	TbuA70	0.66	1.0	0.63	0.10	—	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	Te1Ca29	0.015	1.0	0.27	0.31	0.82	—	0.62	0.21	0.84	0.95	0.61	0.084
	TbuA27	0.18	1.0	0.044	0.65	0.14	1.0	—	0.74	0.79	0.17	0.33	0.81
	TbuA74	0.57	1.0	0.79	0.12	0.20	0.87	0.62	—	1.0	0.69	0.39	0.70
	TbuB12	0.26	1.0	0.44	0.028	0.73	0.52	0.14	0.94	—	0.88	1.0	0.71
	Nsµ3	0.97	1.0	0.79	0.94	0.55	0.98	0.12	0.62	0.57	—	0.40	0.73
	Ts2	0.21	1.0	0.35	0.45	0.90	0.84	0.083	0.54	0.40	0.58	—	1.0
	3Ts	1.0	1.0	0.13	0.77	0.34	0.89	0.53	0.074	0.769	0.47	0.84	—

Table A3. continued

		Fighting Island											
		TbuA04a	TbuA09	TbuA49	TbuA64	TbuA70	Te1Ca29	TbuA27	TbuA74	TbuB12	Ns μ 3	Ts2	3Ts
Belle Isle	TbuA04a	—	1.0	0.36	0.083	0.029	0.60	0.91	0.92	0.78	0.060	0.059	0.25
	TbuA09	1.0	—	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	TbuA49	0.42	1.0	—	0.70	0.19	0.62	0.10	0.69	0.17	0.72	0.17	0.86
	TbuA64	0.42	1.0	0.60	—	0.21	0.10	0.51	0.55	0.73	0.98	0.57	0.19
	TbuA70	0.90	1.0	0.53	0.24	—	0.96	0.069	0.56	0.66	0.59	0.22	0.79
	Te1Ca29	0.74	1.0	0.86	0.073	0.39	—	0.089	0.23	0.55	0.89	0.92	0.19
	TbuA27	1.0	1.0	1.0	1.0	1.0	1.0	—	0.085	0.058	0.54	1.0	0.70
	TbuA74	0.41	1.0	0.16	0.73	0.57	0.81	1.0	—	0.17	0.39	0.51	0.75
	TbuB12	0.66	1.0	0.16	0.70	0.76	0.97	1.0	0.13	—	0.99	0.91	0.76
	Ns μ 3	0.42	1.0	0.37	0.90	0.69	0.89	1.0	0.39	0.27	—	0.31	0.60
	Ts2	1.0	1.0	0.94	0.81	1.0	0.74	1.0	0.86	0.55	0.37	—	0.53
	3Ts	0.88	1.0	0.96	0.37	0.12	0.34	1.0	0.55	0.91	0.69	0.61	—
			South Essex										
		TbuA04a	TbuA09	TbuA49	TbuA64	TbuA70	Te1Ca29	TbuA27	TbuA74	TbuB12	Ns μ 3	Ts2	3Ts
North Essex	TbuA04a	—	1.0	0.59	0.31	0.15	0.86	0.16	0.55	0.38	0.78	0.85	0.75
	TbuA09	1.0	—	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	TbuA49	0.015	1.0	—	0.25	0.80	0.19	0.91	0.47	0.026	0.11	0.28	0.087
	TbuA64	0.042	1.0	0.11	—	0.97	0.091	0.20	0.43	0.22	0.45	0.47	0.67
	TbuA70	0.90	1.0	0.070	0.11	—	0.56	1.0	1.0	0.79	0.36	0.064	0.92
	Te1Ca29	0.0030 [†]	1.0	0.16	0.074	0.33	—	0.83	0.12	0.0030 [†]	0.085	0.76	0.072
	TbuA27	0.51	1.0	0.0060 [†]	0.20	0.69	0.68	—	1.0	0.96	0.15	0.36	0.97
	TbuA74	0.19	1.0	0.48	0.087	0.14	0.0020 [†]	0.62	—	0.053	0.25	0.60	0.28
	TbuB12	0.011	1.0	0.0080 [†]	0.18	0.19	0.089	0.88	0.024	—	0.35	0.69	0.011
	Ns μ 3	0.63	1.0	0.18	0.67	0.92	0.12	0.57	0.34	0.23	—	0.64	0.38
	Ts2	0.60	1.0	0.062	0.15	0.99	0.032	0.83	0.20	0.29	0.15	—	0.93
	3Ts	0.18	1.0	0.0001 [‡]	0.30	0.71	0.0030 [†]	0.91	0.044	0.0090 [†]	0.15	0.042	—

Table A3. continued

		South Lambton											
		TbuA04a	TbuA09	TbuA49	TbuA64	TbuA70	Te1Ca29	TbuA27	TbuA74	TbuB12	Ns μ 3	Ts2	3Ts
Aamijwinaang (Lambton)	TbuA04a	—	1.0	0.40	0.52	1.0	0.91	0.11	0.51	0.23	0.46	0.41	0.66
	TbuA09	1.0	—	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	TbuA49	0.17	1.0	—	0.87	0.94	0.84	0.55	0.56	0.72	0.63	0.10	0.47
	TbuA64	0.27	1.0	0.11	—	0.20	0.63	0.48	0.063	0.62	0.34	0.56	0.82
	TbuA70	0.16	1.0	0.65	0.54	—	0.77	1.0	0.41	0.31	0.69	0.35	0.64
	Te1Ca29	0.024	1.0	0.090	0.12	0.15	—	0.84	0.15	0.26	0.17	0.21	0.48
	TbuA27	0.45	1.0	0.27	0.63	0.95	0.45	—	0.46	0.38	0.50	1.0	0.80
	TbuA74	0.0080[†]	1.0	0.29	0.094	0.84	0.33	0.25	—	0.72	0.65	0.79	1.0
	TbuB12	0.25	1.0	0.64	0.61	0.035	0.012	0.45	0.26	—	0.27	0.52	0.31
	Ns μ 3	0.94	1.0	0.034	0.44	0.39	0.16	0.41	0.36	0.61	—	0.015	0.25
	Ts2	0.61	1.0	0.027	0.41	0.52	0.084	0.62	0.40	0.43	0.14	—	0.069
	3Ts	0.17	1.0	0.90	0.17	0.26	0.091	0.25	0.32	0.56	0.68	0.18	—
		North Lambton											
		TbuA04a	TbuA09	TbuA49	TbuA64	TbuA70	Te1Ca29	TbuA27	TbuA74	TbuB12	Ns μ 3	Ts2	3Ts
Luther Marsh	TbuA04a	—	1.0	0.34	0.59	0.64	0.91	0.15	0.46	0.87	0.90	0.62	0.51
	TbuA09	1.0	—	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	TbuA49	0.060	1.0	—	0.29	0.93	0.48	0.014	0.12	0.15	0.23	0.58	0.44
	TbuA64	0.70	1.0	0.47	—	0.66	0.66	0.91	0.51	0.79	0.64	0.26	0.17
	TbuA70	1.0	1.0	1.0	1.0	—	0.61	0.58	0.10	0.80	0.73	0.34	0.83
	Te1Ca29	0.51	1.0	0.72	0.49	1.0	—	0.46	0.29	0.44	0.18	0.78	0.77
	TbuA27	1.0	1.0	1.0	1.0	1.0	1.0	—	0.49	0.29	0.67	0.94	0.088
	TbuA74	0.35	1.0	0.23	0.63	1.0	0.82	1.0	—	0.70	0.53	0.41	0.25
	TbuB12	0.22	1.0	0.021	0.81	1.0	0.69	1.0	0.76	—	0.15	0.22	0.85
	Ns μ 3	0.051	1.0	0.80	0.22	1.0	0.94	1.0	0.14	0.94	—	0.44	0.027
	Ts2	0.94	1.0	0.34	0.0050[†]	1.0	0.79	1.0	0.55	0.56	0.41	—	0.10
	3Ts	0.91	1.0	0.66	0.66	1.0	0.79	1.0	0.10	0.98	0.23	0.75	—

Table A4. Resulting p values from tests for linkage disequilibrium between all pairs of *Thamnophis s. sirtalis* loci at 9 sampling locations (Table 2.2) in Michigan and Ontario. Significant p values are bolded, indicating linkage disequilibrium between those two loci. To account for multiple testing, corrected significance is also shown: † indicates significant p values for a Benjamini-Yekutieli (B-Y) adjustment ($\alpha = 0.012$); ‡ indicates significance for both a B-Y adjustment and a Bonferroni correction ($\alpha = 0.0014$).

