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The influence of habitat composition and configuration on the genetic structure of the pitcher plant midge

Katie Millette
The University of Western Ontario

Supervisor
Dr. Nusha Keyghobadi
The University of Western Ontario

Graduate Program in Biology
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science
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THE INFLUENCE OF HABITAT COMPOSITION AND CONFIGURATION ON THE GENETIC STRUCTURE OF THE PITCHER PLANT MIDGE

(Spine title: Habitat structure and patterns of genetic differentiation)

(Thesis format: Monograph)

by

Katie Millette

Graduate Program in Biology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

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THE UNIVERSITY OF WESTERN ONTARIO
School of Graduate and Postdoctoral Studies

CERTIFICATE OF EXAMINATION

Supervisor

Dr. Nusha Keyghobadi

Supervisory Committee

Dr. Marc-André Lachance

Dr. Yolanda Morbey

Examiners

Dr. Beth MacDougall-Shackleton

Dr. Sheila Macfie

Dr. Yolanda Morbey

The thesis by

Katie Millette

entitled:

The influence of habitat composition and configuration on the genetic structure of the pitcher plant midge

is accepted in partial fulfillment of the
requirements for the degree of
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Chair of the Thesis Examination Board

Abstract

A major question in landscape genetics is how habitat structure influences spatial patterns of genetic differentiation. In this study, I evaluate the relative importance and effects of aspects of habitat composition (habitat amount) and configuration (patch size and isolation) on the spatial genetic structure of the pitcher plant midge, *Metriocnemus knabi*, whose larvae are found exclusively within the water-filled leaves of pitcher plants (*Sarracenia purpurea*) in a system that is naturally patchy at multiple spatial scales (i.e., leaf, plant, cluster, bog). I estimated genetic differentiation (F_{ST}) among leaves, plants, and clusters using 11 microsatellite loci, and measured the amount of habitat, patch size, and patch isolation at each spatial scale. Multi-model inference analyses indicate that the amount of habitat in the surrounding landscape (i.e., bog) and broad-scale patch isolation are the strongest predictors of genetic differentiation at local spatial scales (i.e., plant, cluster), and habitat amount and isolation have an interactive effect on F_{ST} estimates at the broader bog scale. These results reinforce the value of considering how ecological and evolutionary processes (i.e., behaviour, dispersal, gene flow, drift) occurring across multiple spatial scales may influence patterns of genetic differentiation.

Keywords: Landscape genetics, habitat composition, habitat configuration, isolation, dispersal, genetic structure, spatial scale, microsatellite, *Metriocnemus knabi*

Co-Authorship Statement

This thesis was completed under the supervision and financial support of Nusha Keyghobadi. Nusha and I jointly designed the study and formulated the hypotheses and predictions. I collected the samples and performed the experimental procedures (i.e., genomic extraction, microsatellite amplification, genotyping). I measured variables describing habitat amount, patch size, and patch isolation in the field. I used GPS data collected by Gordana Rasic in 2009-2010 on plant density and bog size to determine the amount of habitat surrounding the clusters I sampled. I performed the genetic analyses and statistical procedures, and drafted the thesis with suggestions and editorial comments from Nusha.

Acknowledgments

I would like to express my sincerest gratitude to my supervisor, Nusha Keyghobadi, for all of her academic and research guidance throughout my MSc. studies. Nusha's research approach to ecology and population genetics has strongly influenced the way I see ecological systems for the better. She has provided me with an exceptional first-hand introduction to the field of landscape genetics and I shall never observe organisms in nature without considering the importance of their surroundings.

I am extremely grateful to Marc-André Lachance and Yolanda Morbey who served as my committee advisors during my studies. Their advice and constructive criticism helped me interpret my results and greatly improved my writing. I thank Yolanda, Beth MacDougall-Shackleton, and Sheila Macfie for serving as my thesis examiners, and I am very appreciative for the time they devoted to my thesis.

Special thanks to Lindsay Crawford, Daria Koscinski, Gordana Rasic, and Kristina Zilic for their friendship, advice, and assistance both inside and outside of the lab. I greatly cherish our many research-based discussions, as well as our research-unrelated ones. I am particularly grateful to Kevin Schreiber for his assistance in the field. His driving and portaging skills, and overall positive outlook were invaluable while collecting samples. Thank you to all the members of the Harkness Laboratory of Fisheries Research Station for sharing their knowledge about Algonquin Provincial Park and their generosity during my stay.

I gratefully acknowledge the institutional support that I have received while working on my degree. I thank Western University, the Province of Ontario, and the Natural Sciences and Engineering Research Council of Canada (NSERC) for supporting me with generous scholarships.

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List of Symbols and Abbreviations

ΔAIC_c	difference of Akaike information criterion value between model *x* and the model with the lowest AIC value
β_j	estimated parameter coefficient of parameter *j*
$\hat{\bar{\beta}}_j$	weighted average parameter estimate of parameter *j*
$\hat{\beta}_{j,i}$	maximum likelihood estimate of parameter β_j
A	amount of habitat; composition metric
A_{bog}	amount of habitat in bog
A_{cl}	amount of habitat in cluster
A_{pl}	amount of habitat in plant
A_R	allelic richness
AIC	Akaike information criterion
AIC_c	second order Akaike information criterion corrected for small sample size
AIC_{cmin}	lowest Akaike information criterion
AMOVA	analysis of molecular variance
F_{ST}	measure of genetic structure or differentiation among subpopulations
F_{STbin}	F_{ST} values converted to binary integers
GIS	geographical information system
GLMM	generalized linear mixed model
GPS	geographical positioning system
H_O	observed heterozygosity
H_E	expected heterozygosity
I	patch isolation; configuration metric
I_{bog}	isolation of bog
I_{cl}	isolation of cluster
I_{lf}	isolation of leaf
I_{pl3}	isolation of plant
N_A	mean number of alleles
PCR	polymerase chain reaction
S	patch size; configuration metric
S_{cl}	patch size of cluster
S_{lf}	patch size of leaf
S_{pl}	patch size of plant
w_i	Akaike model weight
$w_+(i)$	relative variable importance

List of Software Packages

AICcmodavg	http://CRAN.R-project.org/package=AICcmodavg
ArcGIS 9.3	ESRI (Redlands, CA)
FSTAT	http://www2.unil.ch/popgen/softwares/fstat.htm
GenAlEx version 6.4.1	http://www.anu.edu.au/BoZo/GenAlEx/
GoogleEarth 6.2	http://www.google.com/earth/index.html
hierfstat	http://www2.unil.ch/popgen/softwares/hierfstat.htm
lme4	http://lme4.r-forge.r-project.org/
LOSITAN	http://popgen.eu/soft/lositan/
MICRO-CHECKER version 2.2.3	http://www.microchecker.hull.ac.uk/
ML-RELATE	http://www.montana.edu/kalinowski/Software/MLRelate.htm
MuMIn	http://r-forge.r-project.org/projects/mumin/
R version 2.14.1	R Development Core Team, http://www.r-project.org/

Chapter 1.0

Introduction

The relative abundance and distribution of different types of habitat in a landscape is one of the most influential factors determining biodiversity and driving regional patterns of biodiversity change over both space and time (Turner et al. 2001). Habitat structure refers to the abundance and distribution of suitable habitat for any given species within a landscape. Two distinct and quantifiable components comprise habitat structure: composition and configuration. Habitat composition describes the relative amount of suitable habitat in a landscape and is a key determinant of the quality of the landscape based on the resource requirements of a particular species. Habitat configuration describes the spatial characteristics and arrangement of habitat patches within a landscape and can define the connectedness of populations or sub-populations in the landscape. Both habitat composition and configuration can influence ecological (e.g., behaviour, dispersal, reproduction) and evolutionary processes (e.g., genetic drift, gene flow), which in turn contribute to the long-term sustainability of natural populations and biodiversity (MacArthur and Wilson 1963, 1967; Diamond 1975).

Landscapes with more suitable habitat and larger patch sizes (an aspect of habitat configuration) can accommodate larger populations and residents are expected to spend more time in habitat obtaining and allocating resources for survival and reproduction than residents in landscapes with less habitat and smaller patch sizes (MacArthur and Wilson 1963, 1967). When the distance between habitat patches is small (an aspect of habitat configuration), individuals moving among patches experience a reduced risk of dispersal mortality (MacArthur and Wilson 1963, 1967). Thus, by affecting landscape- and patch-

scale carrying capacity, as well as rates of emigration and immigration among patches, habitat composition and configuration influence population density, patch re-colonization rates, and the dynamics of local population persistence and extinction (MacArthur and Wilson 1963, 1967; Shaffer 1981; Wiegand et al. 1999, 2005; Hanski and Ovaskainen 2000; Revilla and Wiegand 2008). As such, large amounts of habitat and large, well-connected habitat patches have been considered critical reserve requirements because of their positive relationship with species richness and abundance (MacArthur and Wilson 1967; Shaffer 1981). Furthermore, small populations that experience frequent extinction-re-colonization events have lower effective population sizes and experience high levels of genetic drift (Frankham et al. 2004). Drift can erode genetic diversity if it is not countered by gene flow which acts to homogenize allelic patterns among populations and introduce new genetic variation into populations. Thus, small amounts of habitat, and/or small and isolated habitat patches can lead to decreased levels of population genetic diversity, elevated fitness costs as a result of inbreeding, and increased risk of regional extinction (Vario et al. 1986; Fahrig and Merriam 1994; Gibbs 1998; Nieminem et al. 2001; Reed and Frankham 2003; Keyghobadi 2007). Overall, understanding how habitat structure influences ecological and genetic patterns and processes is key to the science and practice of conservation, as it determines our ability to predict how species populations respond to changes in land use over space and time, and to manage reserves for long-term persistence of populations (Fahrig 2002; Ovaskainen 2002; Fahrig 2003).

The relative importance of habitat configuration versus composition for ecological processes is a long-standing issue in landscape ecology (Turner 2005), particularly within the context of understanding the effects of habitat loss and

fragmentation on species and ecosystems (reviewed in Fahrig 2003). This issue traces its roots to the 'single large or several small' (SLOSS) debate, which focused on whether it is preferable to maintain fewer, large tracts of habitat or numerous, small patches of habitat for conservation (Diamond 1975; Simberloff and Abele 1976, 1982; Wilcox and Murphy 1985). Typically, habitat fragmentation, which results from the transformation of a contiguous expanse of habitat into a number of smaller patches, occurs in such a way that habitat loss and the physical breaking up of habitat patches (fragmentation *per se*, *sensu* Fahrig 2003) occur simultaneously, and thus the effects of changes in habitat composition and configuration are confounded. As a result, it is very difficult to assess to what extent the changes in species abundances and species diversity that occur in response to habitat fragmentation are driven simply by the loss of habitat, versus changes in the spatial configuration of habitat patches (Fahrig 1997, 1998, 2001, 2003). Some ecological field studies have been able to measure habitat composition and configuration independently (e.g., Fahrig 1997; Trzcinski et al. 1999; Villard et al. 1999; Schmiegelow and Monkkonen 2002; Cushman and McGarigal 2004) and more recent studies have manipulated composition and configuration experimentally (Bonin et al. 2011; With and Pavuk 2011). Overall, the results of these studies indicate that the spatial arrangement of a species' habitat often contributes little to species occupancy, abundance, and distribution patterns, particularly when the amount of habitat in the landscape is high. However, when a species' habitat becomes less abundant (e.g., 10–30%; Radford et al. 2005), the spatial arrangement of the habitat becomes increasingly important (McGarigal and McComb 1995; Fahrig 1997, 1998; Trzcinski et al. 1999, Villard et al. 1999). Thus, there can be a strong, but highly context-dependent influence of habitat configuration on

the distribution and abundance of species above and beyond the effect of habitat composition. Theoretical and simulation studies support these general conclusions regarding the relative importance of habitat amount and configuration on species occupancy and abundance (With and Crist 1995; Fahrig 1997, 1998; Hill and Caswell 1999; With and King 1999; Fahrig 2001, 2002; Flather and Bevers 2002).

The field of landscape genetics is concerned with how characteristics of a landscape can influence the genetic diversity or genetic structure of populations, primarily through effects on dispersal and gene flow among populations (Manel et al. 2003). Genetic diversity refers to the proportion of loci that are polymorphic, or the mean number of individuals that are polymorphic at targeted loci. Example measures of population genetic diversity include allelic richness (i.e., the number of alleles per locus) or heterozygosity (the proportion of individuals that have two different alleles at a particular locus). Changes in the level of genetic diversity, such as the loss of alleles, may impede adaptation of populations to changes in environmental conditions (Reed and Frankham 2003). Genetic structure, or genetic differentiation, describes patterns in allele frequencies at a single locus or multiple loci, between individuals, groups of individuals, or populations. Common measures of genetic structure assess the partitioning of genetic diversity between groups of individuals, or subpopulations, within the greater population (e.g., F_{ST}). Measures of genetic diversity and genetic structure can be used to indirectly quantify the level of gene flow among individuals of different populations. For example, one may infer limited gene flow among individuals of different subpopulations if they have few alleles in common, or if a large amount of population genetic diversity is contained within subpopulations rather than among subpopulations. Understanding the

degree of genetic connectivity of populations across landscapes is critical for species conservation as gene flow maintains local genetic variation by counteracting genetic drift and introduces potentially adaptive alleles. Many landscape genetics studies focus particularly on the role of potential barriers in the landscape (e.g., roads, water bodies, unsuitable habitats) as impediments to gene flow, and characterize the 'connectedness' of populations based on their degree of genetic similarity (Storfer et al. 2010). Physical barriers to movement between populations can reduce gene flow, in turn reducing genetic diversity within local populations and increasing genetic differentiation among them (e.g., Segelbacher and Storch 2002; Keller and Largiader 2003).

Understanding how genetic diversity and spatial genetic structure change in response to habitat fragmentation is also a key area of research in landscape genetics (Manel et al. 2003; Storfer et al. 2010). The increased isolation and reduced size of habitat patches in fragmented landscapes are expected to leave populations smaller and more isolated than populations in unfragmented landscapes. As a result, populations in fragmented landscapes are predicted to experience both reduced gene flow and increased genetic drift (Keyghobadi 2007). Reduced gene flow and increased levels of drift work in combination to generate greater genetic divergence among populations and a loss of genetic diversity within local populations (e.g., Van Dongen et al. 1998; Knutsen et al. 2000; Arnaud et al. 2003).

Despite significant interest in the effects of habitat fragmentation on the genetics of populations, investigations that have explicitly tested the interaction of habitat composition and configuration on spatial patterns of genetic structure, or examined configuration effects on genetic structure while controlling for composition, are limited to

simulation experiments (Bruggeman et al. 2010; Cushman et al. 2012). In contrast to the ecological studies examining habitat composition and configuration effects of species abundances and distributions, genetic simulation studies conclude that habitat configuration can be more important than habitat area in determining genetic differentiation among populations. In simulation modeling of red-cockaded woodpecker data, habitat fragmentation *per se* strongly affected effective population size, F_{ST} patterns, as well as species abundance (Bruggeman et al. 2010). Cushman et al.'s (2012) results similarly indicate that habitat configuration variables, particularly habitat patch cohesion, correlation length, and aggregation index, are stronger determinants of population genetic differentiation than is habitat area (Cushman et al. 2012).