		Wayne, MI								
		TbuA04a	TbuA09	TbuA49	TbuA64	TbuA70	Te1Ca29	Nsµ2	Ts010	Te1Ca3
Macomb, MI	TbuA04a	—	0.54	0.65	0.027	0.047	0.38	0.14	0.0080 †	0.84
	TbuA09	0.064	—	0.031	0.53	0.74	0.030	0.83	0.018	0.38
	TbuA49	0.77	0.74	—	0.41	0.22	0.74	0.98	0.85	0.48
	TbuA64	0.29	0.28	0.80	—	0.32	0.038	0.90	0.56	0.38
	TbuA70	0.93	0.56	0.40	0.29	—	0.21	0.39	0.054	0.13
	Te1Ca29	0.63	0.30	0.86	0.29	0.027	—	0.31	0.60	0.10
	Nsµ2	0.25	0.65	0.67	0.015	0.26	0.91	—	0.065	0.72
	Ts010	0.76	0.73	0.067	0.37	0.12	0.76	0.39	—	0.11
	Te1Ca3	0.12	0.16	0.66	0.086	0.44	0.36	0.54	0.57	—
		TbuA04a	TbuA09	TbuA49	TbuA64	TbuA70	Te1Ca29	Nsµ2	Ts010	
St. Clair, MI	TbuA09	0.38	—	—	—	—	—	—	—	—
	TbuA49	0.59	0.42	—	—	—	—	—	—	—
	TbuA64	0.56	0.47	0.33	—	—	—	—	—	—
	TbuA70	0.11	0.28	0.17	0.73	—	—	—	—	—
	Te1Ca29	0.41	0.13	0.020	0.38	0.27	—	—	—	—
	Nsµ2	0.98	0.35	0.21	0.44	0.99	0.27	—	—	—
	Ts010	0.95	0.22	0.74	0.70	0.41	0.55	0.33	—	—
	Te1Ca3	0.10	0.19	0.25	0.99	0.031	0.10	0.98	0.68	—

Table A4. continued

		South Essex								
		TbuA04a	TbuA09	TbuA49	TbuA64	TbuA70	Te1Ca29	Nsµ2	Ts010	Te1Ca3
North Essex	TbuA04a	—	0.81	0.33	0.42	0.53	0.72	0.45	0.53	0.88
	TbuA09	0.35	—	0.18	0.0040 [†]	0.038	0.18	0.81	0.89	0.94
	TbuA49	0.37	0.81	—	0.094	0.054	0.0000 [‡]	0.0070 [†]	0.0010 [‡]	0.32
	TbuA64	0.18	0.19	0.78	—	0.57	0.0030 [†]	0.034	0.29	0.021
	TbuA70	0.089	0.92	0.52	0.20	—	0.13	0.038	0.0000 [‡]	0.61
	Te1Ca29	0.80	0.77	0.083	0.77	0.34	—	0.0001 [‡]	0.011	0.41
	Nsµ2	0.98	0.69	0.53	0.12	0.0050 [†]	0.27	—	0.58	0.82
	Ts010	0.87	0.85	0.51	0.85	0.64	1.0	0.14	—	0.10
	Te1Ca3	0.037	0.41	0.67	0.14	0.014	0.89	0.58	0.35	—
			South Lambton							
		TbuA04a	TbuA09	TbuA49	TbuA64	TbuA70	Te1Ca29	Nsµ2	Ts010	Te1Ca3
Aamjiwnaang (Lambton)	TbuA04a	—	0.21	0.34	0.84	0.70	0.64	0.42	0.038	0.18
	TbuA09	0.82	—	0.84	0.61	0.020	0.11	0.093	0.52	0.037
	TbuA49	0.27	0.63	—	0.49	0.93	0.67	0.86	0.14	0.56
	TbuA64	0.33	0.022	0.30	—	0.51	0.59	0.083	0.38	0.22
	TbuA70	0.25	0.94	0.034	0.50	—	0.81	0.85	0.39	0.52
	Te1Ca29	0.53	0.025	0.25	0.23	0.29	—	0.54	0.17	0.89
	Nsµ2	0.76	0.33	0.87	0.80	0.12	0.51	—	0.25	0.092
	Ts010	0.31	0.092	0.48	0.10	0.44	0.028	0.27	—	0.83
	Te1Ca3	0.35	0.56	0.30	0.83	0.40	0.90	0.51	0.28	—

Table A4. continued

		North Lambton								
		TbuA04a	TbuA09	TbuA49	TbuA64	TbuA70	Te1Ca29	Nsµ2	Ts010	Te1Ca3
Luther Marsh	TbuA04a	—	0.27	0.75	0.041	0.38	0.23	0.32	0.47	0.48
	TbuA09	1.0	—	0.75	0.31	0.20	0.36	0.77	0.47	0.38
	TbuA49	0.75	0.41	—	0.41	0.13	0.45	0.075	0.10	0.22
	TbuA64	0.16	0.71	0.92	—	0.46	0.26	0.65	0.63	0.21
	TbuA70	0.64	0.41	0.38	0.37	—	0.11	0.47	0.66	0.90
	Te1Ca29	0.39	0.43	0.15	0.73	0.021	—	0.72	0.70	0.59
	Nsµ2	0.63	0.85	0.036	0.90	0.40	0.99	—	0.84	0.56
	Ts010	0.48	0.72	0.97	0.17	0.85	0.92	0.76	—	0.55
	Te1Ca3	0.99	0.22	0.62	0.67	0.27	0.60	0.93	0.77	—

Table A5. Estimates of pairwise F_{ST} (above diagonal) and R_{ST} (below diagonal) between each of the 12 designated *Thamnophis butleri* locations (Table 2.2) in Ontario and Michigan. Significant p values (accounting for multiple testing) are bolded: † indicates significant p values for a Benjamini-Yekutieli (B-Y) adjustment ($\alpha = 0.010$); ‡ indicates significance for both a B-Y adjustment and a Bonferroni correction ($\alpha = 0.00076$).

	Wayne, MI	Macomb, MI	St. Clair, MI	Crystal Bay Is.	Fighting Is.	Belle Isle	Essex South	Essex North	Lambton South	Lambton Aamj.	Lambton North	Luther Marsh
Wayne, MI	—	0.021	0.14 †	0.10 †	0.080 ‡	0.072	0.088 ‡	0.10 ‡	0.14 ‡	0.14 ‡	—	0.021
Macomb, MI	-0.067	—	0.062	0.079	0.053	0.10	0.047	0.057	0.086	0.10	-0.067	—
St. Clair, MI	0.053	-0.11	—	0.18	0.084 †	0.14	0.14 †	0.10 ‡	0.085	0.11 †	0.053	-0.11
Crystal Bay Is.	0.68 ‡	0.70	0.83 †	—	0.11 ‡	0.13	0.15 ‡	0.11 ‡	0.12 ‡	0.17 ‡	0.68 ‡	0.70
Fighting Is.	0.16	0.030	0.10	0.33 †	—	0.078	0.11 ‡	0.069 ‡	0.080 ‡	0.11 ‡	0.16	0.030
Belle Isle	0.089	-0.091	0.013	0.38	-0.10	—	0.12	0.090 †	0.13 †	0.15 ‡	0.089	-0.091
Essex South	0.025	-0.11	-0.0091	0.48 †	0.0090	-0.062	—	0.10 ‡	0.11 ‡	0.11 ‡	0.025	-0.11
Essex North	0.26 ‡	0.15	0.24	0.23 †	0.017	-0.029	0.090	—	0.10 ‡	0.15 ‡	0.26 ‡	0.15
Lambton South	0.010	-0.10	-0.083	0.53 †	0.066	-0.019	-0.019	0.19 ‡	—	0.064 ‡	0.010	-0.10
Lambton Aamj.	0.21 ‡	0.19	0.071	0.82 ‡	0.43 ‡	0.44 †	0.29 ‡	0.46 ‡	0.13 †	—	0.22 ‡	0.19
Lambton North	—	0.021	0.14 †	0.10 †	0.080 ‡	0.072	0.088 ‡	0.10 ‡	0.14 ‡	0.14 ‡	—	0.021
Luther Marsh	-0.067	—	0.062	0.079	0.053	0.10	0.047	0.057	0.086	0.10	-0.067	—

Table A6. Estimates of pairwise F_{ST} (above diagonal) and R_{ST} (below diagonal) between each of the 9 designated locations (Table 2.2) where *Thamnophis s. sirtalis* were found in Ontario and Michigan. Significant p values (accounting for multiple testing) are bolded: † indicates significant p values for a Benjamini-Yekutieli (B-Y) adjustment ($\alpha = 0.012$); ‡ indicates significance for both a B-Y adjustment and a Bonferroni correction ($\alpha = 0.0014$).

	Wayne, MI	Macomb, MI	St. Clair, MI	Essex South	Essex North	Lambton South	Lambton Aamj.	Lambton North	Luther Marsh
Wayne, MI	—	0.0028	0.014	0.028	-0.0012	0.0028	0.012	0.0082	0.034 ‡
Macomb, MI	0.29	—	0.013	0.077 †	0.035	0.023	0.027	0.033	0.093 ‡
St. Clair, MI	0.031	0.29	—	0.031	0.020	0.016	0.011	0.0042	0.058 ‡
Essex South	0.042	0.38	0.061	—	0.017	0.022 ‡	0.019 †	0.035	0.043 †
Essex North	0.025	0.42	0.042	-0.0036	—	0.010	0.0091	0.016	0.034 ‡
Lambton South	-0.014	0.56	0.032	0.022	0.026	—	0.0063	0.011	0.042 ‡
Lambton Aamj.	0.0005	0.54	0.0007	0.0082	0.0051	0.0027	—	0.014	0.045 ‡
Lambton North	-0.024	0.14	-0.083	0.033	0.018	-0.022	-0.048	—	0.053
Luther Marsh	0.063	0.29	0.050	0.0072	0.026	0.059	0.030	0.019	—

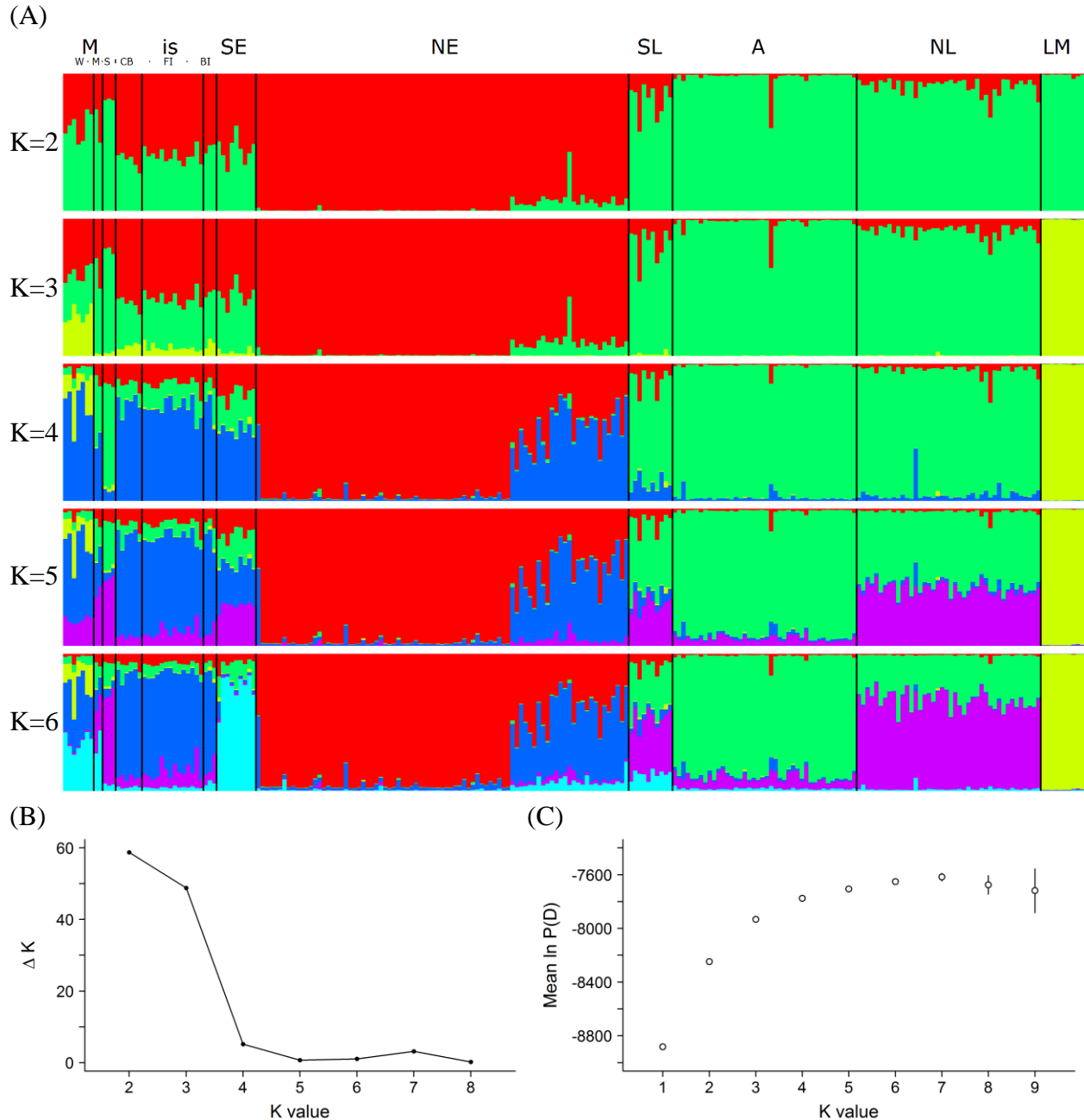


Figure A1. (A) Barplots of *T. butleri* population membership proportions assigned by STRUCTURE using the LOCPRIOR model for $K = 2$ to $K = 6$. The barplots are organized southwest to northeast (with Michigan and the islands grouped on the left), and divided by the designated sampling locations (M_W = Wayne, Michigan; M_M = Macomb County, Michigan; M_S = St. Clair County, Michigan; is_{CB} = Crystal Bay; is_{FI} = Fighting Island; is_{BI} = Belle Isle; SE = South Essex County; NE = North Essex County; SL = South Lambton County; A = Aamjiwnaang; NL = North Lambton County; LM = Luther Marsh WMA). Each vertical bar represents a single snake. For STRUCTURE runs of $K = 2$ to $K = 9$, (B) Evanno's ΔK indicates $K = 2$, with a smaller peak at $K = 3$, and (C) the mean \ln probability of the data plateaus at $K = 3$ or $K = 4$. Overall, this suggests that $K = 3$ may be the most representative number of clusters.

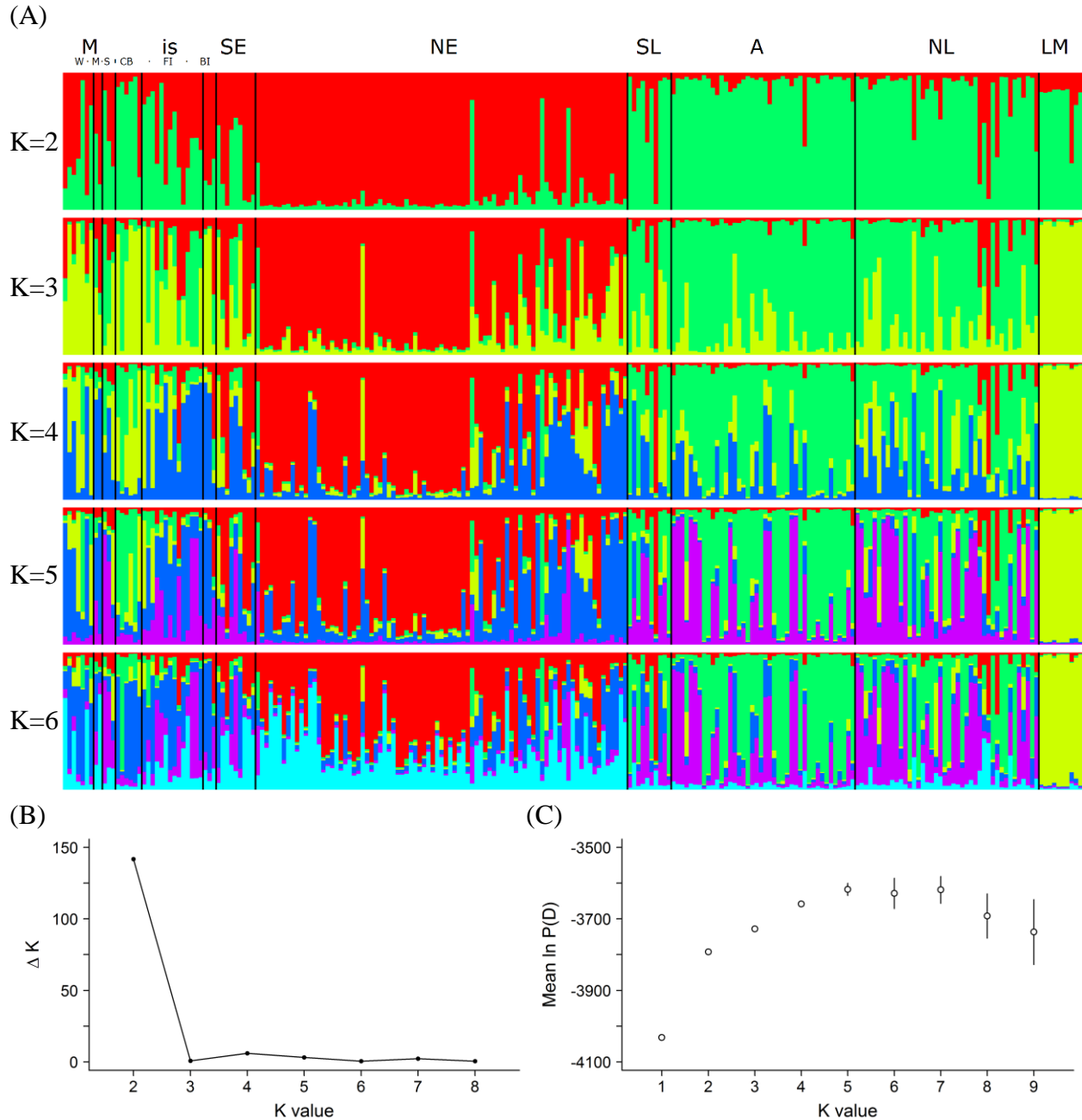


Figure A2. (A) Barplots of *T. butleri* population membership proportions assigned by STRUCTURE using 6 microsatellite loci for $K = 2$ to $K = 6$. The barplots are organized southwest to northeast (with Michigan and the islands grouped on the left), and divided by the designated sampling locations (M_w = Wayne, Michigan; M_m = Macomb County, Michigan; M_s = St. Clair County, Michigan; is_{CB} = Crystal Bay; is_{FI} = Fighting Island; is_{BI} = Belle Isle; SE = South Essex County; NE = North Essex County; SL = South Lambton County; A = Aamjiwnaang; NL = North Lambton County; LM = Luther Marsh WMA). Each vertical bar represents a single snake. For STRUCTURE runs of $K = 2$ to $K = 9$, (B) Evanno's ΔK indicates $K = 2$, and (C) the mean ln probability of the data plateaus between $K = 2$ and $K = 4$. Overall, this suggests that $K = 2$ may be the most representative number of clusters.