While habitat composition and configuration can both affect genetic structure (Cushman et al. 2012), their relative influence may vary with spatial scale. Since the driving processes underlying genetic structure (e.g., mating, reproductive behaviour, dispersal, genetic drift) each operate at unique spatial scales, it is critically important to match the scale of each driving factor with the scale of pattern examined (Wiens 1989; Balkenhol et al. 2009; Anderson et al. 2010). Furthermore, contemporary changes in landscapes rarely result in instantaneously observable changes in the genetic structure of resident populations. A temporal lag of tens to thousands of generations is required for changes in population structure to be detected, and the duration of the lag is highly dependent on standing population genetic variation, effective population sizes, and inherent species dispersal rates (Varvio et al. 1986; Cushman and Landguth 2010; Landguth et al. 2010). Moreover, historical legacies of ancient population genetic structure can confound conclusions regarding the driving factor behind observed

contemporary genetic patterns, and can lead to erroneous inferences regarding the importance of contemporary processes (Thompson and McGarigal 2002; Cushman and Landguth 2010). At increasing spatial scales the requirement of long time lags and likelihood of strong historical genetic signatures is high, further contributing to differences among scales in the underlying processes determining patterns of genetic structure.

My study evaluates the relative influence of aspects of habitat composition and configuration on the population genetic structure of the pitcher plant midge, *Metriocnemus knabi* Coquillett (Diptera, Chironomidae) at multiple spatial scales. *Metriocnemus knabi* (Coquillett 1904) larvae are found exclusively within the fluid-filled leaves of the purple pitcher plant, *Sarracenia purpurea* L., throughout nutrient-poor and patchy bog habitats across eastern North America. The aquatic environment provided by the pitcher plant represents an ecological microcosm that supports an assemblage of invertebrates, protists, rotifers, and bacteria (Giberson and Hardwick 1999). This microcosm has been used extensively in ecological research to address questions related to population regulation, community interactions and patterns, and ecosystem processes (Addicott 1974; Heard 1994b; Cochran-Stafira and von Ende 1998; Srivastava et al. 2004; Kadowaki et al. 2012). In addition to *M. knabi*, larvae of a flesh fly (*Fletcherimyia fletcheri*) and of a mosquito species (*Wyeomyia smithii*) also develop exclusively within *S. purpurea*. The dipteran larvae have a commensal relationship with the plant, whereby the plant provides a suitable aquatic environment and food from trapped decomposing prey. While the plant may not be completely dependent on the larvae, their presence can contribute to enhanced decomposition of dead prey material and to enhanced production

and availability of nitrogenous nutrients for the plant (Gallie and Chang 1977; Bradshaw and Creelman 1984).

At temperate latitudes, *M. knabi* is univoltine and adults emerge in late spring (June-July). Little is known about the adult life stage, although adults are small in size (approximately 3 mm in length) and likely have weak flight abilities (Knab 1905; Wiens 1972; Krawchuk and Taylor 2003; pers. obs.). Females deposit eggs within pitcher leaves and multiple larvae (~ 15 individuals) can be found developing within a single pitcher leaf in late summer (July-August; Heard 1994b; Giberson and Hardwick 1999). Multiple leaves are found in each pitcher plant, and the plants tend to grow in clusters, likely as a result of short seed dispersal (~ 5 cm, Ellison and Parker 2002). Thus the habitat of *M. knabi* is clearly defined by *S. purpurea* and provides a series of discrete habitat patches that are hierarchically nested at several spatial scales (leaf, plant, cluster, and bog). The abundance and distribution of leaves within pitcher plants, plants within clusters, and clusters within bogs vary widely, so that various combinations of habitat composition and configuration occur naturally at each scale. As a result, *M. knabi*, as well as the other obligate inhabitants of the purple pitcher plant, provides a naturally occurring system within which we can observe and independently measure varying amounts of habitat and spatial configurations of habitat within the landscape.

An ecological investigation of all three pitcher plant dipterans (*F. fletcheri, M. knabi, W. smithii*) has shown a significant relationship between larval abundance and habitat structure (Krawchuk and Taylor 2003). In general, habitat configuration had a consistently significant effect on larval species abundance regardless of the amount of habitat in the surrounding landscape, although habitat patch size was found to be more

important than configuration metrics at distances within the dispersal range of individuals (i.e., leaf, plant), while habitat isolation became important at larger scales (i.e., cluster and bog; Krawchuk and Taylor 2003). Previous genetic analyses on *M. knabi* using individual-based measures of genetic differentiation indicate significant genetic structuring among all habitat spatial scales (i.e., leaf, plant, cluster, and bog) and greater partitioning of genetic variability at the higher spatial scales (i.e., cluster, bog) (Rasic and Keyghobadi 2012). In addition, landscape variables in the broader landscape, such as bog size and plant density, accounted for approximately 50% of the genetic differentiation among individuals (Rasic and Keyghobadi 2012). Therefore there is evidence that *M. knabi* responds to metrics of habitat structure, and that the relationship may be scale-dependant.

In this study, I use independent measures of habitat composition and configuration, and measures of genetic differentiation, to evaluate the relative effect of the amount of habitat, patch size, and patch isolation on patterns of genetic structure in *Metriocnemus knabi* across three spatial scales (i.e., plant, cluster, bog). By measuring the genetic differentiation among leaves within plants (plant scale), among plants within clusters (cluster scale), and among clusters within bogs (bog scale), I aim to quantify the relative importance of habitat composition and configuration at each spatial scale and determine whether the effects of habitat composition and configuration on genetic structure changes at different spatial scales. In my study, each plant, cluster, and bog can be thought of as a replicate 'landscape' from which I have sampled multiple habitat patches. At each scale, I estimate the genetic differentiation among the sampled patches within each 'landscape', and relate measures of differentiation to composition and

configuration metrics. The size and distance among sampled habitat patches are key configuration metrics commonly measured in landscape genetic and habitat fragmentation studies, and are highly likely to influence the degree of differentiation among populations. Since patch size influences local population size and potentially genetic drift, while patch isolation influences the rate of gene flow between sites (Bruggeman et al. 2010; Cushman et al. 2012), I predict that habitat configuration (i.e., patch size and isolation) will significantly affect patterns of spatial genetic structure. Furthermore, because genetic differentiation is shaped by evolutionary and ecological processes that operate at multiple spatial scales, I predict that the influence of habitat configuration will depend on the spatial scale of interest. Specifically, genetic differentiation among leaves and among plants are most likely driven by the female oviposition behaviour and stochastic colonization/mortality rates, whereas genetic differentiation among clusters are most likely the result of patterns of dispersal and gene flow, which are limited by increasing spatial distances. Thus, with increasing spatial scale, I predict an elevated importance of habitat isolation above patch size. In addition, I consider whether the amount of habitat at broader spatial scales or the isolation of the 'landscape' itself are also important in determining genetic differentiation. The amount of habitat and isolation of patches within the landscape are expected to influence effective population sizes and contribute to stochastic differences in patterns of genetic differentiation. In addition, habitat patches in the surrounding landscape may provide a source of colonizing individuals and serve as potential stepping stone patches that mitigate the isolation of sampled populations. Thus, my third prediction is that the effect of habitat configuration will depend on the amount of habitat in the broader landscape.

Chapter 2.0

Materials and Methods

2.1 Study area and bogs

The study was conducted in Algonquin Provincial Park, Ontario, Canada (Fig. 1), which is characterized as a transition zone between southern deciduous forest and northern coniferous forest. The predominant land cover type is forest habitat (e.g., species of balsam fir (*Abies balsamea*), tamarack (*Larix laricina*), black spruce (*Picea mariana*), red spruce (*P. rubens*), white spruce (*P. glauca*), red pine (*Pinus resinosa*), white pine (*P. strobus*), white cedar (*Thuja occidentalis*), hemlock (*Tsuga canadensis*), ash (*Fraxinus* spp.), red maple (*Acer rubrum*), silver maple (*A. saccharinum*), sugar maple (*A. saccharum*), oak (*Quercus* spp.), and American beech (*Fagus grandifolia*)) among which bog habitat is patchily distributed. Bog habitat represents a successional land cover type between forest and small water bodies and is described as a type of wetland where the only water input is through precipitation (Gore 1983). As a result of poor drainage and the decay of accumulated plant material, bog habitats are characteristically low in pH and oxygen levels and harbour a distinctive assembly of plant species (e.g., bog cranberry (*Vaccinium* spp.), Labrador tea (*Rhododendron* spp.), leatherleaf (*Chamaedaphne calyculata*), sphagnum mosses (*Sphagnum* spp.), and sundew (*Drosera* spp.)). The carnivorous purple pitcher plant, *Sarracenia purpurea*, is well adapted to grow in these nutrient poor environments and can be found throughout bog habitats in the study area (Ellison and Gotelli 2002; Ellison et al. 2012).

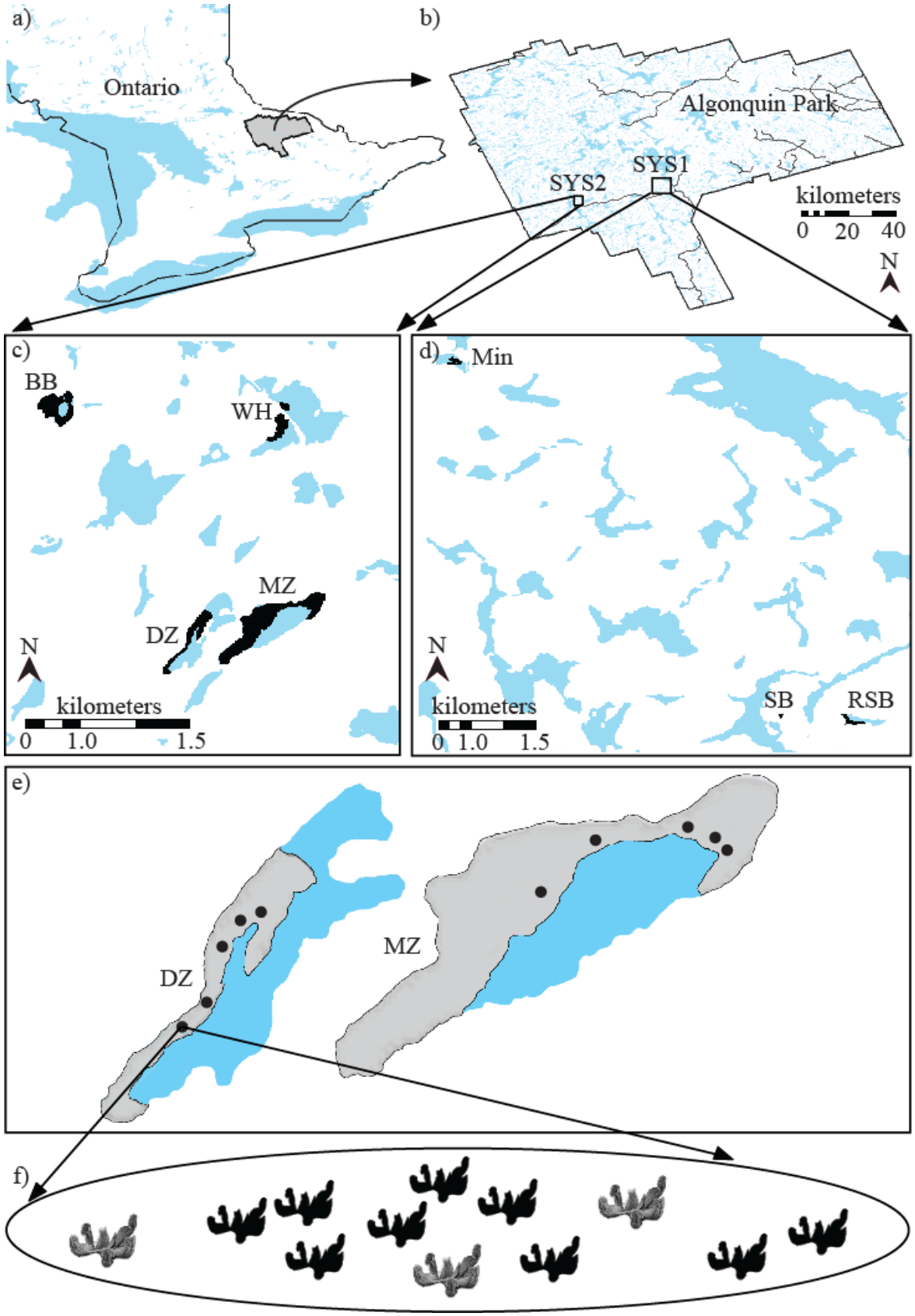

a)
Ontario
b)
Algonquin Park
SYS1
SYS2
kilometers
0 20 40
N
c)
BB
WH
MZ
DZ
N
kilometers
0 1.0 1.5
d)
Min
N
kilometers
0 1.0 1.5
SB
RSB
e)
DZ
MZ
f)

Figure 1.

Figure 1. Sampling map of *Metriocnemus knabi*. (a, b) Larvae were sampled from two systems of bogs (SYS1, SYS2) in Algonquin Provincial Park (Ontario, Canada). (c) System 2 consists of 'Buggy' (BB), Dizzy Lake (DZ), Mizzy Lake (MZ), and Wolf Howl (WH) bogs. (d) System 1 consists of Minor Lake (Min), 'Roadside' (RSB), and Spruce (SB) bogs. (e) Within each bog, 3-5 clusters of plants (i.e., 5 m-radius area containing ≥ 10 plants) were arbitrarily selected. (f) Within each cluster, three plants were haphazardly chosen and larvae (~ 5 individuals) were pipetted from three leaves per plant.

2.2 Sampling

Metriocnemus knabi larvae were sampled at four nested spatial scales: leaf, plant, cluster, and bog, and replicated in two areas or 'systems' approximately 25 km apart (Fig. 1). Within each system, 3-4 bogs were selected and 3-5 clusters were sampled per bog. A cluster was defined as a 5 m-radius area containing ≥10 pitcher plants and its centre was considered to be the point of highest pitcher plant density within the 5 m-radius circle. Three plants were haphazardly selected within a cluster and larvae were removed from three leaves per plant (Fig. 1). The locations of the centre of each cluster and each sampled plant were recorded using a high-accuracy (< 30 cm) GPS receiver (Trimble GeoXH, Sunnyvale, CA, USA; Table 1).

Table 1. Names and codes of bogs with UTM coordinates (zone 17) of sampled clusters and the number of larvae sampled per cluster.

System	Bog name	Code	Cluster	Easting	Northing	Number of larvae
1	Minor Lake	Min	1	701470.35	5057404.66	38
			2	701431.21	5057416.08	32
			3	701492.58	5057439.70	45
			4	701455.00	5057477.32	38
			5	701406.24	5057471.99	37
1	'Roadside'	RSB	1	705952.47	5051985.57	42
			2	705946.24	5051996.82	45
			3	705930.78	5052027.05	45
1	Spruce	SB	1	705175.87	5052062.63	42
			2	705198.36	5052047.58	45
			3	705187.01	5052024.75	40
2	'Buggy'	BB	1	679543.08	5049240.78	35
			2	679534.18	5049207.55	35
			3	679435.99	5049239.82	45
			4	679475.67	5049063.61	37
			5	679535.96	5049087.45	38
2	Dizzy Lake	DZ	1	680239.54	5046841.01	36
			2	680367.22	5047086.61	45
			3	680426.09	5047190.66	42
			4	680382.56	5047150.68	45
			5	680320.50	5046946.62	43
2	Mizzy Lake	MZ	1	681125.51	5047380.16	34
			2	681104.87	5047388.79	20
			3	681075.61	5047398.62	52
			4	680978.63	5047381.92	38
			5	680940.23	5047357.64	30
2	Wolf Howl	WH	1	680310.84	5049817.92	36
			2	680347.46	5049867.28	45
			3	680285.56	5049907.96	40
			4	680247.52	5049928.95	41
			5	680233.54	5049869.09	45

2.3 DNA extraction, amplification, and fragment analysis

Individual larvae were removed from *Sarracenia purpurea* leaves, sorted, and preserved in 95% ethanol and stored at 4°C. Genomic DNA was extracted from single larvae using the DNeasy tissue extraction kit (Qiagen, Germantown, MC, USA). Individuals were analyzed at 11 neutral microsatellite loci (Rasic et al. 2009), such that an association between allele frequencies and habitat structure variables is expected to reflect the effect of limited gene flow and genetic isolation rather than selection. The 10 µL multiplexed polymerase chain reactions (PCR), thermal cycling, and fragment analysis protocols followed that of Rasic and Keyghobadi (2012).

2.4 Genetic data analyses

I tested for the presence of null alleles within systems using MICRO-CHECKER version 2.2.3 (van Oosterhout et al. 2004). Loci were assessed for neutrality using LOSITAN software (Antao et al. 2008), which tests for potentially adaptive loci, or loci under selection, using an F_{ST}-outlier detection method (Antao et al. 2008). The number of alleles (N_A) and the mean observed (H_O) and expected (H_E) heterozygosities were calculated across loci and samples for each plant, cluster, bog, and system using GenAlEx version 6.4.1 (Peakall and Smouse 2006). Allelic richness (A_R) was calculated in FSTAT version 2.9.3.2 (Goudet 1995, 2002) using a rarefaction method (Mousadik and Petit 1996) to compensate for unequal sample sizes among sampling groups.

Full siblings represent individuals that have developed from eggs of a single clutch and the distribution of full-sibling relationships among larvae therefore reflects the oviposition behaviour of adult females. The familial relationships between pairs of larvae

were assessed by calculating maximum-likelihood coefficients from multi-locus genotypes in ML-Relate (Kalinowski et al. 2006). Full-sibling (FS), half-sibling (HS), parent-offspring (PO), and unrelated (U) relationships were tested between individuals sampled within the same leaf, in different leaves of the same plant, in different plants of the same cluster, and in different clusters of the same bog using a 99% confidence set and 1000 randomizations. Since parent-offspring relationships are not possible for larvae collected within a single season, putative PO relationships were treated as FS relationships. If an alternative relationship with a high likelihood was identified by the confidence set for each FS and/or PO relationship, the FS and/or PO relationship was tested against its alternative relationship using a likelihood ratio test and 1000 simulated random genotype pairs (Kalinowski et al. 2006). Pairwise comparisons between individuals from the same lower level (e.g., leaf, plant) were removed in the calculation of full-sibling relationships at higher levels (e.g., cluster). The percentage of full-sibling pairs identified among individuals from the same leaf, between different leaves, between plants, and between clusters was then plotted and compared for each bog in both systems.

A hierarchical analysis of molecular variance (AMOVA) was conducted to assess the distribution of genetic variation across all the spatial scales in both systems. Variance components and hierarchical *F*-statistic coefficients were computed in R (v. 2.14.1, R Development Core Team 2009) using the hierfstat package (Goudet 2005) which allows the permutation of units among any number of levels. For example, it was possible to evaluate the significance of variance components and *F*-statistic values at the plant scale by permutating entire units of leaves among plants, while maintaining plants within their respective bog and system levels. The significance of variance components and *F*-statistic

coefficients was tested among leaves, plants, clusters, and bogs in each system using 1000 permutations and $\alpha = 0.05$. In System 1, genetic variation was assessed across the three bogs, 11 clusters, 33 plants, and 98 leaves (449 individuals). The large sample size of System 2 exceeded the computational limit for AMOVA analysis in the hierfstat package. Thus, a cluster was randomly removed from each bog in System 2 and analysis was conducted across the four bogs, 16 clusters, 49 plants, 143 leaves (641 individuals).

Genetic differentiation was analyzed at the plant, cluster, and bog scales using Weir-Cockerham (1984) estimates of F_{ST} in GenAlEx (v. 6.4.1, Peakeall and Smouse 2006). These estimates of F_{ST} assess the partitioning of genetic diversity among subpopulations and range from 0 to 1, where a value of zero indicates no difference in allele frequencies among subpopulations, whereas a value of 1 indicates subpopulations are completely differentiated and share no alleles in common. At the plant scale, F_{ST} was estimated for each plant by partitioning the variance of genetic diversity among the three sampled leaves. Similarly, at the cluster and bog scales, F_{ST} was estimated among the three sampled plants within each cluster and among the 3-5 sampled clusters within each bog. The statistical significance of F_{ST} values was tested in GenAlEx (v. 6.4.1, Peakeall and Smouse 2006) by permutating individual genotypes among samples, re-calculating F_{ST}, and determining if the observed F_{ST} value fell within the upper tail of the permutated data set. In the plant, cluster, and bog scale tests, significance was assessed using 999 permutations and $\alpha = 0.05$. In downstream statistical analyses, the F_{ST} values at each scale represented the response variable for that scale. For example, at the plant scale the response variable was the differentiation among the three sampled leaves within each plant, and the total sample size was equal to the number of sampled plants. This node-

based approach of estimating genetic differentiation is different from most landscape genetic studies where the response variable is typically a pairwise measure of genetic differentiation. In comparison to a pairwise approach, my approach provides data points that are not inherently dependent on each other and where the sample size is not inflated by multiple pairwise comparisons (Legendre and Fortin 2010).

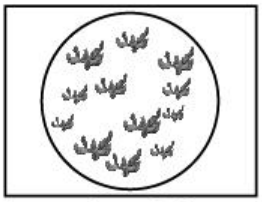
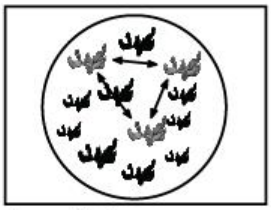
2.5 Habitat composition: Amount of habitat

The amount of habitat (A) represents the quantity of habitat resource available to *M. knabi*. Larger amounts of habitat are expected to support larger populations, exhibit higher levels of genetic diversity, and lower levels of genetic differentiation. At the plant scale, the amount of habitat was quantified as the number of leaves per plant (A_{pl}; Fig. 2). At the cluster scale, the amount of habitat was quantified as the number of plants per cluster (A_{cl}), and at the bog scale, the amount of habitat was quantified as the area of the bog (m^2; A_{bog}). Bog area was measured in ArcGIS version 9.3 (ESRI, Redlands, CA) using a combination of high resolution enhanced Forestry Resource Information (eFRI) imagery and GPS transect points. Considering the nested nature of the sampling structure, I was also interested in understanding whether the amount of habitat beyond the scale of interest had an effect on genetic differentiation. Therefore at the plant scale, not only was A_{pl} considered, but the amount of habitat in the surrounding cluster (A_{cl}) and bog (A_{bog}) were included in statistical models (Fig. 2). Similarly, at the cluster scale, A_{bog} was included to account for the influence of the amount of habitat in the surrounding bog.

(a)

	Habitat composition Amount of habitat	Habitat configuration Patch size	 Patch isolation
Effect of system scale ↓			Distance to nearest bog
Effect of bog scale ↓	Bog area		Number of plants in 10 m buffer around cluster
Effect of cluster scale ↓	Number of plants per cluster		Distance to centre of cluster
Plant scale	 Number of leaves per plant	 Avg. size of sampled leaves	 Avg. distance among sampled leaves

(b)

Effect of system scale ↓			Distance to nearest bog
Effect of bog scale ↓	Bog area		Number of plants in 10 m buffer around cluster
Cluster scale	 Number of plants per cluster	 Avg. number of leaves among sampled plants	 Avg. distance among sampled plants

(c)

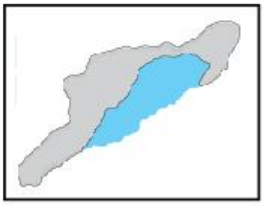
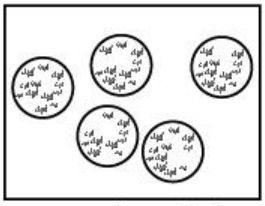
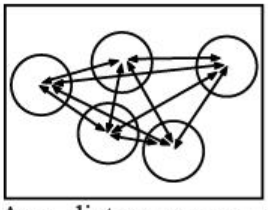
Effect of system scale ↓			Distance to nearest bog
Bog scale	 Bog area	 Avg. number of plants in sampled clusters	 Avg. distance among sampled clusters

Figure 2.

Figure 2. Summary of habitat composition and configuration measurements recorded at the (a) plant, (b) cluster, and (c) bog scales. At the plant scale (a), the amount of habitat was measured as the number of leaves per plant, and patch size and isolation were measured as the average size of the three sampled leaves and average distance among the three sampled leaves. The effect of the amount of habitat in the surrounding cluster and bog was measured as the number of plants per cluster and bog area, while the effect of the isolation of the plant within the cluster, bog, and system was measured as the distance to the centre of the cluster, the number of plants in a 10 m wide buffer area around the cluster, and the distance of the bog to the centre of the nearest neighbouring bog in the system, respectively. At the cluster scale (b), the amount of habitat was measured as the number of plants per cluster, and patch size and isolation were measured as the average number of leaves among the three sampled plants and the average distance among the three sampled plants, respectively. The effect of the amount of habitat in the surrounding bog was measured as bog area, while the effect of the isolation of the cluster within the bog and system was measured as the number of plants in a 10 m wide buffer area around the cluster and the distance of the bog to the centre of the nearest neighbouring bog in the system, respectively. At the bog scale (c), the amount of habitat was quantified using bog area, and patch size and isolation were measured as the average number of plants in the sampled clusters and the average distance among the sampled clusters as measured from the centre of each cluster. The effect of the isolation of the bog in the system was measured as the distance of the bog to the centre of the nearest neighbouring bog in the system.

2.6 Habitat configuration: Patch size and patch isolation

Patch size (S) is a metric that represents the size of a contiguous habitat area. The patch size of the leaf was quantified by measuring the widest part of the pitcher vessel as this metric was found to be a strong predictor of the leaf's potential volume (Fig. A1; Table A6; Fig. A2). The patch size of the plant and cluster were measured as the number of leaves per plant and the number of pitcher plants per cluster, respectively. For plant scale analysis, the patch size of the leaf was averaged among the three sampled leaves to give a single patch size metric (S_{lf}). Similarly, for cluster scale analysis, plant patch size was averaged among the three sampled plants (S_{cl}) and for bog scale analysis the patch size of the cluster was averaged among the sampled clusters (S_{bog}; Fig. 2).

Patch isolation (I) describes the relative spatial arrangement of habitat patches and may be positively correlated with the degree of genetic differentiation as gene flow among patches declines with distance. At the plant scale, patch isolation was measured as the average distance among the three sampled leaves (I_{lf}; Fig. 2). At the cluster and bog scales, patch isolation was measured as the average distance among the sampled plants (I_{pl3}), and the average distance among the sampled clusters (I_{cl3}) using the distance from the centre of each cluster, respectively. I was interested in understanding how the isolation of the patch of interest in a broader context may influence patterns of genetic differentiation. Thus, in addition to I_{lf} at the plant scale, I evaluated how isolated each plant was within the cluster by measuring the distance from the plant to the centre of the cluster (I_{pl}). Since the centre of each cluster was positioned to represent the area of highest pitcher plant density, plants along the cluster periphery were more isolated. Also for the plant scale, I evaluated how isolated the cluster was within the bog by quantifying

the number of plants within a 10 m wide buffer area around the cluster (I_{cl}). The buffer was created in ArcGIS version 9.3 (ESRI, Redlands, CA) and the number of plants was determined using maps of interpolated plant-count data that was collected in 2009-2010 (Rasic and Keyghobadi 2012). To generate the plant-count data, the density and distribution of pitcher plants within each bog has been recorded along linear transects, where plants were counted within a 2 m-radius area at 10 m intervals and transects were repeated every 5 m (Rasic and Keyghobadi 2012). Plant-count maps were created in ArcGIS using the spherical semivariogram kriging method to interpolate the number of plants between data collection points. The isolation of each bog (I_{bog}) within the landscape was evaluated by measuring the distance of the centre of the bog to the centre of the nearest neighbouring bog in the system using GoogleEarth 6.2 (Fig. 2). At the cluster scale, I_{cl} and I_{bog} were included in statistical models to account for the isolation of the cluster in the bog and the isolation of the bog within the system, while I_{bog} was included at the bog scale to evaluate the isolation of the bog within the system (Fig. 2).

2.7 Statistical analyses

Separate datasets containing predictor and response variables were constructed for each of the three spatial scales (plant, cluster, bog). At the plant scale, eight predictor variables were included in the models: patch size of the leaf (S_{lf}), patch isolation of the leaf (I_{lf}), patch isolation of the plant (I_{pl}), patch isolation of the cluster (I_{cl}), patch isolation of the bog (I_{bog}), and the amount of habitat in the plant, cluster, and bog (i.e., A_{pl}, A_{cl}, A_{bog}; Tables 2, A3). The predictor variables S_{lf}, I_{lf}, and A_{pl} represent the most local habitat

Table 2. Predictor variable codes and descriptions used in models at the plant, cluster, and bog scales.

Code	Description	Measurement
Plant scale		
S_{lf}	Patch size of leaf	Average size of the three sampled leaves
I_{lf}	Isolation of leaf	Average distance among the three sampled leaves
I_{pl}	Isolation of plant	Distance of plant to centre of cluster
I_{cl}	Isolation of cluster	Number of plants in 10 m-wide buffer around sampled cluster
I_{bog}	Isolation of bog	Distance to nearest bog
A_{pl}	Amount of habitat in plant	Number of leaves per plant
A_{cl}	Amount of habitat in cluster	Number of plants per cluster
A_{bog}	Amount of habitat in bog	Bog area
Cluster scale		
S_{pl}	Patch size of plant	Average number of leaves per plant for the three sampled plants
I_{pl3}	Isolation of plant	Average distance among the three sampled plants
I_{cl}	Isolation of cluster	Number of plants in 10 m-wide buffer around sampled cluster
I_{bog}	Isolation of bog	Distance to nearest bog
A_{cl}	Amount of habitat in cluster	Number of plants per cluster
A_{bog}	Amount of habitat in bog	Bog area
Bog scale		
S_{cl}	Patch size of cluster	Average number of plants in the sampled clusters
I_{cl3}	Isolation of cluster	Average distance among the sampled clusters
I_{bog}	Isolation of bog	Distance to nearest bog
A_{bog}	Amount of habitat in bog	Bog area

metrics acting on the leaf scale, whereas I_{pl} and A_{cl}, and I_{cl} and A_{bog} represent potential habitat variables in the broader cluster and bog scales that may also affect patterns of genetic differentiation of *M. knabi* measured at the plant scale. The genetic response variable was measured as the F_{ST} value among the three sampled leaves within each individual plant (sample size = 94 plants). *F*-statistic values have the potential to be highly stochastic when calculated among leaf units considering the small sample size (~ 5 individuals per leaf) and likelihood of full-sibling relationships within single leaves. As a result, F_{ST} values were also treated as a binomial response, where F_{ST} values that were significantly greater than zero ($p < 0.05$) were coded as 1 and non-significant values were coded as 0. Concordance among plant scale analyses using F_{ST} values and binomial integers (F_{STbin}) is expected to provide strong support for estimates of relative variable importance and parameter effects.