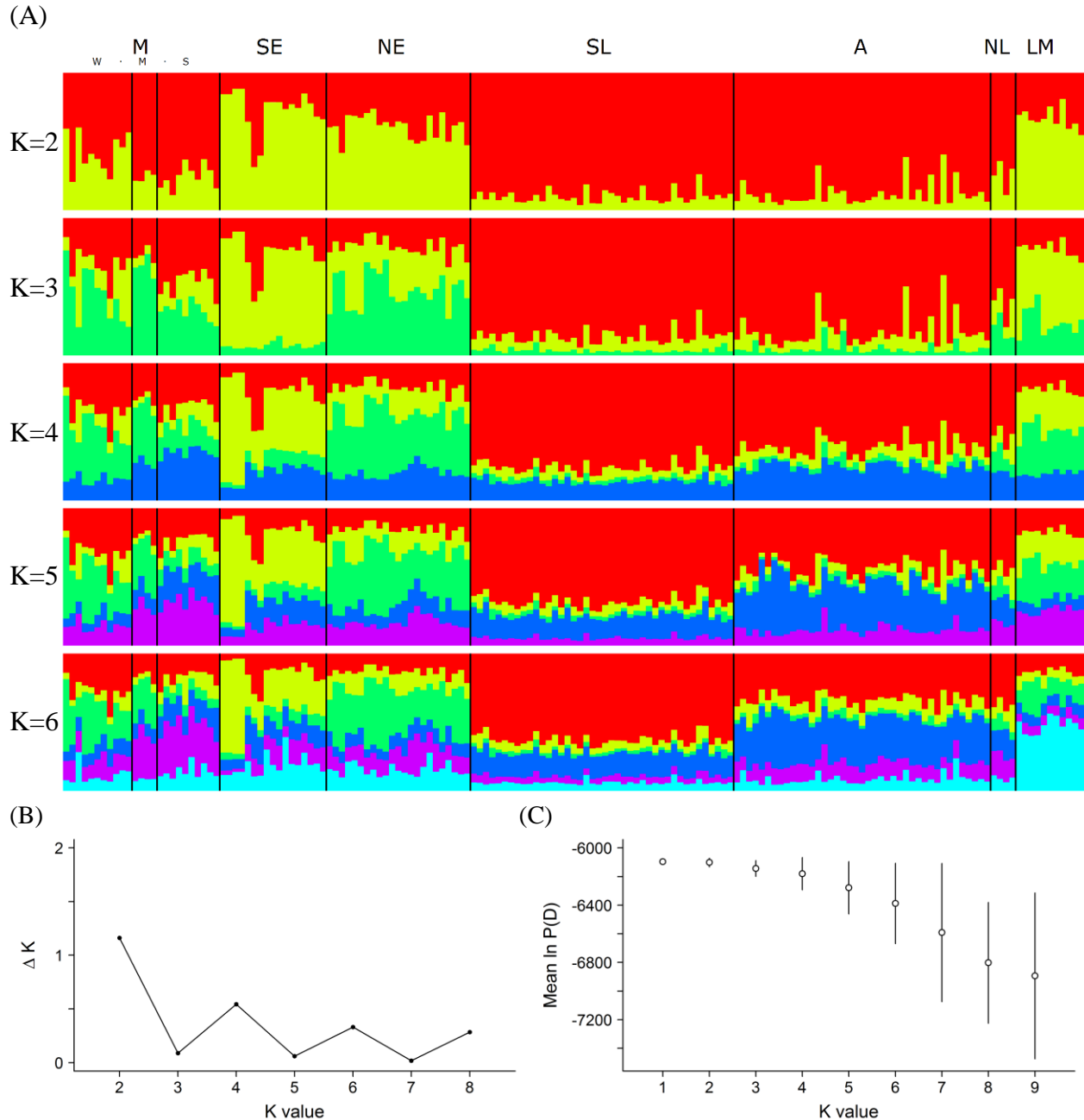


Figure A3. (A) Barplots of *T. s. sirtalis* population membership proportions assigned by STRUCTURE using the LOCPRIOR model for $K = 2$ to $K = 6$. The barplots are organized southwest to northeast (with Michigan areas grouped on the left), and divided by the designated sampling locations (M_W = Wayne, Michigan; M_M = Macomb County, Michigan; M_S = St. Clair County, Michigan; is_{CB} = Crystal Bay; is_{FI} = Fighting Island; is_{BI} = Belle Isle; SE = South Essex County; NE = North Essex County; SL = South Lambton County; A = Aamjiwnaang; NL = North Lambton County; LM = Luther Marsh WMA). Each vertical bar represents a single snake. For STRUCTURE runs of $K = 2$ to $K = 9$, (B) Evanno's ΔK does not strongly indicate any K -value, and (C) the mean \ln probability of the data plateaus at $K = 1$, with high variability between runs at larger values of K . Overall, this suggests that $K = 1$ may be the most representative number of clusters.

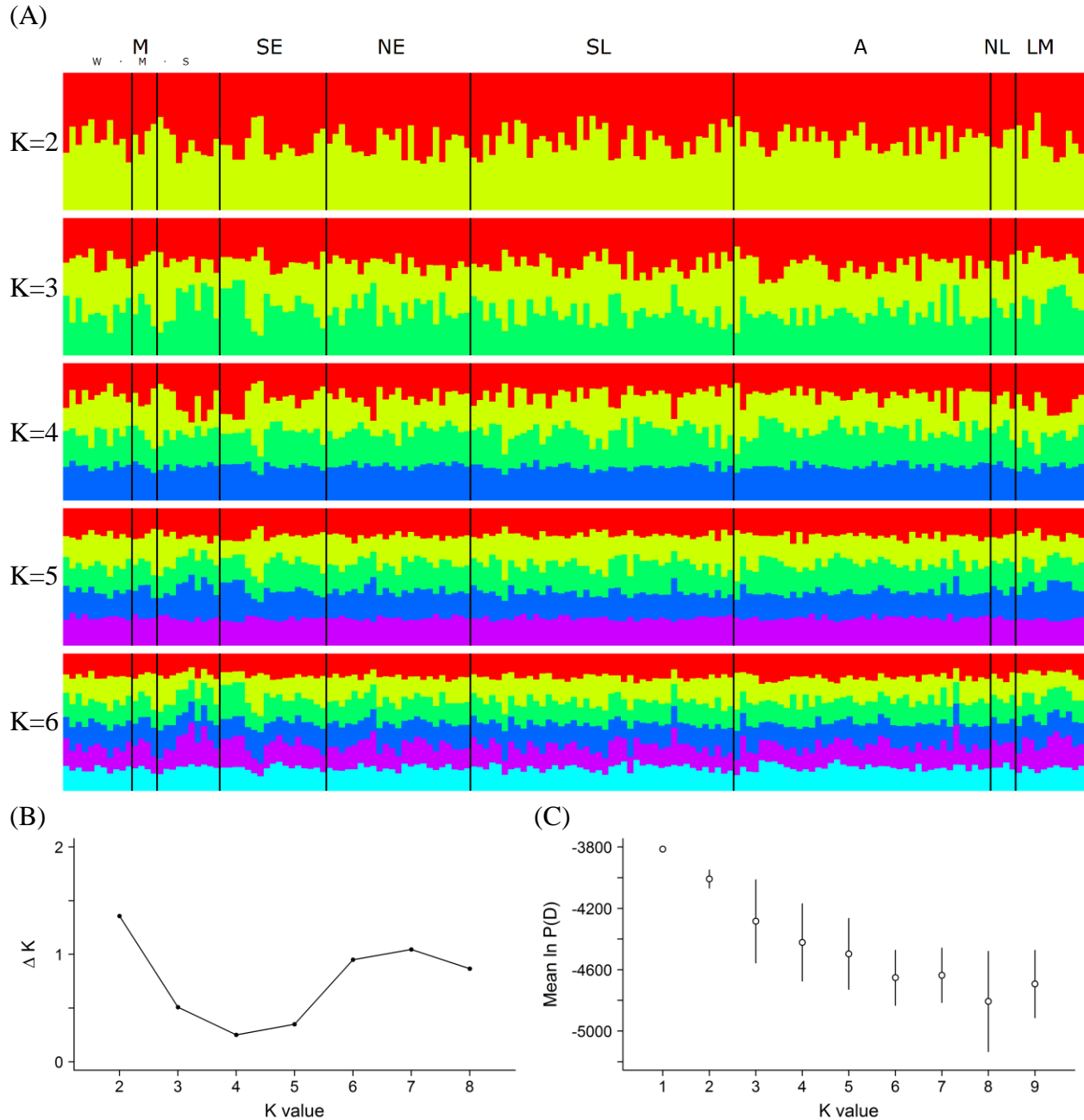


Figure A4. (A) Barplots of *T. s. sirtalis* population membership proportions assigned by STRUCTURE using 6 microsatellite loci for $K = 2$ to $K = 6$. The barplots are organized southwest to northeast (with Michigan areas grouped on the left), and divided by the designated sampling locations (M_W = Wayne, Michigan; M_M = Macomb County, Michigan; M_S = St. Clair County, Michigan; i_{SCB} = Crystal Bay; i_{SFI} = Fighting Island; i_{SBI} = Belle Isle; SE = South Essex County; NE = North Essex County; SL = South Lambton County; A = Aamjiwnaang; NL = North Lambton County; LM = Luther Marsh WMA). Each vertical bar represents a single snake. For STRUCTURE runs of $K = 2$ to $K = 9$, (B) Evanno's ΔK does not strongly indicate any K -value, and (C) the mean \ln probability of the data plateaus at $K = 1$, with high variability between runs at larger values of K . Overall, this suggests that $K = 1$ may be the most representative number of clusters.

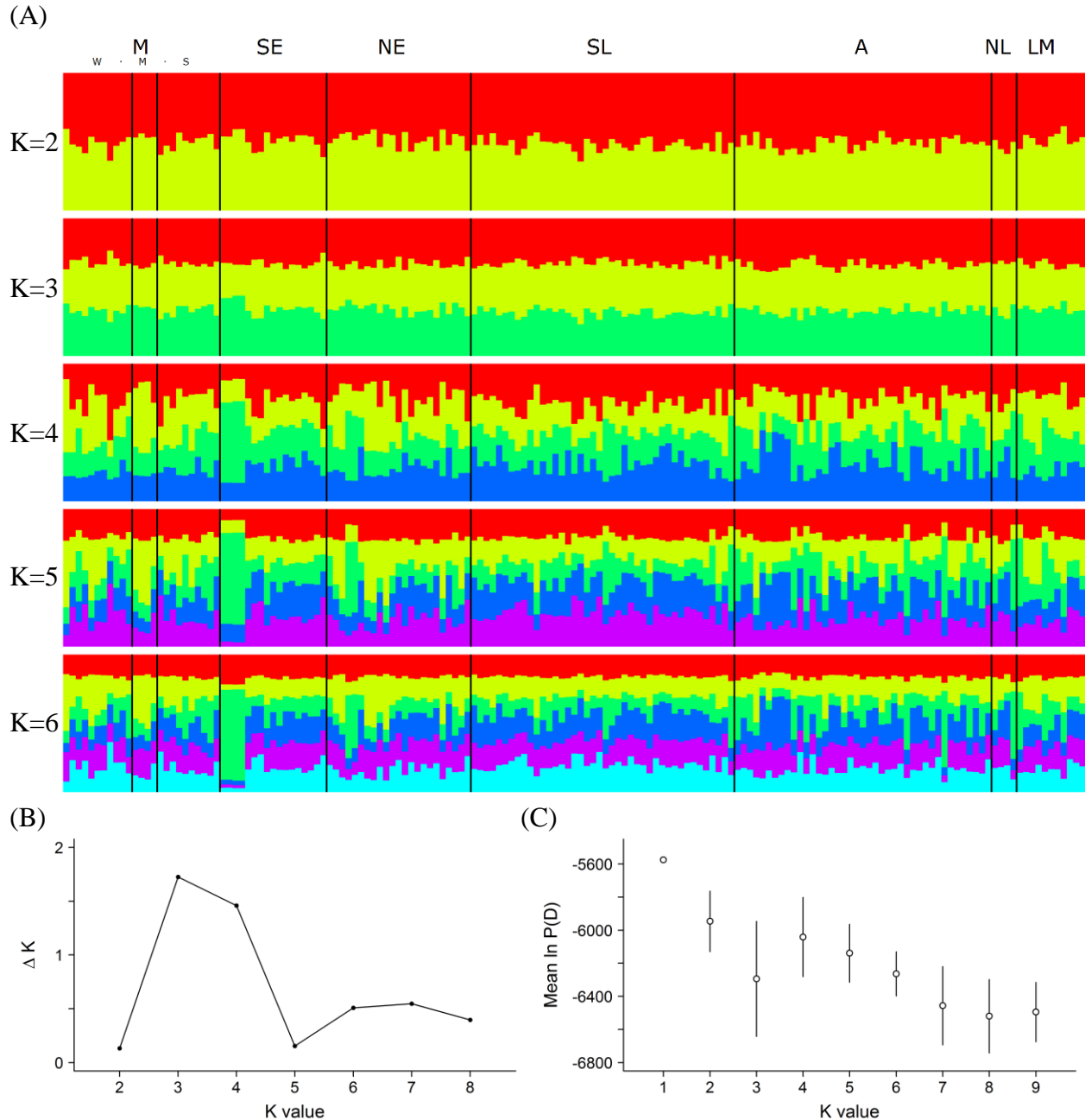


Figure A5. (A) Barplots of *T. s. sirtalis* population membership proportions assigned by STRUCTURE using 8 microsatellite loci for $K = 2$ to $K = 6$. The barplots are organized southwest to northeast (with Michigan areas grouped on the left), and divided by the designated sampling locations (M_W = Wayne, Michigan; M_M = Macomb County, Michigan; M_S = St. Clair County, Michigan; is_{CB} = Crystal Bay; is_{FI} = Fighting Island; is_{BI} = Belle Isle; SE = South Essex County; NE = North Essex County; SL = South Lambton County; A = Aamjiwnaang; NL = North Lambton County; LM = Luther Marsh WMA). Each vertical bar represents a single snake. For STRUCTURE runs of $K = 2$ to $K = 9$, (B) Evanno's ΔK does not strongly indicate any K -value, and (C) the mean \ln probability of the data plateaus at $K = 1$, with high variability between runs at larger values of K . Overall, this suggests that $K = 1$ may be the most representative number of clusters.

Appendix B: Supplemental Material for Chapter 3

Table B1. Mantel tests for correlations between matrices of genetic distance and cost distances of each IBD and IBR model (Gen~Res), partial Mantel tests controlling for the effect of geographical distance from the IBD model (Gen~Res+Dist), and partial Mantel tests controlling for the effect of resistance from each IBR model (Gen~Dist+Res) for the Lambton County region including open water as a non-resistant land class. Significant p-values are bolded.

Model	Gen~Res				Gen~Res+Dist				Gen~Dist+Res			
	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI
UD10	0.21	0.0010	0.17	0.25								
LS10_5A	0.22	0.0010	0.19	0.27	0.087	0.027	0.051	0.12	-0.042	0.79	-0.069	-0.0080
LS10_5B	0.23	0.0010	0.19	0.27	0.097	0.017	0.063	0.12	-0.036	0.78	-0.063	0.0010
LS10_5C	0.23	0.0010	0.20	0.27	0.097	0.035	0.058	0.13	-0.0010	0.50	-0.027	0.033
LS10_5D	0.22	0.0010	0.19	0.26	0.090	0.054	0.059	0.11	0.027	0.29	-0.0010	0.061
LS10_20A	0.18	0.0010	0.14	0.22	-0.080	0.91	-0.12	-0.042	0.13	0.012	0.095	0.17
LS10_20B	0.17	0.0010	0.14	0.21	-0.068	0.87	-0.10	-0.028	0.14	0.0040	0.11	0.18
LS10_20C	0.13	0.0050	0.094	0.16	-0.051	0.80	-0.085	-0.022	0.17	0.0010	0.14	0.21
LS10_20D	0.11	0.016	0.07	0.14	-0.050	0.78	-0.087	-0.015	0.19	0.0010	0.15	0.23
UD5	0.21	0.0010	0.18	0.25								
LS5_5A	0.22	0.001	0.18	0.26	0.075	0.10	0.037	0.11	-0.026	0.67	-0.061	0.010
LS5_5B	0.22	0.0010	0.19	0.26	0.089	0.068	0.053	0.12	-0.021	0.63	-0.047	0.012
LS5_5C	0.23	0.0010	0.20	0.27	0.10	0.043	0.073	0.13	0.027	0.30	-0.004	0.061
LS5_5D	0.22	0.0010	0.20	0.25	0.10	0.059	0.073	0.12	0.064	0.10	0.029	0.10
LS5_20A	0.19	0.0010	0.16	0.23	-0.030	0.73	-0.067	0.0060	0.092	0.063	0.059	0.13
LS5_20B	0.18	0.0010	0.15	0.22	-0.019	0.63	-0.054	0.016	0.11	0.036	0.065	0.14
LS5_20C	0.15	0.0010	0.12	0.19	0.0010	0.48	-0.029	0.036	0.14	0.0050	0.11	0.18
LS5_20D	0.14	0.0070	0.11	0.17	0.0050	0.48	-0.029	0.041	0.16	0.0030	0.12	0.20
UD3	0.21	0.0010	0.17	0.25								
LS3_5A	0.21	0.0010	0.17	0.25	0.027	0.32	-0.012	0.063	0.042	0.27	0.01	0.078
LS3_5B	0.20	0.0010	0.17	0.23	0.028	0.30	-0.0080	0.053	0.070	0.12	0.039	0.11
LS3_5C	0.16	0.0040	0.13	0.20	0.029	0.32	-0.0080	0.066	0.13	0.0060	0.095	0.18
LS3_5D	0.14	0.0040	0.11	0.18	0.029	0.30	-0.0020	0.059	0.16	0.0040	0.12	0.20
LS3_20A	0.19	0.0010	0.16	0.23	-0.0040	0.51	-0.041	0.028	0.07	0.12	0.04	0.11
LS3_20B	0.19	0.0010	0.16	0.22	0.0020	0.51	-0.035	0.035	0.10	0.058	0.059	0.13
LS3_20C	0.15	0.0010	0.12	0.18	0.013	0.43	-0.020	0.041	0.14	0.0040	0.11	0.18
LS3_20D	0.14	0.0040	0.11	0.17	0.014	0.40	-0.021	0.044	0.16	0.0020	0.13	0.20

Table B2. Mantel tests for correlations between matrices of genetic distance and cost distances of each IBD and IBR model (Gen~Res), partial Mantel tests controlling for the effect of geographical distance from the IBD model (Gen~Res+Dist), and partial Mantel tests controlling for the effect of resistance from each IBR model (Gen~Dist+Res) for the Essex County region including open water as a non-resistant land class. Significant p-values are bolded.