At the cluster scale, six predictors were included in the models: patch size of the plant (S_{pl}), patch isolation of the plant (I_{pl}), patch isolation of the cluster (I_{cl}), patch isolation of the bog (I_{bog}), and the amount of habitat in the cluster (A_{cl}) and bog (A_{bog}) (Tables 2, A4). Here, S_{pl}, I_{pl}, and A_{cl} are the local habitat metrics at the cluster scale, while I_{cl} and A_{bog} represent the potential effect of variables of the broader bog scale. The response variable was calculated as F_{ST} among the three sampled plants within each individual cluster (sample size = 31 clusters).

At the bog scale, four predictors were included in the models: patch size of the cluster (S_{cl}), patch isolation of the cluster (I_{cl}), patch isolation of the bog (I_{bog}), and the amount of habitat in the bog (A_{bog}; Tables 2, A5). Broader habitat metrics were not included beyond the bog scale considering the increasing influence of environmental

processes (e.g. wind) and genetic drift at these scales. The response variable was calculated as F_{ST} among the 3-5 sampled clusters within each individual bog (sample size = 7 bogs). All predictor variables within each dataset were screened for inter-correlations ($r > 0.6$) to prevent model selection on redundant predictor variables. Standardization (i.e., scaled with mean = 0 and standard deviation = 1) was carried out on each habitat metric variable within each dataset (i.e., at each spatial scale) to aid in comparisons of predictor variable estimates (Tables A3-A5).

2.8 Generalized linear mixed models (GLMMs)

The influence of habitat structure on genetic differentiation at each spatial scale was analyzed using generalized linear mixed models (GLMMs). Generalized linear mixed models offer a flexible approach for evaluating data with nested random effects (Bolker et al. 2008). Random effects serve to quantify variation among units, where observations may be replicated in space, time, or individuals. Because individuals are grouped in space (e.g., individuals from leaves, within a plant, within a cluster), the assumption of independence is not valid. Therefore, I accounted for the nested structure of the data and the potential of covariance among nested units by coding the nested random effects with a random intercept varying among systems, among bogs within systems, and among clusters within bogs. I justified the use of nested effects parameters using likelihood ratio tests, by fitting a model with and without the variance component and comparing the quality of the fits (Baayen et al. 2008).

Models for the plant, cluster, and bog scale were fitted using the respective datasets and consisted of additive effects of habitat metrics as fixed effects, as well as

interactions among composition and configuration metrics. In particular, the effect of metrics of isolation may be dependent on the amount of habitat in the broader spatial scale. Thus, interactions between leaf isolation and the amount of habitat in the plant (I_{lf}:A_{pl}), plant isolation and the amount of habitat in the cluster (I_{pl}:A_{cl}), and cluster isolation and the amount of habitat in the bog (I_{cl}:A_{bog}) were included in the plant scale models, I_{pl}:A_{cl} and I_{cl}:A_{bog} were included in the cluster scale models, and I_{cl}:A_{bog} was included in the bog scale models. Generalized linear mixed models were fitted using the lme4 package (Bates and Maechler 2010) in R (v. 2.14.1, R Development Core Team 2009).

2.9 Model selection and multi-model inference

A multi-model inference approach was used to examine the relative effects of predictor variables on genetic differentiation at each spatial scale (Burnham and Anderson 2002). This approach consists of generating a candidate set of models based on all possible combinations of parameters present in a global model (Table 3). The global model represents the most parameterized prediction of the effect of habitat metrics on genetic differentiation. For the plant scale, the global model contained all predictors (Table 2) and three interaction variables, resulting in a model set with 2^{11} models. Similarly, the candidate sets for the cluster and bog scales contained 2^8 and 2^5 models, respectively (Table 3).

Table 3. Global models used to generate candidate model sets in the plant, cluster, and bog scale datasets. Variables with colons denote interaction terms between patch isolation (I) and amount of habitat (A) metrics. Nested random effects were included in each global model, where at the plant scale, data are nested in clusters, within bogs, within systems. At the cluster scale, data are nested in bogs within systems. At the bog scale, data are nested within systems.

Scale	Global model
Plant	$F_{ST} = S_{lf} + I_{lf} + I_{pl} + I_{cl} + I_{bog} + A_{pl} + A_{cl} + A_{bog} + I_{lf}{:}A_{pl} + I_{pl}{:}A_{cl} + I_{cl}{:}A_{bog}$ + (nested random effects)
Cluster	$F_{ST} = S_{pl} + I_{pl3} + I_{cl} + I_{bog} + A_{cl} + A_{bog} + I_{pl}{:}A_{cl} + I_{cl}{:}A_{bog}$ + (nested random effects)
Bog	$F_{ST} = S_{cl} + I_{cl3} + I_{bog} + A_{bog} + I_{cl3}{:}A_{bog}$ + (nested random effects)

Models were ranked separately for each scale according to corrected Akaike information criterion values (AIC_c; Akaike 1973, Sugiura 1978), a criterion recommended when the number of observations (n) relative to the number of parameters (K) is small ($n/K < 40$; Burnham and Anderson 2002). The model with the lowest AIC_c value (i.e., AIC_{cmin}) is considered the top model in the set, while those within 2 AIC_c values of the top model ($\Delta AIC_c < 2$) are essentially as good as the top model (Burnham and Anderson 2002). Akaike model weights (w_i) were calculated and interpreted as the probability that model M_i is the true model explaining genetic structure, given that the true model is in the model set,

$$w_i = \frac{e^{-\frac{1}{2}\left(\mathrm{AIC_{c_i}} - \mathrm{AIC_{c_{min}}}\right)}}{\sum_{r=1}^{R} e^{-\frac{1}{2}\left(\mathrm{AIC_{c_r}} - \mathrm{AIC_{c_{min}}}\right)}}$$

where w_i is the model weight, AIC_{ci} is the AIC value for the *i*th model, and AIC_{cmin} is the value of the top model in the set at the target spatial scale (Burnham and Anderson 2002; Link and Barker 2006). A top-ranked model with $w_i > 0.9$ and AIC_c four units less than the second-ranked model is strong evidence in support of the best model (Burnham and Anderson 2002). When the best model in the set was not clear, model averaging was conducted using all models in the set. Relative variable importance ($w_+(i)$) was assessed for each predictor to identify the most important habitat metric at each scale by summing the Akaike weights of the target predictor across the models in which the variable was present. Model averaged parameter estimates and their unconditional standard errors were calculated for each parameter in the plant, cluster, and bog scale models using the weighted average of the parameter estimates from the models in which the target parameter is explicitly present,

$$\hat{\bar{\beta}}_j = \frac{\sum_{i=1}^{R} w_i I_j(g_i) \hat{\beta}_{j,i}}{\sum_{i=1}^{R} w_i I_j(g_i)}$$

where $\hat{\bar{\beta}}_j$ is the weighted average parameter estimate, w_i is the Akaike weight of model *i*, $I_j(g_i) = 1$ if the parameter is included in model *i* and $I_j(g_i) = 0$ if the parameter is not included in the model, and $\hat{\beta}_{j,i}$ is the maximum likelihood estimate of parameter *j* in model *i* (Burnham and Anderson 2002). The significance of parameter estimates was evaluated by the exclusion of zero from the 90% and 95% confidence intervals. Model averaging and the calculation of parameter estimates were conducted using the MuMIn (Bartoń 2009) and AICcmodavg packages (Mazerolle 2012) in R (v. 2.14. 1, R Core Development Team 2009).

Chapter 3.0

Results

3.1 Genetic diversity and structure

A total of 1,231 individuals were genotyped from 7 bogs, 31 clusters, 94 plants, and 276 leaves (approximately 5 individuals per leaf; Tables A1, A2). All 11 loci were included in the analysis, as null alleles and loci under selection were not detected. At the plant scale, mean number of alleles (N_A) and allelic richness (A_R) ranged from 2.091 – 3.364 and 3.100 – 4.296, respectively, while the range of mean observed (H_O) and expected heterozygosities (H_E) were 0.371 – 0.636 and 0.361 – 0.548, respectively. At the cluster scale, N_A = 3.030 – 4.515 and A_R = 2.774 – 4.515, whereas H_E = 0.449 – 0.509 and H_O = 0.437 – 0.580. At the bog scale, N_A = 5.309 – 6.109, A_R = 4.564 – 6.024, H_O = 0.501 – 0.552, and H_E = 0.485 – 0.519. Overall, the average number of alleles and allelic richness across loci and bogs was, respectively, 7.33 (SE = 0.043) and 7.171 in System 1 and 8.84 (SE = 0.952) and 8.731 in System 2. Observed and expected values of heterozygosity were not significantly different between the systems (System 1: H_O = 0.540 (SE = 0.048), H_E =0.528 (SE = 0.043); System 2: H_O = 0.518 (SE = 0.040), H_E = 0.538 (SE = 0.033)).

The incidence of full-sibling pairs was highest for individuals from the same leaf and decreased steadily in between-leaf, -plant, and -cluster comparisons in System 1 (Fig. 3). A similar pattern was observed in System 2, except in Dizzy Lake bog (DZ), where the percentage of full-sibling pairs increased slightly in between-cluster comparisons (0.131%) as compared to between-plant comparisons (0.046%, Fig. 4).

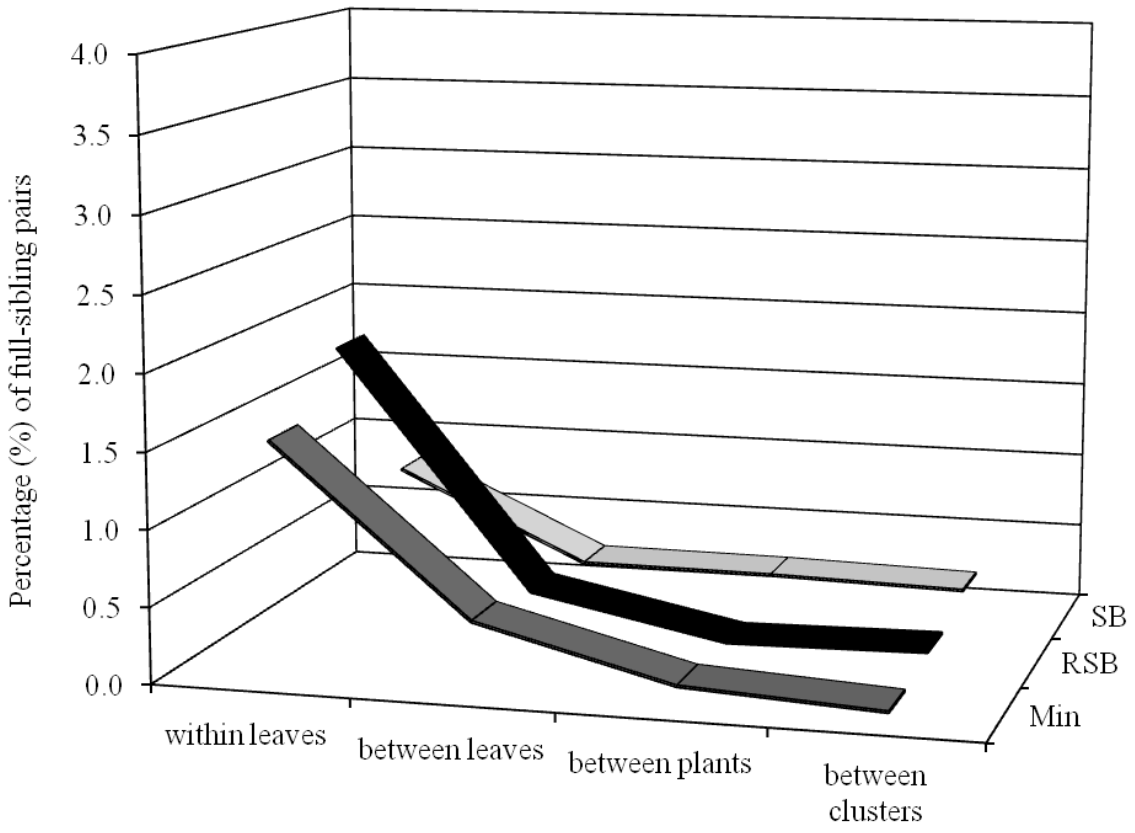


Figure 3. Percentage of full-sibling relationships among individuals within leaves, between leaves within plants, between plants within clusters, and between clusters within Minor Lake (Min), Roadside (RSB), and Spruce (SB) bogs for System 1.

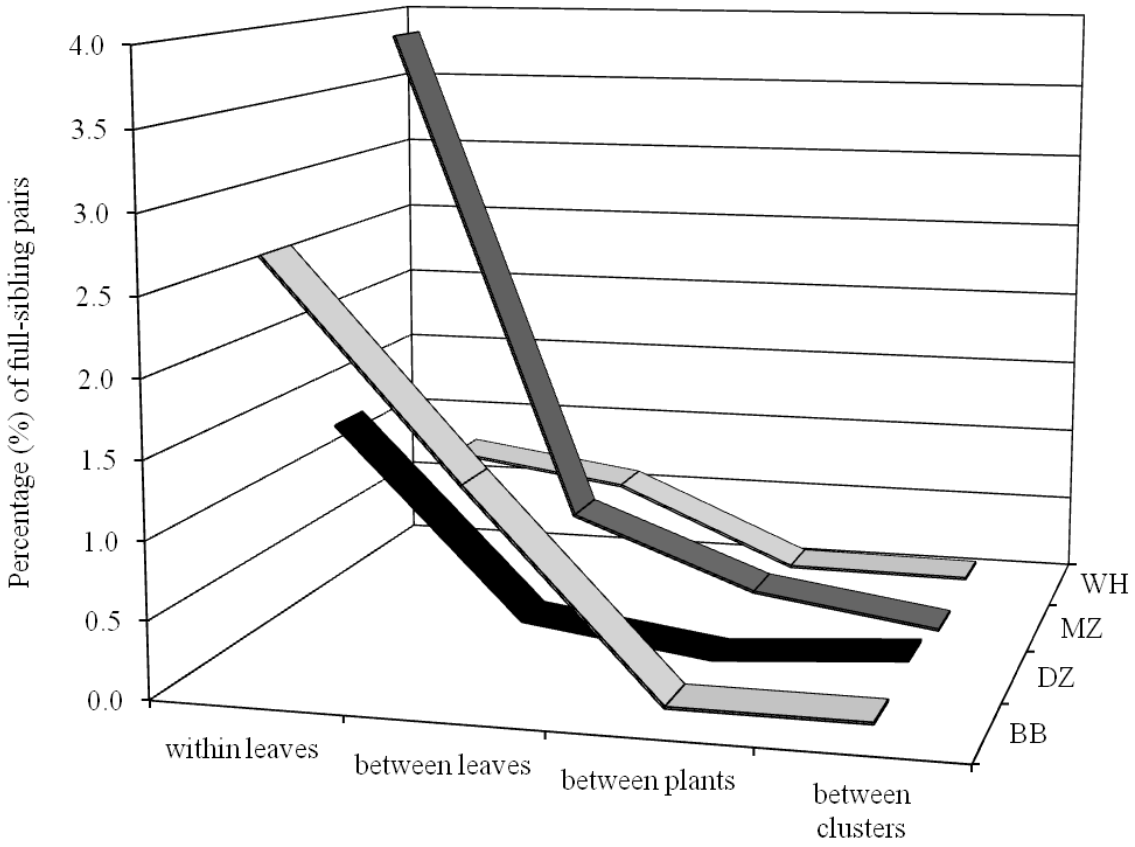


Figure 4. Percentage of full-sibling relationships among individuals within leaves, between leaves within plants, between plants within clusters, and between clusters within Buggy (BB), Dizzy Lake (DZ), Mizzy Lake (MZ), and Wolf Howl (WH) bogs for System 2.