Model	Gen~Res				Gen~Res+Dist				Gen~Dist+Res			
	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI
UD	0.43	0.0010	0.40	0.45								
LS10_5A	0.36	0.0010	0.33	0.39	0.025	0.30	-0.015	0.060	0.25	0.0010	0.21	0.28
LS10_5B	0.30	0.0010	0.27	0.34	0.029	0.30	-0.0060	0.064	0.32	0.0010	0.28	0.34
LS10_5C	0.20	0.0010	0.17	0.24	0.036	0.25	0.0000	0.067	0.39	0.0010	0.36	0.41
LS10_5D	0.17	0.0020	0.14	0.20	0.037	0.25	0.0030	0.072	0.40	0.0010	0.37	0.42
LS10_20A	0.35	0.0010	0.32	0.38	0.0040	0.52	-0.044	0.039	0.27	0.0010	0.23	0.31
LS10_20B	0.29	0.0010	0.26	0.32	0.0030	0.49	-0.038	0.043	0.33	0.0010	0.28	0.36
LS10_20C	0.19	0.0010	0.15	0.23	0.012	0.41	-0.024	0.051	0.39	0.0010	0.36	0.42
LS10_20D	0.17	0.0010	0.13	0.20	0.014	0.43	-0.019	0.048	0.40	0.0010	0.37	0.43
UD5	0.43	0.0010	0.40	0.46								
LS5_5A	0.36	0.0010	0.32	0.38	0.017	0.36	-0.026	0.054	0.26	0.0010	0.22	0.29
LS5_5B	0.30	0.0010	0.28	0.34	0.018	0.35	-0.022	0.058	0.32	0.0010	0.28	0.35
LS5_5C	0.21	0.0020	0.17	0.24	0.019	0.36	-0.018	0.053	0.39	0.0010	0.36	0.41
LS5_5D	0.18	0.0010	0.14	0.21	0.018	0.34	-0.018	0.055	0.40	0.0010	0.37	0.43
LS5_20A	0.36	0.0010	0.33	0.40	0.021	0.34	-0.015	0.068	0.24	0.0010	0.19	0.28
LS5_20B	0.32	0.0010	0.29	0.35	0.024	0.32	-0.014	0.071	0.30	0.0010	0.26	0.34
LS5_20C	0.24	0.0010	0.20	0.28	0.032	0.27	-0.010	0.070	0.37	0.0010	0.33	0.40
LS5_20D	0.22	0.0010	0.19	0.26	0.034	0.26	-0.0040	0.075	0.38	0.0010	0.34	0.40
UD3	0.43	0.0010	0.40	0.45								
LS3_5A	0.35	0.0010	0.32	0.39	0.027	0.32	-0.011	0.070	0.26	0.0010	0.22	0.29
LS3_5B	0.30	0.0010	0.27	0.34	0.031	0.27	-0.0050	0.071	0.32	0.0010	0.28	0.35
LS3_5C	0.22	0.0010	0.18	0.26	0.041	0.22	0.0020	0.077	0.38	0.0010	0.35	0.40
LS3_5D	0.19	0.0010	0.16	0.23	0.043	0.23	0.0080	0.078	0.39	0.0010	0.36	0.42
LS3_20A	0.35	0.0010	0.32	0.38	0.018	0.35	-0.016	0.060	0.26	0.0010	0.22	0.29
LS3_20B	0.30	0.0010	0.27	0.34	0.023	0.34	-0.016	0.059	0.32	0.0010	0.28	0.35
LS3_20C	0.21	0.0010	0.18	0.25	0.032	0.28	-0.0020	0.073	0.38	0.0010	0.35	0.41
LS3_20D	0.19	0.0010	0.16	0.22	0.034	0.25	-0.0030	0.070	0.39	0.0010	0.36	0.42

Table B3. Mantel tests for correlations between matrices of genetic distance and cost distances of each IBD and IBR model (Gen~Res), partial Mantel tests controlling for the effect of geographical distance from the IBD model (Gen~Res+Dist), and partial Mantel tests controlling for the effect of resistance from each IBR model (Gen~Dist+Res) for the Luther Marsh region including open water as a non-resistant land class. Significant p-values are bolded.

Model	Gen~Res				Gen~Res+Dist				Gen~Dist+Res			
	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI
UD10	0.40	0.028	0.21	0.69								
LS10_5A	0.40	0.023	0.24	0.71	0.044	0.40	-0.076	0.15	0.015	0.50	-0.14	0.19
LS10_5B	0.40	0.027	0.21	0.69	0.044	0.38	-0.11	0.16	0.031	0.49	-0.087	0.19
LS10_5C	0.40	0.023	0.21	0.69	0.044	0.39	-0.10	0.15	0.052	0.40	-0.11	0.24
LS10_5D	0.40	0.022	0.21	0.67	0.044	0.38	-0.082	0.15	0.055	0.41	-0.095	0.23
LS10_20A	0.40	0.034	0.22	0.71	0.057	0.37	-0.097	0.16	0.038	0.44	-0.095	0.23
LS10_20B	0.40	0.026	0.24	0.68	0.058	0.37	-0.078	0.16	0.064	0.37	-0.12	0.25
LS10_20C	0.39	0.036	0.22	0.66	0.059	0.37	-0.11	0.20	0.096	0.29	-0.027	0.33
LS10_20D	0.39	0.047	0.21	0.66	0.059	0.37	-0.096	0.17	0.10	0.28	-0.086	0.33
UD5	0.39	0.020	0.15	0.71								
LS5_5A	0.39	0.030	0.18	0.71	0.075	0.36	-0.13	0.20	0.0010	0.55	-0.15	0.21
LS5_5B	0.39	0.041	0.19	0.70	0.075	0.32	-0.084	0.21	0.026	0.46	-0.15	0.26
LS5_5C	0.39	0.030	0.19	0.68	0.075	0.32	-0.077	0.19	0.061	0.38	-0.12	0.38
LS5_5D	0.39	0.031	0.18	0.69	0.075	0.34	-0.050	0.21	0.068	0.35	-0.10	0.36
LS5_20A	0.39	0.031	0.18	0.73	0.080	0.34	-0.069	0.21	0.028	0.47	-0.15	0.27
LS5_20B	0.39	0.030	0.19	0.71	0.080	0.33	-0.082	0.19	0.057	0.37	-0.12	0.39
LS5_20C	0.39	0.037	0.19	0.68	0.083	0.34	-0.070	0.21	0.090	0.29	-0.064	0.42
LS5_20D	0.39	0.042	0.17	0.66	0.084	0.33	-0.12	0.21	0.096	0.28	-0.081	0.40
UD3	0.45	0.0080	0.24	0.72								
LS3_5A	0.44	0.0080	0.26	0.71	0.032	0.39	-0.078	0.14	0.078	0.37	-0.058	0.24
LS3_5B	0.44	0.013	0.26	0.70	0.032	0.41	-0.073	0.15	0.11	0.29	-0.027	0.30
LS3_5C	0.43	0.025	0.24	0.67	0.034	0.41	-0.088	0.15	0.15	0.18	-0.0060	0.34
LS3_5D	0.43	0.029	0.24	0.69	0.035	0.38	-0.076	0.14	0.16	0.22	-0.015	0.32
LS3_20A	0.44	0.014	0.26	0.71	0.030	0.40	-0.085	0.14	0.089	0.30	-0.048	0.25
LS3_20B	0.43	0.016	0.24	0.70	0.031	0.40	-0.059	0.16	0.12	0.23	-0.011	0.30
LS3_20C	0.42	0.015	0.24	0.69	0.035	0.38	-0.062	0.16	0.16	0.16	-0.0060	0.37
LS3_20D	0.42	0.026	0.24	0.68	0.036	0.40	-0.066	0.15	0.17	0.16	-0.015	0.40

Table B4. Mantel tests for correlations between matrices of genetic distance and log-transformed cost distances of each IBD and IBR model (Gen~Res), partial Mantel tests controlling for the effect of geographical distance from the IBD model (Gen~Res+Dist), and partial Mantel tests controlling for the effect of resistance from each IBR model (Gen~Dist+Res) for the Lambton County region. Significant p-values are bolded.

Model	Gen~Res				Gen~Res+Dist				Gen~Dist+Res			
	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI
UD10	0.16	0.0010	0.13	0.18								
LS10_5A	0.18	0.0010	0.15	0.20	0.21	0.0010	0.18	0.24	-0.20	1.0	-0.22	-0.17
LS10_5B	0.18	0.0010	0.16	0.21	0.21	0.0010	0.18	0.24	-0.19	1.0	-0.21	-0.16
LS10_5C	0.20	0.0010	0.17	0.23	0.20	0.0010	0.18	0.23	-0.16	1.0	-0.19	-0.14
LS10_5D	0.21	0.0010	0.18	0.24	0.20	0.0010	0.18	0.23	-0.15	1.0	-0.18	-0.12
LS10_20A	0.16	0.0010	0.14	0.19	0.076	0.031	0.051	0.099	-0.064	0.95	-0.086	-0.041
LS10_20B	0.17	0.0010	0.14	0.19	0.071	0.040	0.046	0.092	-0.054	0.92	-0.078	-0.031
LS10_20C	0.17	0.0010	0.14	0.19	0.061	0.055	0.035	0.083	-0.033	0.84	-0.055	-0.0080
LS10_20D	0.17	0.0010	0.14	0.19	0.058	0.057	0.035	0.080	-0.026	0.75	-0.051	0.0010
UD5	0.14	0.0010	0.12	0.16								
LS5_5A	0.15	0.0010	0.13	0.18	0.17	0.0010	0.15	0.19	-0.16	1.0	-0.18	-0.13
LS5_5B	0.16	0.0010	0.14	0.18	0.17	0.0010	0.15	0.20	-0.15	1.0	-0.18	-0.13
LS5_5C	0.18	0.0010	0.16	0.20	0.19	0.0010	0.16	0.21	-0.15	1.0	-0.18	-0.13
LS5_5D	0.19	0.0010	0.16	0.21	0.19	0.0010	0.17	0.22	-0.15	1.0	-0.17	-0.13
LS5_20A	0.15	0.0010	0.12	0.17	0.084	0.032	0.057	0.11	-0.071	0.96	-0.10	-0.041
LS5_20B	0.15	0.0010	0.13	0.17	0.084	0.020	0.059	0.11	-0.064	0.94	-0.090	-0.038
LS5_20C	0.15	0.001	0.13	0.18	0.079	0.029	0.052	0.10	-0.046	0.86	-0.073	-0.021
LS5_20D	0.15	0.0010	0.13	0.17	0.076	0.027	0.051	0.11	-0.038	0.84	-0.064	-0.012
UD3	0.13	0.0010	0.11	0.15								
LS3_5A	0.14	0.0010	0.11	0.15	0.12	0.0050	0.087	0.15	-0.11	1.0	-0.14	-0.082
LS3_5B	0.14	0.0010	0.12	0.16	0.12	0.0020	0.093	0.15	-0.10	0.99	-0.13	-0.073
LS3_5C	0.15	0.0010	0.13	0.17	0.12	0.0020	0.096	0.15	-0.091	0.99	-0.12	-0.065
LS3_5D	0.16	0.0010	0.13	0.18	0.12	0.0010	0.098	0.15	-0.086	0.99	-0.11	-0.061
LS3_20A	0.14	0.0010	0.11	0.16	0.11	0.011	0.083	0.13	-0.095	0.98	-0.12	-0.069
LS3_20B	0.14	0.0010	0.12	0.16	0.11	0.0090	0.080	0.13	-0.089	0.98	-0.12	-0.064
LS3_20C	0.15	0.0010	0.13	0.17	0.10	0.0080	0.077	0.13	-0.069	0.94	-0.096	-0.046
LS3_20D	0.15	0.0010	0.13	0.17	0.10	0.0050	0.070	0.12	-0.060	0.91	-0.088	-0.033

Table B5. Mantel tests for correlations between matrices of genetic distance and log-transformed cost distances of each IBD and IBR model (Gen~Res), partial Mantel tests controlling for the effect of geographical distance from the IBD model (Gen~Res+Dist), and partial Mantel tests controlling for the effect of resistance from each IBR model (Gen~Dist+Res) for the Essex County region. Significant p-values are bolded.

Model	Gen~Res				Gen~Res+Dist				Gen~Dist+Res			
	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI
UD10	0.25	0.0010	0.23	0.27								
LS10_5A	0.28	0.0010	0.26	0.30	0.22	0.0010	0.18	0.25	-0.18	1.0	-0.21	-0.14
LS10_5B	0.29	0.0010	0.27	0.31	0.21	0.0010	0.18	0.25	-0.16	1.0	-0.19	-0.12
LS10_5C	0.31	0.0010	0.28	0.33	0.21	0.0010	0.18	0.25	-0.11	0.99	-0.14	-0.071
LS10_5D	0.31	0.0010	0.29	0.33	0.21	0.0010	0.18	0.25	-0.089	0.98	-0.12	-0.056
LS10_20A	0.26	0.0010	0.24	0.27	0.060	0.10	0.027	0.10	-0.039	0.80	-0.075	-0.0020
LS10_20B	0.26	0.0010	0.24	0.27	0.049	0.13	0.015	0.079	-0.020	0.67	-0.056	0.015
LS10_20C	0.25	0.0010	0.24	0.27	0.032	0.22	0.0010	0.064	0.014	0.37	-0.015	0.045
LS10_20D	0.25	0.0010	0.23	0.27	0.028	0.23	-0.0050	0.062	0.024	0.27	-0.0040	0.055
UD5	0.23	0.0010	0.21	0.24								
LS5_5A	0.26	0.0010	0.24	0.27	0.23	0.0010	0.20	0.27	-0.20	1.0	-0.24	-0.17
LS5_5B	0.27	0.0010	0.25	0.29	0.23	0.0010	0.19	0.27	-0.18	1.0	-0.22	-0.15
LS5_5C	0.29	0.0010	0.27	0.32	0.24	0.0010	0.20	0.28	-0.15	1.0	-0.18	-0.12
LS5_5D	0.30	0.0010	0.28	0.32	0.25	0.0010	0.21	0.28	-0.14	1.0	-0.17	-0.11
LS5_20A	0.25	0.0010	0.23	0.27	0.22	0.0010	0.19	0.26	-0.19	1.0	-0.22	-0.16
LS5_20B	0.26	0.0010	0.24	0.28	0.22	0.0010	0.18	0.26	-0.17	1.0	-0.21	-0.14
LS5_20C	0.28	0.0010	0.25	0.30	0.21	0.0010	0.17	0.24	-0.14	1.0	-0.17	-0.10
LS5_20D	0.28	0.0010	0.26	0.30	0.21	0.0010	0.17	0.24	-0.12	1.0	-0.15	-0.092
UD3	0.20	0.0010	0.18	0.21								
LS3_5A	0.23	0.0010	0.22	0.25	0.26	0.0010	0.22	0.29	-0.23	1.0	-0.26	-0.20
LS3_5B	0.25	0.0010	0.23	0.27	0.26	0.0010	0.22	0.29	-0.21	1.0	-0.24	-0.18
LS3_5C	0.28	0.0010	0.26	0.30	0.27	0.0010	0.23	0.30	-0.18	1.0	-0.21	-0.14
LS3_5D	0.29	0.0010	0.27	0.31	0.27	0.0010	0.23	0.31	-0.17	1.0	-0.20	-0.14
LS3_20A	0.23	0.0010	0.21	0.25	0.25	0.0010	0.21	0.28	-0.22	1.0	-0.26	-0.19
LS3_20B	0.24	0.0010	0.22	0.26	0.25	0.0010	0.21	0.28	-0.20	1.0	-0.24	-0.17
LS3_20C	0.26	0.0010	0.24	0.28	0.24	0.0010	0.21	0.28	-0.16	1.0	-0.19	-0.13
LS3_20D	0.27	0.0010	0.25	0.29	0.24	0.0010	0.20	0.27	-0.15	1.0	-0.18	-0.12

Table B6. Mantel tests for correlations between matrices of genetic distance and log-transformed cost distances of each IBD and IBR model (Gen~Res), partial Mantel tests controlling for the effect of geographical distance from the IBD model (Gen~Res+Dist), and partial Mantel tests controlling for the effect of resistance from each IBR model (Gen~Dist+Res) for the Luther Marsh region. Significant p-values are bolded.