Hierarchical *F*-statistics (i.e., AMOVA) in both systems were significant ($p < 0.01$) at all spatial scales. The highest values of variance components were measured among individuals, which is commonly observed in microsatellite data (Hedrick 1999; Tables 4, 5). F_{ST} values at the individual level are similar to inbreeding coefficients, and negative values imply individual genotypes are highly heterozygous (Table 5). At the plant scale, 29.23% of F_{ST} values computed among leaves were significantly greater than zero ($p < 0.05$) and ranged from –0.060 to 0.231. At the cluster scale, 72.22% of F_{ST}

Table 4. Summary of hierarchical analysis of variance components among the spatial scales in System 1 and System 2.

System	Bog	Cluster	Plant	Leaf	Individual	Error
1	0.1372	0.2386	0.0213	0.1332	–0.4075	5.9603
2	0.0317	0.2178	0.0741	0.1452	–0.3904	5.7487

Table 5. Matrix of hierarchical *F*-statistics among bogs, clusters, plants, and leaves within System 1 (SYS1) and System 2 (SYS2). Values represent F_{ST} values among the 'column' scale within the 'row' scale. Statistical significance of values along the diagonal were obtained by permutating whole units of the scale below within the scale of interest, while maintaining the nested structure of the scales above. For example, in the plant column, whole units of the leaf were permutated among plants, but retained within respective clusters (all values $p < 0.01$).

	Bog		Cluster		Plant		Leaf		Individual	
	SYS1	SYS2	SYS1	SYS2	SYS1	SYS2	SYS1	SYS2	SYS1	SYS2
Total	0.0211	0.0054								
Bog			0.0391	0.0375						
Cluster					0.0035	0.0132				
Plant							0.0219	0.0264		
Leaf									–0.0794	–0.0728

values among plants were significantly greater than zero and ranged from –0.009 to 0.086, and 85.71% of F_{ST} values among clusters at the bog scale were significantly greater than zero and ranged from 0.082 to 0.103.

3.2 Habitat structure

Habitat structure was assessed at each spatial scale using variables that represented independent measures of habitat composition and configuration (Table 6).

Table 6. Summary of habitat metrics measured at the plant, cluster, and bog scales with mean, minimum, and maximum recorded values.

Scale	Metric	Mean	Min.	Max.
Plant	S_{lf}	32.01 mm	24.33 mm	44.67 mm
	I_{lf}	11.28 cm	4.17 cm	31.33 cm
	I_{pl}	3.42 m	1.00 m	8.28 m
	I_{cl}	17.07	1.00	53.00
	I_{bog}	250.54 m	131.52 m	532.11 m
	A_{pl}	8.05	4.00	17.00
	A_{cl}	120.60	15.00	346.00
	A_{bog}	32,014.46 m^2	2,046.44 m^2	77,983.01 m^2
Cluster	S_{pl}	8.03	4.67	11.33
	I_{pl3}	4.34 m	1.82 m	7.32 m
	I_{cl}	17.16	1.00	53.00
	I_{bog}	251.81 m	131.52 m	532.11 m
	A_{cl}	121.74	15.00	346.00
	A_{bog}	31,520.17 m^2	2,046.44 m^2	77,983.01 m^2
Bog	S_{cl}	122.95	46.99	217.77
	I_{cl3}	45.99 m	19.53 m	91.14 m
	I_{bog}	262.64 m	131.52 m	532.11 m
	A_{bog}	28,649.93 m^2	2,046.44 m^2	77,983.01 m^2

3.3 Model selection

Inter-correlation analysis among predictor variables in the plant, cluster, and bog datasets did not recover strong ($r > 0.6$) correlations (Tables A7-A9). Within the plant scale model set of estimated F_{ST} values among leaves, no single model had a high probability of being the 'best', as five models were within $\Delta AIC_c < 2$ and Akaike weight (w_i) ranged from 0.062 to 0.023 (Table 7). The cumulative sum of the Akaike model weights (0.172) among the top five models suggests considerable model uncertainty. All models within the top-ranking set ($\Delta AIC_c < 2$) contained predictors for the amount of habitat in the surrounding bog (A_{bog}) and bog isolation (I_{bog}), while leaf isolation (I_{lf}) appeared in three models, cluster isolation (I_{cl}) appeared in two models, and the amount of habitat in the plant (A_{pl}) and the patch size of the leaf (S_{lf}) were each in one of the top five models (Table 7). Model-averaging of all models in the plant scale set indicates that A_{bog} and I_{bog} had the highest relative variable importance with model-averaged parameter weights ($w_+(i)$) of 0.840 and 0.613, respectively (Table 8; Fig. 5). Although A_{bog} and I_{bog} may be considered the most important predictors of genetic differentiation of *M. knabi* calculated at the leaf scale, their estimated effect as well as the effect of all other plant scale predictor variables was not significant at $\alpha = 0.05$ (Tables 9, 10; Fig. 6). However, the effect of A_{bog} at the plant scale was significant at $\alpha = 0.10$ (Tables 9; Fig.6).

Analysis at the plant scale using binomial F_{ST} (F_{STbin}) values recovered nine top models ($\Delta AIC_c < 2$) with w_i range = 0.042 – 0.016 and cumulative w_i sum = 0.236 (Table 7). The best model in the set was the random intercept, while S_{lf} was present in three of the top nine models, I_{bog}, I_{lf}, and I_{cl} were each present in two models, and plant isolation (I_{pl}) was present in one of the top models. Model-averaging among all models in the

Table 7. Summary of model selection statistics for candidate models at the plant, cluster, and bog scales, with log likelihood (logLik) statistics, corrected Akaike information criterion (AIC_c), $\Delta_i AIC_c$, and Akaike weights (w_i). Models are ranked according to AIC_c and may be compared by $\Delta_i AIC_c$. Models with $\Delta_i AIC_c < 4$ are presented and all models include nested random effects. Variables with colons denote interaction terms between patch isolation (I) and amount of habitat (A) metrics.

Model	logLik	AIC_c	$\Delta_i AIC_c$	w_i
Plant scale				
$I_{lf} + A_{bog} + I_{bog}$	118.078	–251.957	0.000	0.062
$I_{cl} + A_{bog} + I_{bog}$	120.712	–250.849	1.108	0.036
$I_{lf} + I_{cl} + A_{bog} + I_{bog}$	114.341	–250.179	1.778	0.026
$S_{lf} + A_{bog} + I_{bog}$	117.346	–250.121	1.836	0.025
$I_{lf} + A_{pl} + A_{bog} + I_{bog}$	114.056	–249.967	1.990	0.023
$S_{lf} + I_{lf} + A_{bog} + I_{bog}$	114.225	–249.940	2.017	0.023
$I_{lf} + I_{pl} + A_{bog} + I_{bog}$	113.796	–249.479	2.478	0.018
$I_{lf} + A_{cl} + A_{bog} + I_{bog}$	113.931	–249.320	2.637	0.017
$A_{pl} + A_{bog} + I_{bog}$	116.820	–249.225	2.732	0.016
$I_{cl} + A_{bog} + I_{bog}$	117.074	–249.211	2.746	0.016
$I_{lf} + A_{bog}$	120.221	–249.018	2.939	0.014
$I_{lf} + I_{cl} + A_{bog}$	116.981	–248.900	3.057	0.013
$I_{cl} + A_{bog}$	119.811	–248.577	3.380	0.011
$I_{pl} + A_{bog} + I_{bog}$	116.420	–248.480	3.477	0.011
$A_{cl} + I_{cl} + A_{bog}$	116.729	–248.466	3.491	0.011
A_{bog}	122.968	–248.436	3.521	0.011
$A_{cl} + A_{bog} + I_{bog}$	116.639	–248.399	3.558	0.011
$S_{lf} + A_{pl} + A_{bog} + I_{bog}$	113.433	–248.382	3.575	0.010
$S_{llf} + I_{cl} + A_{bog} + I_{bog}$	113.623	–248.285	3.672	0.010
$I_{lf} + I_{cl} + A_{bog} + I_{bog} + I_{cl}{:}A_{bog}$	110.688	–248.260	3.697	0.010
$I_{lf} + A_{pl} + I_{cl} + A_{bog} + I_{bog}$	110.292	–248.096	3.861	0.009
$S_{lf} + I_{lf} + I_{cl} + A_{bog} + I_{bog}$	110.461	–248.036	3.921	0.008
Plant scale ($F_{STbin.}$*)*				
Intercept	–44.557	97.620	0.000	0.042
S_{lf}	–43.611	97.991	0.371	0.035
I_{bog}	–43.644	98.057	0.437	0.034
$S_{lf} + I_{bog}$	–42.789	98.669	1.048	0.025
I_{lf}	–43.978	98.725	1.105	0.024
I_{cl}	–44.086	98.940	1.320	0.022
$I_{lf} + I_{bog}$	–43.127	99.345	1.724	0.018
I_{pl}	–44.310	99.389	1.769	0.017

Model	logLik	AIC_c	$\Delta_i AIC_c$	w_i
$S_{lf} + I_{cl}$	–43.201	99.492	1.872	0.016
$S_{lf} + I_{pl}$	–43.290	99.671	2.051	0.015
A_{cl}	–44.463	99.696	2.076	0.014
A_{pl}	–44.541	99.850	2.230	0.014
A_{bog}	–44.556	99.881	2.261	0.013
$S_{lf} + I_{lf}$	–43.440	99.971	2.350	0.013
$I_{cl} + I_{bog}$	–43.482	100.055	2.435	0.012
$I_{pl} + I_{bog}$	–43.490	100.071	2.451	0.012
$I_{lf} + I_{cl}$	–43.522	100.134	2.514	0.012
$Sl_f + A_{cl}$	–43.576	100.244	2.623	0.011
$S_{lf} + A_{bog}$	–43.579	100.248	2.628	0.011
$A_{bog} + I_{bog}$	–43.585	100.261	2.640	0.011
$A_{pl} + I_{bog}$	–43.603	100.298	2.677	0.011
$S_{lf} + A_{pl}$	–43.605	100.301	2.681	0.011
$A_{cl} + I_{bog}$	–43.643	100.376	2.756	0.010
$S_{lf} + I_{pl} + I_{bog}$	–42.545	100.564	2.943	0.010
$I_{lf} + I_{pl}$	–43.777	100.645	3.024	0.009
$S_{lf} + A_{bog} + I_{bog}$	–42.586	100.646	3.025	0.009
$A_{cl} + I_{cl}$	–43.778	100.646	3.026	0.009
$S_{lf} + I_{cl} + I_{bog}$	–42.635	100.743	3.122	0.009
$S_{lf} + I_{lf} + I_{bog}$	–42.635	100.744	3.124	0.009
$I_{pl} + I_{cl}$	–43.892	100.874	3.254	0.008
$S_{lf} + A_{pl} + I_{bog}$	–42.728	100.930	3.309	0.008
$I_{lf} + A_{bog}$	–43.935	100.960	3.340	0.008
$I_{lf} + A_{cl}$	–43.937	100.964	3.344	0.008
$I_{lf} + A_{pl}$	–43.952	100.994	3.374	0.008
$S_{lf} + A_{cl} + I_{bog}$	–42.781	101.035	3.414	0.008
$A_{pl} + I_{cl}$	–44.076	101.242	3.622	0.007
$I_{cl} + A_{bog}$	–44.077	101.246	3.625	0.007
$I_{lf} + A_{bog} + I_{bog}$	–42.892	101.258	3.637	0.007
$S_{lf} + I_{pl} + I_{cl}$	–42.940	101.354	3.733	0.006
$I_{lf} + I_{cl} + I_{bog}$	–42.979	101.432	3.812	0.006
$I_{lf} + I_{pl} + I_{bog}$	–42.999	101.472	3.852	0.006
$S_{lf} + I_{lf} + I_{cl}$	–43.011	101.495	3.874	0.006
$S_{lf} + A_{cl} + I_{cl}$	–43.019	101.512	3.892	0.006
$I_{pl} + A_{cl}$	–44.231	101.553	3.932	0.006
Cluster scale				
$A_{bog} + I_{bog}$	69.429	–152.200	0.000	0.324
$I_{cla} + A_{bog} + I_{bog}$	64.842	–149.651	2.549	0.090
$A_{cl} + A_{bog} + I_{bog}$	64.890	–149.221	2.978	0.073
$I_{pl3} + A_{bog} + I_{bog}$	64.587	–148.950	3.250	0.064
$S_{pl} + A_{bog} + I_{bog}$	64.857	–148.862	3.337	0.061

Model	logLik	AIC_c	$\Delta_i AIC_c$	w_i
Bog scale				
$I_{cl3} + A_{bog} + I_{bog} + I_{cl3}:A_{bog}$	2.347	–149.508	0.000	0.859
$S_{cl} + I_{cl3} + A_{bog} + I_{cl3}:A_{bog}$	1.792	–145.892	3.616	0.141

Table 8. Relative variable importance ($w_+(i)$) of each predictor variable in the plant, cluster, and bog scale model sets. Values at the plant and cluster scales were averaged across all models in each data set. Values at the bog scale were determined using re-calculated model-averaged Akaike weights from the top two models. Variables with colons denote interaction terms between patch isolation (I) and amount of habitat (A) metrics.

Predictor variable	$w_+(i)$	Predictor variable	$w_+(i)$
Plant scale		*Plant scale (*$F_{STbin.}$*)*	
A_{bog}	0.840	S_{lf}	0.400
I_{bog}	0.613	I_{bog}	0.393
I_{lf}	0.517	I_{lf}	0.329
I_{cl}	0.455	I_{cl}	0.321
A_{cl}	0.329	I_{pl}	0.285
A_{pl}	0.310	A_{bog}	0.269
S_{lf}	0.300	A_{cl}	0.261
I_{pl}	0.272	A_{pl}	0.252
I_{cl}:A_{bog}	0.101	I_{cl}:A_{bog}	0.032
I_{pl}:A_{cl}	0.057	I_{lf}:A_{pl}	0.023
I_{lf}:A_{pl}	0.035	I_{pl}:A_{cl}	0.019
Cluster scale			
A_{bog}	0.815		
I_{bog}	0.775		
I_{cl}	0.234		
A_{cl}	0.175		
I_{pl3}	0.166		
S_{pl}	0.158		
I_{cl}:A_{bog}	0.029		
I_{pl3}:A_{cl}	0.009		
Bog scale			
A_{bog}	1.000		
I_{cl3}	1.000		
I_{cl3}:A_{bog}	1.000		
I_{bog}	0.859		
S_{cl}	0.141		

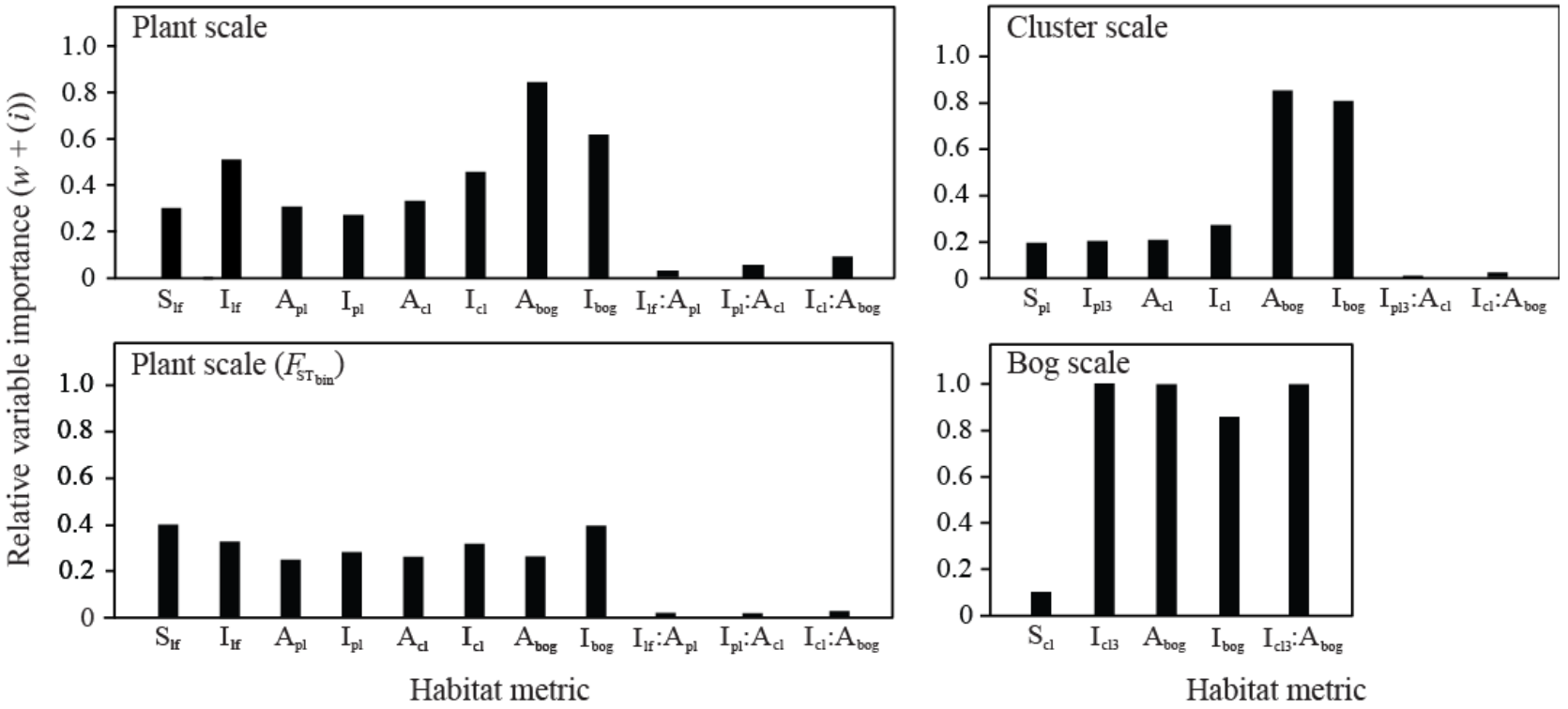


Figure 5. Model-averaged relative variable importance ($w_+(i)$) for each parameter in the plant, cluster, and bog scale models. Variables with colons denote interaction terms between patch isolation (I) and amount of habitat (A) metrics. The importance of plant and cluster scale parameters were averaged across all models in each model set and bog scale parameters were averaged across the top two models.

Table 9. Model-averaged Akaike weights ($w_+(i)$), parameter estimates ($\hat{\bar{\beta}}_j$), and standard errors (SE) for metrics of habitat composition at the plant, cluster, and bog scales. At the plant and cluster scales, Akaike weights and parameter estimates were averaged across all models in the model set, whereas values at the bog scale were calculated using the top two models. Parameter estimates noted in bold represent significant values where unconditional 90% confidence intervals exclude zero, and parameter estimates noted with an asterisk (*) represent significant values where unconditional 95% confidence intervals exclude zero.

Scale	Parameter	$w_+(i)$	$\hat{\bar{\beta}}_j$	SE
Plant	A_{pl}	0.310	0.005	0.006
	A_{cl}	0.329	–0.005	0.007
	A_{bog}	0.840	**0.014**	0.008
Plant (F_{STbin})	A_{pl}	0.252	0.004	0.295
	A_{cl}	0.261	–0.104	0.361
	A_{bog}	0.269	0.103	0.355
Cluster	A_{cl}	0.175	0.001	0.003
	A_{bog}	0.815	0.008	0.005
Bog	A_{bog}	1.000	**–0.014**	0.008

Table 10. Model-averaged Akaike weights ($w_+(i)$), parameter estimates ($\hat{\bar{\beta}}_j$), and standard errors (SE) for metrics of habitat configuration (i.e., patch size and patch isolation) at the plant, cluster, and bog scales. At the plant and cluster scales, Akaike weights and parameter estimates were averaged across all models in the model set, whereas values at the bog scale were calculated using the top two models. Parameter estimates noted in bold represent significant values where unconditional 90% confidence intervals exclude zero, and parameter estimates noted with an asterisk (*) represent significant values where unconditional 95% confidence intervals exclude zero.

	Patch size				Patch isolation			
Scale	Parameter	$w_+(i)$	$\hat{\bar{\beta}}_j$	SE	Parameter	$w_+(i)$	$\hat{\bar{\beta}}_j$	SE
Plant	S_{lf}	0.300	–0.006	0.006	I_{lf}	0.517	–0.009	0.006
					I_{pl}	0.272	–0.002	0.005
					I_{cl}	0.455	0.009	0.007
					I_{bog}	0.613	0.008	0.009
Plant (F_{STbin})	S_{lf}	0.400	–0.419	0.341	I_{lf}	0.329	–0.318	0.376
					I_{pl}	0.285	–0.192	0.301
					I_{cl}	0.321	0.248	0.304
					I_{bog}	0.393	0.365	0.286
Cluster	S_{pl}	0.158	0.000	0.004	I_{pl3}	0.166	0.000	0.003
					I_{cl}	0.234	0.003	0.003
					I_{bog}	0.775	0.007	0.005
Bog	S_{cl}	0.014	0.001	0.006	I_{cl3}	1.000	**0.036***	0.007
					I_{bog}	0.859	–0.006	0.005

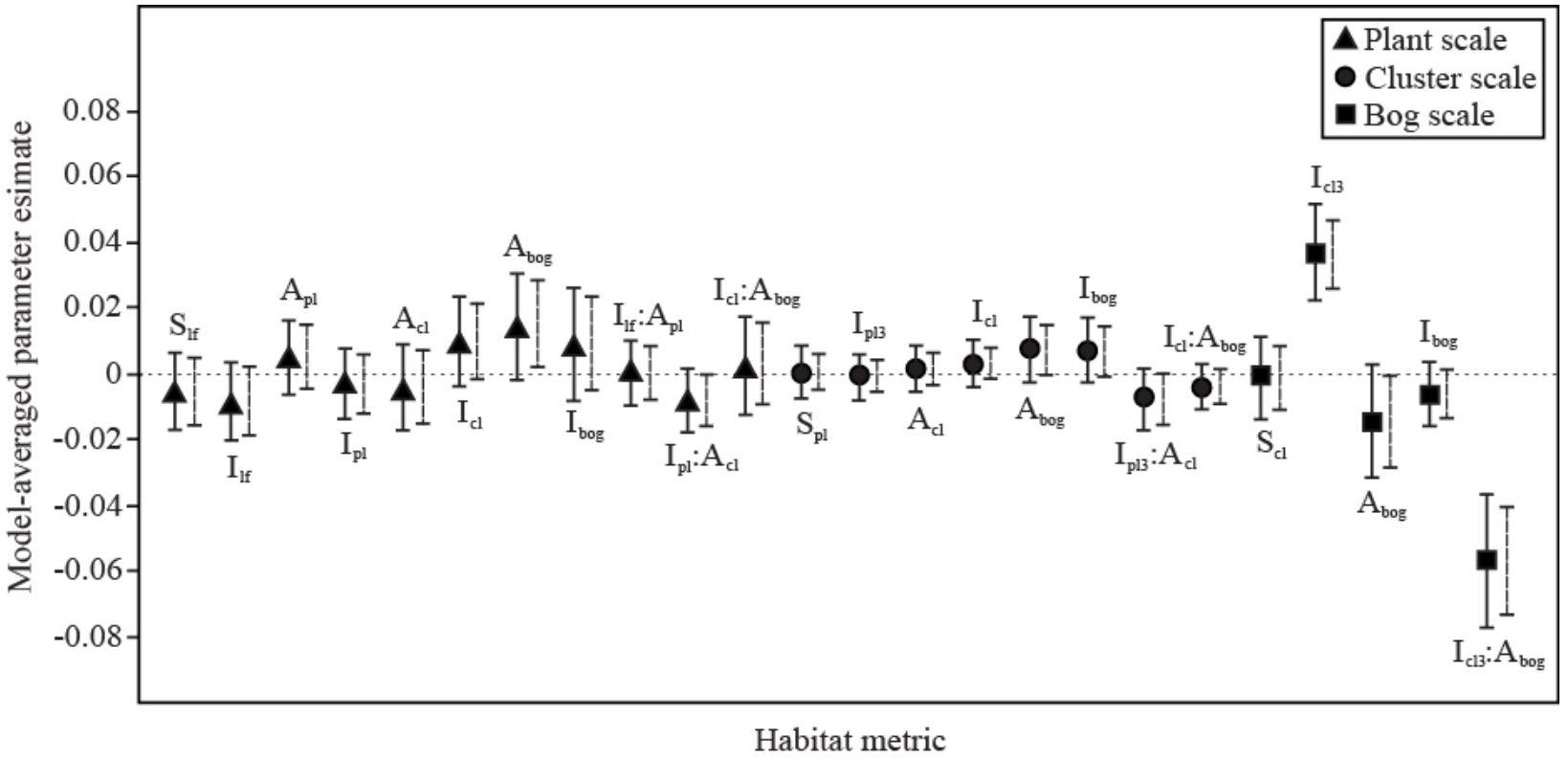


Figure 6. Model-averaged parameter estimates ($\hat{\bar{\beta}}_j$) for predictor variables at the plant, cluster, and bog scales. Points are the averaged coefficients from all models in the plant and cluster scale models sets and top two models in the bog scale model set. Broken-line and solid error bars represent the associated unconditional 90% and 95% confidence limits, respectively. Variables with colons denote interaction terms between patch isolation (I) and amount of habitat (A) metrics.

binomial F_{ST} plant scale data set indicates that S_{lf}, I_{bog}, I_{lf}, I_{cl} have relatively equal importance ($w_+(i)$ = 0.400-0.321; Table 8; Fig. 5). The estimated effect of each parameter on binomial F_{ST} values was non-significant (Tables 9-11).

Table 11. Model-averaged Akaike weights ($w_+(i)$), parameter estimates ($\hat{\bar{\beta}}_j$), and standard errors (SE) for interaction metrics of patch isolation (I) and the amount of habitat (A) at the plant, cluster, and bog scales. At the plant and cluster scales, Akaike weights and parameter estimates were averaged across all models in the model set, whereas values at the bog scale were calculated using the top two models. Parameter estimates noted in bold represent significant values where unconditional 90% confidence intervals exclude zero, and parameter estimates noted with an asterisk (*) represent significant values where unconditional 95% confidence intervals exclude zero.

Scale	Parameter	$w_+(i)$	$\hat{\bar{\beta}}_j$	SE
Plant	I_{lf}:A_{pl}	0.035	0.000	0.005
	I_{pl}:A_{cl}	0.058	–0.008	0.005
	I_{cl}:A_{bog}	0.101	0.002	0.007
Plant (F_{STbin})	I_{lf}:A_{pl}	0.023	0.139	0.298
	I_{pl}:A_{cl}	0.019	–0.094	0.324
	$I_{cl:}A_{bog}$	0.032	0.343	0.363
Cluster	I_{pl3}:A_{cl}	0.009	–0.007	0.004
	I_{cl}:A_{bog}	0.029	–0.004	0.003
Bog	$I_{cl3:}A_{bog}$	1.000	**–0.056***	0.010

At the cluster scale, a single model containing A_{bog} and I_{bog} predictors was ranked the top model according to $\Delta AIC_c < 2$ values and w_i = 0.324 (Table 7). Interestingly, all models within $\Delta AIC_c < 4$ contained A_{bog} and I_{bog} and had a cumulative Akaike weight sum of 0.661. Model-averaged Akaike weights of the predictor variables indicate that

A_{bog} ($w_+(i)$ = 0.815) and I_{bog} ($w_+(i)$ = 0.775) had the highest relative importance (Table 8; Fig. 5) although the effects of parameters on *M. knabi* F_{ST} values at the cluster scale were not significant (Tables 9, 10; Fig. 6).

At the bog scale, a model including cluster isolation (I_{cl3}), A_{bog}, I_{bog}, and an interaction among the isolation of the cluster and the amount of habitat in the bog (I_{cl3}:A_{bog}) predictor variables was identified as the best model, with Akaike weight of 0.859 (Table 7). The second-ranked model had an ΔAIC_c of 3.616 and Akaike weight of 0.141, and included patch size of the cluster (S_{cl}), I_{cl3}, A_{bog}, and I_{cl3}:A_{bog} predictor variables. Considering the strong support for the top model, the cumulative Akaike weight sum of the top two models (1.000), and the similarity of their predictor variables, model-averaging was conducted on the top two models. Relative variable importance indicates that A_{bog}, I_{cl3}, and I_{cl3}:A_{bog} have equally high parameter importance ($w_+(i)$ = 1.000), followed by Ibog ($w_+(i)$ = 0.859; Table 8; Fig. 5). The estimated effect of A_{bog} ($\hat{\bar{\beta}}_j$ = –0.014) on F_{ST} values was significant at $\alpha = 0.10$, while the estimated effects of I_{cl3} ($\hat{\bar{\beta}}_j$ = 0.036) and I_{cl3}:A_{bog} ($\hat{\bar{\beta}}_j$ = –0.056) on F_{ST} values were significant at $\alpha = 0.05$ (Tables 9-11; Fig. 6).

Chapter 4.0

Discussion

In this study, I examined the relative importance and effect of aspects of habitat composition and habitat configuration on patterns of genetic differentiation in *M. knabi* larvae at three spatial scales (plant, cluster, bog). Multi-model inference indicates that both habitat composition and configuration affect the genetic structure of *M. knabi*, and the relative importance of the amount of habitat in the landscape (A), patch size (S), and patch isolation (I) varies with the scale of analysis.

4.1 Plant scale patterns and processes

The plant scale represents the finest spatial scale of habitat for *M. knabi* considering that the leaf is the smallest measureable habitat unit. Hierarchical AMOVA among leaves within plants revealed that plants contain moderate levels of genetic variance and that there is significant differentiation even among leaves within a single plant (Tables 4, 5). Significant genetic structure detected among leaves within plants, despite the extremely small average distance between leaves (11.28 cm) (Table 6), is very likely to be driven by the occurrence of highly related individuals, or family groups, within the leaf samples (Anderson and Dunham 2008; Goldberg and Waits 2010). Indeed, I found that individuals sampled from the same leaf were on average approximately three times more likely to be full-siblings than individuals sampled from different leaves, and 16 and 21 times more likely to be full-siblings than individuals sampled from different plants and clusters, after removing comparisons of individuals at the lower spatial scales (Figs. 3, 4). These findings corroborate previous work indicating that leaves harbour the highest

proportion of full sibling relationships relative to the plant, cluster, and bog scales (Rasic and Keyghobadi 2012).