Model	Gen~Res				Gen~Res+Dist				Gen~Dist+Res			
	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI	Mantel r	P	lower CI	upper CI
UD10	0.32	0.039	0.14	0.59								
LS10_5A	0.33	0.038	0.15	0.60	0.32	0.057	0.19	0.51	-0.31	0.94	-0.50	-0.20
LS10_5B	0.33	0.040	0.14	0.61	0.32	0.054	0.18	0.51	-0.32	0.93	-0.50	-0.18
LS10_5C	0.33	0.038	0.15	0.64	0.32	0.062	0.19	0.52	-0.32	0.93	-0.53	-0.19
LS10_5D	0.33	0.040	0.16	0.60	0.32	0.067	0.18	0.52	-0.31	0.94	-0.50	-0.18
LS10_20A	0.33	0.027	0.14	0.60	0.34	0.046	0.21	0.54	-0.34	0.94	-0.54	-0.19
LS10_20B	0.33	0.035	0.15	0.60	0.34	0.059	0.20	0.56	-0.33	0.95	-0.53	-0.20
LS10_20C	0.34	0.033	0.14	0.63	0.34	0.054	0.19	0.54	-0.33	0.93	-0.52	-0.19
LS10_20D	0.34	0.031	0.15	0.61	0.34	0.059	0.20	0.57	-0.32	0.95	-0.51	-0.19
UD5	0.23	0.065	-0.025	0.59								
LS5_5A	0.24	0.061	0.0000	0.60	0.39	0.055	0.22	0.59	-0.38	0.96	-0.57	-0.21
LS5_5B	0.25	0.064	-0.049	0.57	0.39	0.059	0.26	0.59	-0.38	0.96	-0.57	-0.21
LS5_5C	0.25	0.055	-0.023	0.60	0.39	0.048	0.26	0.59	-0.38	0.95	-0.56	-0.26
LS5_5D	0.25	0.045	-0.017	0.62	0.39	0.057	0.23	0.59	-0.38	0.95	-0.56	-0.21
LS5_20A	0.24	0.060	-0.011	0.61	0.38	0.038	0.26	0.57	-0.38	0.96	-0.58	-0.21
LS5_20B	0.25	0.051	-0.018	0.61	0.39	0.038	0.26	0.60	-0.38	0.96	-0.54	-0.22
LS5_20C	0.26	0.065	-0.0060	0.61	0.39	0.029	0.22	0.59	-0.38	0.96	-0.56	-0.27
LS5_20D	0.26	0.049	-0.016	0.61	0.39	0.028	0.27	0.59	-0.38	0.96	-0.56	-0.23
UD3	0.38	0.0050	0.15	0.58								
LS3_5A	0.38	0.0050	0.17	0.59	0.33	0.060	0.13	0.61	-0.32	0.93	-0.62	-0.15
LS3_5B	0.39	0.0060	0.17	0.58	0.33	0.077	0.15	0.62	-0.31	0.93	-0.60	-0.16
LS3_5C	0.39	0.0080	0.18	0.59	0.34	0.064	0.12	0.63	-0.31	0.93	-0.60	-0.15
LS3_5D	0.40	0.0050	0.15	0.58	0.34	0.059	0.18	0.64	-0.31	0.93	-0.60	-0.14
LS3_20A	0.39	0.0070	0.14	0.59	0.33	0.058	0.16	0.61	-0.31	0.94	-0.60	-0.13
LS3_20B	0.39	0.0060	0.14	0.56	0.33	0.061	0.18	0.63	-0.31	0.92	-0.61	-0.15
LS3_20C	0.40	0.0030	0.15	0.59	0.34	0.055	0.16	0.63	-0.32	0.93	-0.61	-0.16
LS3_20D	0.40	0.0050	0.14	0.59	0.34	0.045	0.14	0.64	-0.31	0.94	-0.61	-0.14

Table B7. Comparison of R^2 values of the relationship between genetic and cost distances in the Lambton County region for untransformed and log-transformed cost distances.

Model	Gen~Res		Gen~Res+Dist		Gen~Dist+Res	
	R^2 , untransf.	R^2 , log transf.	R^2 , untransf.	R^2 , log transf.	R^2 , untransf.	R^2 , log transf.
UD10	0.043	0.025				
LS10_5A	0.058	0.031	0.061	0.068	0.061	0.068
LS10_5B	0.059	0.034	0.059	0.067	0.059	0.067
LS10_5C	0.053	0.040	0.056	0.065	0.056	0.065
LS10_5D	0.049	0.043	0.055	0.065	0.055	0.065
LS10_20A	0.047	0.027	0.047	0.031	0.047	0.031
LS10_20B	0.047	0.025	0.047	0.030	0.047	0.030
LS10_20C	0.046	0.028	0.047	0.029	0.047	0.029
LS10_20D	0.045	0.028	0.047	0.028	0.047	0.028
UD5	0.043	0.019				
LS5_5A	0.059	0.023	0.066	0.047	0.066	0.047
LS5_5B	0.062	0.025	0.065	0.049	0.065	0.049
LS5_5C	0.064	0.032	0.064	0.053	0.064	0.053
LS5_5D	0.063	0.035	0.064	0.056	0.064	0.056
LS5_20A	0.053	0.021	0.056	0.026	0.056	0.026
LS5_20B	0.056	0.022	0.058	0.026	0.058	0.026
LS5_20C	0.059	0.023	0.059	0.025	0.059	0.025
LS5_20D	0.059	0.024	0.059	0.025	0.059	0.025
UD3	0.043	0.016				
LS3_5A	0.054	0.019	0.056	0.030	0.056	0.030
LS3_5B	0.056	0.020	0.057	0.030	0.057	0.030
LS3_5C	0.059	0.023	0.059	0.031	0.059	0.031
LS3_5D	0.059	0.024	0.060	0.031	0.060	0.031
LS3_20A	0.057	0.019	0.062	0.028	0.062	0.028
LS3_20B	0.061	0.020	0.064	0.027	0.064	0.027
LS3_20C	0.067	0.021	0.067	0.026	0.067	0.026
LS3_20D	0.067	0.022	0.067	0.025	0.067	0.025

Table B8. Comparison of R^2 values of the relationship between genetic and cost distances in the Essex County region for untransformed and log-transformed cost distances.

Model	Gen~Res		Gen~Res+Dist		Gen~Dist+Res	
	R^2 , untransf.	R^2 , log transf.	R^2 , untransf.	R^2 , log transf.	R^2 , untransf.	R^2 , log transf.
UD10	0.18	0.063				
LS10_5A	0.14	0.078	0.18	0.11	0.18	0.11
LS10_5B	0.11	0.084	0.18	0.11	0.18	0.11
LS10_5C	0.057	0.094	0.18	0.11	0.18	0.11
LS10_5D	0.043	0.097	0.18	0.10	0.18	0.10
LS10_20A	0.17	0.065	0.18	0.067	0.18	0.067
LS10_20B	0.15	0.065	0.18	0.064	0.18	0.065
LS10_20C	0.12	0.064	0.18	0.061	0.18	0.064
LS10_20D	0.12	0.063	0.18	0.064	0.18	0.064
UD5	0.18	0.051				
LS5_5A	0.15	0.066	0.19	0.10	0.19	0.10
LS5_5B	0.12	0.072	0.18	0.10	0.18	0.10
LS5_5C	0.080	0.085	0.18	0.11	0.18	0.11
LS5_5D	0.069	0.091	0.18	0.11	0.18	0.11
LS5_20A	0.17	0.063	0.19	0.097	0.19	0.097
LS5_20B	0.15	0.068	0.19	0.096	0.19	0.096
LS5_20C	0.13	0.076	0.19	0.093	0.19	0.093
LS5_20D	0.12	0.078	0.19	0.092	0.19	0.092
UD3	0.18	0.038				
LS3_5A	0.16	0.054	0.18	0.10	0.18	0.10
LS3_5B	0.14	0.061	0.18	0.10	0.18	0.10
LS3_5C	0.098	0.077	0.19	0.11	0.19	0.11
LS3_5D	0.088	0.083	0.19	0.11	0.19	0.11
LS3_20A	0.16	0.053	0.18	0.098	0.18	0.098
LS3_20B	0.15	0.058	0.18	0.097	0.18	0.097
LS3_20C	0.11	0.069	0.19	0.094	0.19	0.094
LS3_20D	0.088	0.083	0.19	0.11	0.19	0.11

Table B9. Comparison of R² values of the relationship between genetic and cost distances in the Luther Marsh region for untransformed and log-transformed cost distances.

Model	Gen~Res		Gen~Res+Dist		Gen~Dist+Res	
	R ² , untransf.	R ² , log transf.	R ² , untransf.	R ² , log transf.	R ² , untransf.	R ² , log transf.
UD10	0.16	0.10				
LS10_5A	0.17	0.11	0.19	0.20	0.19	0.20
LS10_5B	0.18	0.11	0.19	0.20	0.19	0.20
LS10_5C	0.18	0.11	0.19	0.20	0.19	0.20
LS10_5D	0.18	0.11	0.19	0.20	0.19	0.20
LS10_20A	0.18	0.11	0.20	0.21	0.20	0.21
LS10_20B	0.19	0.11	0.20	0.21	0.20	0.21
LS10_20C	0.19	0.11	0.20	0.21	0.20	0.21
LS10_20D	0.19	0.11	0.20	0.21	0.20	0.21
UD5	0.15	0.054				
LS5_5A	0.17	0.058	0.19	0.20	0.19	0.20
LS5_5B	0.18	0.060	0.19	0.20	0.19	0.20
LS5_5C	0.18	0.063	0.19	0.20	0.19	0.20
LS5_5D	0.18	0.064	0.19	0.20	0.19	0.20
LS5_20A	0.17	0.059	0.19	0.19	0.19	0.19
LS5_20B	0.18	0.062	0.19	0.20	0.19	0.20
LS5_20C	0.19	0.066	0.19	0.20	0.19	0.20
LS5_20D	0.19	0.067	0.19	0.20	0.19	0.20
UD3	0.20	0.14				
LS3_5A	0.21	0.15	0.21	0.23	0.21	0.23
LS3_5B	0.21	0.15	0.21	0.23	0.21	0.23
LS3_5C	0.21	0.16	0.22	0.24	0.22	0.24
LS3_5D	0.21	0.16	0.22	0.24	0.22	0.24
LS3_20A	0.21	0.15	0.21	0.23	0.21	0.23
LS3_20B	0.22	0.15	0.22	0.24	0.22	0.24
LS3_20C	0.22	0.16	0.22	0.24	0.22	0.24
LS3_20D	0.22	0.14	0.22	0.21	0.22	0.21