Despite the difficulty of directly observing adult *M. knabi* behaviour in the field, the distribution of full-sibling pairs can serve as a proxy for female oviposition behaviour. Based on the highly clumped distribution of full siblings, I infer oviposition behaviour to be the dominant process influencing genetic structure among leaves. As such, one might expect that leaf patch size (S_{lf}) would have the strongest relative parameter importance at this scale. The average distance among the leaves (I_{lf}) would not be expected to influence genetic structure as the distance among leaves is most likely well within the dispersal ability of adult *M. knabi* (Wiens 1972; Krawchuk and Taylor 2003). All else being equal, however, larger leaves and more leaves per plant would accommodate more larvae. It has been suggested that leaf size determines habitat accessibility, and that large leaves are also selected by adults because large leaves tend to capture more insects and provide more resources for the developing larvae (Wolf 1981; Naeem 1988; Cresswell 1993; Heard 1998; Krawchuk and Taylor 2003; Trzcinski et al. 2003). Therefore, one might expect that female midges would cluster their eggs within the preferred leaves, leading to larger family groups within the largest leaves, and consequently high levels of genetic differentiation among those leaves. On the other hand, previous studies suggest that the tendency for female pitcher plant midges to be 'choosy' and to cluster their eggs within single leaves is highest where pitcher plants are sparse (Trzcinski et al. 2003; Rasic and Keyghobadi 2012). My analyses corroborate these latter findings, as multi-model inference identified the amount of habitat in the surrounding bog (A_{bog}) and bog isolation (I_{bog}) as the most important predictor variables

of F_{ST} at the plant scale, rather than S_{lf} or any other plant-scale variables. These results indicate that broad scale habitat structure influences female oviposition and therefore genetic differentiation patterns at fine scales.

The positive relationship with A_{bog} and F_{ST}, and A_{bog} and F_{STbin} suggests that genetic differentiation among leaves in plants varies with the amount of habitat in the surrounding bog, manifested as increased genetic structure among leaves in large bogs (Tables 9, 10; Fig. 6). The marginally significant and positive coefficient of I_{bog} suggests that F_{ST} among leaves increases with bog isolation (Table 10). In another genetic study, plant density within bogs and isolation of clusters explained a large proportion of the variation in pairwise genetic distances among *M. knabi* samples from leaves (Rasic and Keyghobadi 2012). Given the strict habitat requirements for developing larvae, it seems advantageous for females to evaluate habitat structure at more than one spatial scale and be selective of larval habitat during egg-laying, and it was suggested that low plant density and elevated isolation at the bog scale may lead females to aggregate their eggs at fine scales, within leaves (Rasic and Keyghobadi 2012). While the positive effect of I_{bog} recovered in my study supports this interpretation, the positive effect of A_{bog} appears contradictory. However, it is very likely that the discordance is an artefact of A_{bog} being inversely related to pitcher plant density. While the total number of available pitcher plants increases with bog size (A_{bog}), the density of plants (number per unit area) actually declines, and density rather than total number of plants appears to be the key factor influencing *M. knabi* oviposition (Rasic and Keyghobadi 2012). Therefore, the density of pitcher plants within the bog may be an alternative or additional bog-scale predictor that could be included in the statistical models.

By converting estimated F_{ST} data to F_{STbin}, I aimed to remove falsely inflated F_{ST} values resulting from the stochasticity of small sample sizes, and to assess a less variable genetic response against habitat metrics. However, model averaging results of F_{STbin} analysis indicated that habitat metrics were uninformative predictors of genetic differentiation (Table 8; Figs. 5, 6). The discordance among estimated F_{ST} and F_{STbin} results may be driven by the binomial response values being too coarse. Although the estimated F_{ST} values among leaves may be somewhat noisy and stochastic, there is meaningful information in the observed variation of estimated F_{ST} values that is lost when collapsed into a binomial response. The fact that conclusions similar to those I obtained using the estimated F_{ST} values, regarding the influence of broad-scale habitat variables on differentiation of midge samples taken from leaves, were reached by Rasic and Keyghobadi (2012), using different data sets and analyses, offers support for the importance of the amount of habitat in the surrounding bog, and bog isolation, on estimates of population genetic structure at the plant scale.

Additional metrics of habitat composition and configuration at the plant scale may potentially be important predictors of genetic structure among leaves. The actual amount of fluid within pitcher leaves rather than the leaf's potential volume may be a better metric of patch size. In comparisons of pitcher plant characteristics (e.g., pitcher age, pitcher size, maximum and actual fluid volume, hood size, degree of red venation), the actual amount of fluid was most positively correlated with midge abundance (Nastase et al. 1995). However, pitcher plant characteristics do not seem to be the only relevant leaf factor influencing midge abundance as pitcher plant characteristics explained less than half of the abundance variation (Nastase et al. 1995). Additional evidence suggests that

the pitcher leaf community, including interactions between *M. knabi*, *F. fletcheri*, and *W. smithii* larvae, bacteria, protists, as well as the level of detritus, chemical cues, and wind exposure may affect the likelihood of female oviposition and/or the survivability of *M. knabi* larvae (Paterson and Cameron 1982; Bradshaw 1983; Istock et al. 1983; Heard 1994a; Heard 1994b; Nastase et al. 1995; Harvey and Miller 1996; Trzcinski et al. 2003). The relevance of these additional factors to genetic structure among leaves deserves further testing.

In summary, I infer that female oviposition behaviour appears to be the primary process determining patterns of genetic differentiation at the plant scale, and this in turn may be influenced by broad-scale habitat composition (A_{bog}) and configuration metrics (I_{bog}).

4.2 Cluster scale patterns and processes

In this study, the cluster scale represents an intermediate spatial scale where genetic drift, oviposition behaviour, and dispersal may all interact and affect patterns of genetic structure. Adults colonize *S. purpurea* plants through oviposition, and abundance patterns are determined by this process and any subsequent mortality of developing larvae (Trzcinski et al. 2003). The number of individuals within plants may also be affected by fine-scale factors, including leaf size, and the presence/absence of other taxa or captured prey (Paterson and Cameron 1982; Bradshaw 1983). Owing to fluctuations and variation in plant occupancy and population sizes, the genetic structure of larvae among plants in clusters may be strongly influenced by genetic drift. Indeed this is supported by the low level of allelic richness (3.100 – 4.296) and wide range of observed and expected

heterozygosities (H_O = 0.371 – 0.636; H_E = 0.361 – 0.548) of larvae among plants. A low proportion of full-sibling pairs were measured between plants within clusters, but this was still higher than the proportion of full siblings observed among clusters within bogs (Figs. 3, 4). This suggests that females will sometimes move among plants within clusters when laying their eggs, and that female oviposition may have some influence on genetic differentiation at the cluster scale. Movement distances have not been directly observed in *M. knabi*, although low variance component values in larval abundance data at the cluster scale suggest that individual midges have limited dispersal potential, and aggregate around plants and clusters (Krawchuk and Taylor 2003). Thus, it is possible that dispersal and gene flow among plants within clusters might be limited. However, I found that in System 1, the cluster scale contained the lowest genetic variance component (0.0213) and hierarchical *F*-statistic values (0.0035), while in System 2, the cluster scale variance component (0.0741) and *F*-statistic values (0.0132) were the second lowest among the spatial scales (Tables 4, 5). These results indicate lower genetic structuring and differentiation of *M. knabi* among plants within clusters than among leaves in plants and clusters in bogs, and suggest that gene flow is not generally restricted among plants within clusters.

With an increase in spatial scale, habitat isolation (I_{pl3}) was expected to become a more important predictor of genetic structure relative to the plant scale, as gene flow becomes increasingly restricted among individuals from distant habitat patches. I also expected important effects of the amount of habitat in the cluster (A_{cl}) and an interaction between I_{pl3} and A_{cl}, as plant colonization relationships indicate that females respond to plant density and that the relationship weakens when there are many plants close together

(Trzcinski et al. 2003). However, multi-model inference identified the amount of habitat in the surrounding bog (A_{bog}) and bog isolation (I_{bog}) as the most important predictor variables of F_{ST} structure among plants at the cluster scale, and ascribed low importance to $I_{pl3,}$ A_{cl}, I_{pl3}:$A_{cl,}$ and S_{pl} parameters (Table 8; Fig. 5). Model-averaged parameter estimates of A_{bog} and I_{bog} were both positive for the response variables of F_{ST} among plants within clusters (Tables 9, 10; Fig. 6). Therefore, similar to the plant scale, habitat structure in the broader bog scale is a better predictor of genetic differentiation at the cluster scale, and genetic differentiation among plants may be greater in large and isolated bogs. Although I did not measure spatial genetic autocorrelation in this study, evidence from Rasic and Keyghobadi (2012) indicates that individuals sampled from within the same cluster are not genetically independent, and that distances among plants within clusters do not exceed the range of spatial genetic autocorrelation. In other words, gene flow between plants is not limited within the spatial extent of clusters. This is consistent with the low parameter importance of I_{pl3} and my observation that variance among plants within clusters was generally low relative to other scales. Genetic differentiation at the cluster scale is likely driven primarily by patterns of plant colonization by females and founder events. Much of female oviposition among leaves within plants is influenced by broad-scale habitat variables (Rasic & Keyghobadi 2012), and the high relative importance of A_{bog} and I_{bog} suggests colonization patterns among plants within clusters is similarly influenced by broad-scale variables.

In summary, I infer that dispersal and gene flow do not appear to be strongly limited at the cluster scale. Genetic structure at the cluster scale may be determined primarily by genetic drift, which reflects underlying population sizes, and demographic

processes, as well as oviposition behaviour. Broad-scale habitat composition (A_{bog}) and configuration (I_{bog}) metrics are the most important variables explaining genetic differentiation at the cluster scale, most likely because of their influence on oviposition.

4.3 Bog scale patterns and processes

In this study, the bog scale represents the largest spatial scale at which genetic differentiation among samples was estimated. Since clusters contained the lowest proportion of full-sibling pairs (Figs. 3, 4), female oviposition behaviour was not expected to be a significant process influencing gene differentiation among clusters within bogs. Genetic drift may affect F_{ST} values among clusters as allelic variation within clusters is driven by changes in the number of *M. knabi* larvae and the introduction of new or private alleles by colonizing females (i.e., founder effects). At this scale, dispersal and gene flow are also most likely to be limited because of the greater absolute distances among sampled patches. Overall, bog scale samples had the greatest proportion of significant F_{ST} values (85.71%), and the highest variance component and hierarchical *F*-statistics values, indicating greater genetic structure and differentiation among clusters than among leaves and among plants (Tables 4, 5). This supports the hypothesis that there is limited dispersal of *M. knabi* occurring at this scale, and that plant and cluster distances represent the extent of individual movement distances. It also suggests that patterns of genetic structure at this scale are driven by a balance between genetic drift and spatially constrained gene flow.

Since the distance among clusters (average distance = 46.0 m) (Table 6) is an order of magnitude greater than that among plants within a cluster, a stronger negative

effect of patch isolation was expected at the bog scale (i.e., among clusters). I also predicted a significant interaction effect of patch isolation with the amount of habitat in the bog (I_{cl3}:A_{bog}). Cluster patch size (S_{cl}) could also affect genetic drift by influencing the recruitment of individuals to an area, accommodating more individuals, and contributing to the long-term stability of residents. The relative importance of parameters indicates that A_{bog}, I_{cl3}, and I_{cl3}:A_{bog} were the strongest predictors of genetic differentiation among clusters (Table 8; Fig. 5). Interestingly, this is the only instance where a parameter of habitat structure measured at the same spatial scale at which genetic structure was analyzed was identified as a parameter of high importance. Cluster isolation (I_{cl3}) had a significantly positive effect on F_{ST} estimates, such that as the average distance among clusters increased, genetic differentiation increased (Table 10; Fig. 6). Greater genetic differentiation associated with greater patch isolation is analogous to isolation by distance, where genetic differentiation among groups increases with distance (Wright 1943, 1946, 1951). In analyses of midge abundance data, patch isolation was also found to be an important habitat metric at the bog scale (Krawchuk and Taylor 2003). The negative relationship between A_{bog} and F_{ST} values in this study suggests that the amount of habitat in the bog may buffer the posi effect of cluster isolation on F_{ST} estimates (Table 9; Fig. 6). Thresholds have been demonstrated where the influence of habitat configuration on the distribution or abundance of a species appears to increase strongly as the amount of habitat in the surrounding environment decreases (e.g., Fahrig 1997; Trzcinski et al. 1999; Villard et al. 1999; Smith et al. 2011). The significant effect of the interaction between I_{cl3} and A_{bog} (I_{cl3}:A_{bog}) makes it difficult to differentiate whether a similar phenomenon is occurring in estimates of F_{ST} structure, yet supports the idea that

habitat composition and configuration can interact simultaneously to influence patterns of genetic differentiation (Smith et al. 2011; Rasic and Keyghobadi 2012; Table 11; Fig. 6).

In summary, I infer that patterns of genetic differentiation at the bog scale are driven primarily by the processes of drift and spatially restricted gene flow. In accordance with factors that are likely to influence these processes, cluster isolation, bog size and their interaction are the most important habitat composition and configuration variables affecting patterns of genetic differentiation at the bog scale.

4.4 Composition versus configuration in genetic studies

There are a considerable number of studies and an equal amount of debate focused on the relative importance of habitat composition and habitat configuration for ecological processes. Many empirical studies, mostly on bird species, generally suggest that habitat loss has a stronger effect on species occupancy, distribution, and abundance than configuration, except when considerable amounts of habitat are lost (reviewed in Fahrig 2003; Cushman and McGarigal 2004). In contrast, simulation modeling approaches to quantify the influence of habitat area and configuration on genetic structure conclude that habitat configuration is more important than habitat area in predicting genetic differentiation (Bruggeman et al. 2010; Cushman et al. 2012) and that patch characteristics such as patch cohesion, correlation length, and aggregation index are among the strongest individual predictor variables of genetic structure (Cushman et al. 2012).

Simulation studies can allow unique insights into the effects of habitat composition and configuration on genetic structure, through precise manipulation of

landscape variables and tests of their relative effects on genetic differentiation. However, despite the use of simulated landscapes, Cushman et al. (2012) indicate that the configuration metrics used in their analyses were still correlated with habitat amount, making it impossible to separate formally and unequivocally the relative influences of habitat area and configuration on genetic differentiation. The authors maintain that configuration has more influence on genetic differentiation because the magnitudes of marginal and independent explained variance were highest for configuration metrics (Cushman et al. 2012). In addition, simulation studies may suffer from lack of realism. For example, population size remained fixed throughout the simulation experiment of Cushman et al. (2012) such that the effect of differential rates of genetic drift on the genetic differentiation was not included. Furthermore, a sufficiently large number of generations were simulated to ensure that migration-drift equilibrium was reached (Cushman et al. 2012). However, stable environmental conditions required to reach equilibrium may rarely be achieved within (i.e., inbreeding, genetic drift) and between (i.e., gene flow) sample units (Nei 1986; Whitlock 1992) in nature. Habitats that have been recently colonized or undergone a recent population bottleneck may not have had sufficient time for migration-drift equilibrium to be reached, making F_{ST} estimates biased toward previous population conditions (Whitlock and McCauley 1999).

My study is novel in attempting empirically to measure the influence and interaction of independent aspects of habitat composition and configuration on genetic differentiation. My work is quite different in approach from previous simulation work (Cushman et al. 2012) in that I did not experimentally manipulate habitat composition and configuration metrics. Indeed, such empirical experiments would be extremely

difficult to conduct, even in a laboratory setting, because of the long time frames required for spatial genetic patterns to establish. Furthermore, it is also very difficult to manipulate habitat composition and configuration truly independently, even with simulated landscapes, as suggested by Cushman et al. (2012). Nonetheless, I was able to assess the relative influence of some independent aspects of habitat composition and configuration in a natural system, subject to variable population size and potentially non-equilibrium conditions.

4.5 Conclusion

By investigating the effect of habitat structure at more than one spatial scale, I aimed to evaluate whether the relative importance of habitat composition and configuration was scale-dependent. A key finding of my study is that the amount of habitat and patch isolation of habitat structure in the surrounding landscape are consistently strong predictors of genetic structure measured at fine scales. I observed high relative parameter importance for the amount of habitat in the bog (A_{bog}) and bog isolation (I_{bog}) when measuring genetic differentiation among leaves and among plants. I was able to infer a strong influence of female oviposition behaviour at the plant scale through the elevated proportion of full-sibling pairs within leaves and a likely influence of oviposition, drift, and founder events at the cluster scale. My study provides evidence that when significant genetic structure is measured among samples beyond the expected dispersal distance of the study organism, in this case at the bog scale, habitat amount, patch isolation, and their interaction have important effects on genetic differentiation (I_{bog}:A_{bog}). Although I did not directly detect a threshold at which patch isolation becomes more important than habitat

amount, the interactive effect of habitat amount and isolation on genetic structure is consistent with the ecological work on the relative effects of habitat composition and configuration on species' abundance and distribution patterns. My findings suggest that in landscape genetic and habitat fragmentation studies of natural systems, habitat structure beyond the scale of genetic sampling could be important and should be included in models explaining patterns of genetic differentiation.

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Table A1. Sampling summary of the number of systems, bogs, clusters per bog, plants per cluster, and leaves per plant from which individual *Metriocnemus knabi* were collected.

System (n = 2)	Bog name (n = 7)	Code	Cluster (n = 31)	Plant (n = 94)	Leaf (n = 276)	Number of individuals (n = 1,231)
1	Minor Lake	Min	1	1	1	5
					2	3
					3	3
				2	1	4
					2	5
					3	5
				3	1	3
					2	5
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			4	1	1	3
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			5	1	1	5
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					3	5
				2	1	3
					2	5
					3	5
				3	1	4
					2	4
					3	4
1	'Roadside'	RSB	1	1	1	5
					2	2
					3	5
				2	1	5
					2	5
					3	5
				3	1	5
					2	5
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					3	5
				2	1	5
					2	5
					3	5
				3	1	5
					2	5
					3	5
1	Spruce	SB	1	1	1	5
					2	5
					3	5
				2	1	2
					2	5
					3	5

				3	1	5
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			2	1	1	5
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					2	5
					3	5
				2	1	5
					2	7
					3	5
				3	1	5
					2	1
					3	4
2	'Buggy'	BB	1	1	1	4
					2	4
					3	3
				2	1	5
					2	5
					3	5
				3	1	3
					2	2
					3	4
			2	1	1	2
					2	5
					3	4
				2	1	2
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			4	1	1	5
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			5	1	1	3
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				2	1	3
					2	5
					3	5
				3	1	3
					2	5
					3	5
2	Dizzy Lake	DZ	1	1	1	5
					2	1
					3	6
				2	1	6
					2	1
					3	5
				3	1	5
					2	5
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			2	1	1	5
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				2	1	5
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				3	1	5
					2	5
					3	5
2	Mizzy Lake	MZ	1	1	1	6
					2	6
				2	1	2
					2	6
				3	1	2
					2	8
					3	4
			2	1	1	2
					2	1
					3	6
				2	1	2
					2	4
				3	1	1
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			5	1	1	5
					2	5
				2	1	5
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					3	1
				3	1	5
					2	5
					3	1
2	Wolf Howl	WH	1	1	1	3
					2	5
					3	5
				2	1	5
					2	4
					3	4
				3	1	2
					2	5
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			5	1	1	5
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Table A2. Standardized plant scale landscape data measured among leaves per plant (Pl) from the corresponding cluster (Cl), bog, and system (Sys), where S_{lf} = patch size of the leaf (mm), A_{pl} = amount of habitat in the plant, A_{cl} = amount of habitat in the cluster, A_{bog} = amount of habitat in the bog (m^2), I_{lf} = isolation of the leaf (cm), I_{pl} = isolation of the plant (m), I_{cl} = isolation of the cluster, and I_{bog} = isolation of the bog (m).

ID	Sys	Bog	Cl	Pl	S_{lf}	A_{pl}	A_{cl}	A_{bog}	I_{lf}	I_{pl}	I_{cl}	I_{bog}
Min	1	1	1	1	-1.4920	-0.6757	-0.1893	-0.9648	-0.9583	0.5950	-0.2525	-0.2213
Min	1	1	1	2	-0.5250	0.3116	-0.1893	-0.9648	-1.3531	-0.9698	-0.2525	-0.2213
Min	1	1	1	3	-1.7160	1.6279	-0.1893	-0.9648	-1.3531	0.1039	-0.2525	-0.2213
Min	1	1	2	4	-1.4180	-0.3466	-0.7138	-0.9648	0.0108	1.4353	-0.4168	-0.2213
Min	1	1	2	5	-0.9710	-0.3466	-0.7138	-0.9648	-1.4967	-0.4080	-0.4168	-0.2213
Min	1	1	2	6	-0.3010	-0.6757	-0.7138	-0.9648	-0.4917	0.6962	-0.4168	-0.2213
Min	1	1	3	7	-0.3760	-0.6757	-1.0558	-0.9648	-0.2763	-1.6750	-0.2525	-0.2213
Min	1	1	3	8	-1.4180	-1.0048	-1.0558	-0.9648	0.0826	1.0495	-0.2525	-0.2213
Min	1	1	3	9	2.2300	-0.3466	-1.0558	-0.9648	1.3030	1.6459	-0.2525	-0.2213
Min	1	1	4	10	0.5170	-1.0048	-0.0411	-0.9648	0.0827	0.4655	-0.0061	-0.2213
Min	1	1	4	11	-0.4500	-0.6757	-0.0411	-0.9648	-0.6353	-0.5369	-0.0061	-0.2213
Min	1	1	4	12	-1.4180	-0.6757	-0.0411	-0.9648	-0.6353	-0.5237	-0.0061	-0.2213
Min	1	1	5	13	-0.5990	-0.0175	-0.3261	-0.9648	-0.6711	-0.1060	0.1581	-0.2213
Min	1	1	5	14	-1.1950	-0.0175	-0.3261	-0.9648	-1.2814	0.5895	0.1581	-0.2213
Min	1	1	5	15	-0.7480	-0.3466	-0.3261	-0.9648	-1.0660	-1.4180	0.1581	-0.2213
RSB	1	2	6	16	-0.7480	-0.6757	-0.9988	-0.8467	0.4416	-1.3896	-0.0061	1.2886
RSB	1	2	6	17	-1.7160	-1.0048	-0.9988	-0.8467	-0.9942	1.6223	-0.0061	1.2886
RSB	1	2	6	18	-1.1950	-1.3339	-0.9988	-0.8467	-0.3481	0.5098	-0.0061	1.2886
RSB	1	2	7	19	-0.4500	-1.0048	-0.6111	-0.8467	0.2621	-0.2466	2.2113	1.2886
RSB	1	2	7	20	0.3690	-0.6757	-0.6111	-0.8467	-0.3481	-0.4433	2.2113	1.2886
RSB	1	2	7	21	0.5920	-1.0048	-0.6111	-0.8467	-0.5635	0.8867	2.2113	1.2886
RSB	1	2	8	22	-0.2270	1.6279	-0.9076	-0.8467	-0.8506	-1.6750	-0.5810	1.2886
RSB	1	2	8	23	-0.5990	0.3116	-0.9076	-0.8467	-0.4917	0.1947	-0.5810	1.2886
RSB	1	2	8	24	1.1870	-0.6757	-0.9076	-0.8467	-0.3840	0.2390	-0.5810	1.2886

SB	1	3	9	25	0.4430	-0.3466	1.0419	-1.1945	-0.2548	-1.0218	-0.4989	-0.0389
SB	1	3	9	26	-0.1520	-0.6757	1.0419	-1.1945	0.3698	-0.1108	-0.4989	-0.0389
SB	1	3	9	27	1.7090	-1.3339	1.0419	-1.1945	1.7696	0.4704	-0.4989	-0.0389
SB	1	3	10	28	0.1450	-1.3339	2.5696	-1.1945	2.0926	-1.2718	-0.4989	-0.0389
SB	1	3	10	29	-0.2270	1.2988	2.5696	-1.1945	0.0826	1.6230	-0.4989	-0.0389
SB	1	3	10	30	1.1130	0.9698	2.5696	-1.1945	0.5133	1.2242	-0.4989	-0.0389
SB	1	3	11	31	-1.3430	-1.3339	-0.2919	-1.1945	-0.4199	-0.4468	-1.0738	-0.0389
SB	1	3	11	32	-0.8220	-1.0048	-0.2919	-1.1945	-0.2763	1.3917	-1.0738	-0.0389
SB	1	3	11	33	0.9640	0.6407	-0.2919	-1.1945	2.7387	2.6392	-1.0738	-0.0389
BB	2	4	12	34	-0.5990	-0.6757	-0.6453	0.0049	-0.4199	-0.9726	-0.1704	1.8283
BB	2	4	12	35	0.0710	0.9698	-0.6453	0.0049	-0.4199	-0.8700	-0.1704	1.8283
BB	2	4	12	36	-0.5990	-1.0048	-0.6453	0.0049	-0.1327	-0.0928	-0.1704	1.8283
BB	2	4	13	37	0.0710	0.3116	-0.7822	0.0049	0.6569	-1.4131	-0.8274	1.8283
BB	2	4	13	38	0.8900	-0.0175	-0.7822	0.0049	0.2980	-1.3723	-0.8274	1.8283
BB	2	4	13	39	-0.6740	-0.6757	-0.7822	0.0049	-0.4917	1.2427	-0.8274	1.8283
BB	2	4	14	40	0.0710	-0.6757	0.2781	0.0049	-0.9583	-0.6387	1.1437	1.8283
BB	2	4	14	41	-0.5250	-0.6757	0.2781	0.0049	-0.6352	-0.7959	1.1437	1.8283
BB	2	4	14	42	-0.3760	0.3116	0.2781	0.0049	0.0826	0.5071	1.1437	1.8283
BB	2	4	15	43	0.6660	-1.3339	0.0045	0.0049	-0.1327	-1.0425	2.9504	1.8283
BB	2	4	15	44	1.4110	-0.6757	0.0045	0.0049	-0.2763	-1.4859	2.9504	1.8283
BB	2	4	15	45	-1.1200	-1.3339	0.0045	0.0049	-0.5276	-0.8001	2.9504	1.8283
BB	2	4	16	46	-0.0040	-0.0175	-0.8050	0.0049	-0.0609	-0.4281	-0.3346	1.8283
BB	2	4	16	47	-0.4500	0.6407	-0.8050	0.0049	-0.2046	1.4284	-0.3346	1.8283
BB	2	4	16	48	-0.5990	-1.0048	-0.8050	0.0049	2.0926	1.0308	-0.3346	1.8283
DZ	2	5	17	49	-0.8220	0.3116	0.1755	0.5504	-0.2045	0.6269	-0.7453	-0.7658
DZ	2	5	17	50	2.8250	-0.6757	0.1755	0.5504	0.8005	-0.3186	-0.7453	-0.7658
DZ	2	5	17	51	1.4110	-0.0175	0.1755	0.5504	0.7287	-1.0813	-0.7453	-0.7658
DZ	2	5	18	52	0.2940	0.3116	-0.2691	0.5504	-0.7788	0.1746	-0.8274	-0.7658
DZ	2	5	18	53	0.1450	-0.0175	-0.2691	0.5504	0.2262	-0.6179	-0.8274	-0.7658
DZ	2	5	18	54	1.3360	-1.0048	-0.2691	0.5504	1.1594	-1.5330	-0.8274	-0.7658
DZ	2	5	19	55	0.5170	1.9570	-0.6909	0.5504	-0.2045	0.7357	-1.3201	-0.7658
DZ	2	5	19	56	-0.2270	-0.3466	-0.6909	0.5504	-0.3481	-0.8950	-1.3201	-0.7658
DZ	2	5	19	57	-0.1520	-0.6757	-0.6909	0.5504	-0.5635	-0.3651	-1.3201	-0.7658

DZ	2	5	20	58	0.4430	-1.0048	-0.6681	0.5504	1.2312	-0.1288	-1.3201	-0.7658
DZ	2	5	20	59	0.8150	0.6407	-0.6681	0.5504	0.5492	0.1101	-1.3201	-0.7658
DZ	2	5	20	60	1.1130	-1.3339	-0.6681	0.5504	1.4466	-0.0326	-1.3201	-0.7658
DZ	2	5	21	61	-1.0460	-0.0175	-0.6111	0.5504	1.4466	0.6976	0.0760	-0.7658
DZ	2	5	21	62	0.3690	0.9698	-0.6111	0.5504	0.2621	-0.1850	0.0760	-0.7658
DZ	2	5	21	63	-0.0780	-0.0175	-0.6111	0.5504	0.0827	0.1621	0.0760	-0.7658
MZ	2	6	22	64	0.7410	-0.0175	-1.2040	1.8315	1.0159	-0.5999	-0.6631	-0.7658
MZ	2	6	22	65	1.2620	2.6152	-1.2040	1.8315	0.3698	0.9359	-0.6631	-0.7658
MZ	2	6	22	66	-1.0080	-0.3466	-1.2040	1.8315	-0.7070	-0.6782	-0.6631	-0.7658
MZ	2	6	22	67	0.5920	0.3116	-1.2040	1.8315	-0.5634	0.0042	-0.6631	-0.7658
MZ	2	6	23	68	0.7410	1.6279	-0.8506	1.8315	0.2987	-1.3459	-0.5810	-0.7658
MZ	2	6	23	69	2.2300	-0.3466	-0.8506	1.8315	1.8062	0.3824	-0.5810	-0.7658
MZ	2	6	23	70	-0.3760	1.2988	-0.8506	1.8315	0.8716	-0.4191	-0.5810	-0.7658
MZ	2	6	24	71	-0.0040	2.2861	2.2048	1.8315	1.4466	0.1663	1.8828	-0.7658
MZ	2	6	24	72	-0.1520	1.6279	2.2048	1.8315	4.3173	0.2300	1.8828	-0.7658
MZ	2	6	24	73	1.5600	-0.6757	2.2048	1.8315	1.2312	0.1538	1.8828	-0.7658
MZ	2	6	25	74	-0.0040	0.9698	0.3921	1.8315	0.3698	0.9192	-0.1704	-0.7658
MZ	2	6	25	75	-0.0780	-0.6757	0.3921	1.8315	-0.9224	1.2039	-0.1704	-0.7658
MZ	2	6	25	76	0.8150	-0.0175	0.3921	1.8315	-0.5634	-0.7980	-0.1704	-0.7658
MZ	2	6	26	77	-1.5670	1.9570	1.3498	1.8315	-0.0609	0.0249	1.6364	-0.7658
MZ	2	6	26	78	0.7410	-0.6757	1.3498	1.8315	1.1595	0.0727	1.6364	-0.7658
MZ	2	6	26	79	2.1550	0.3116	1.3498	1.8315	-0.6353	0.7855	1.6364	-0.7658
WH	2	7	27	80	-0.0780	0.9698	0.5631	-0.3194	-0.4199	-1.6750	-0.3346	-0.7741
WH	2	7	27	81	0.2200	1.6279	0.5631	-0.3194	-1.5326	-0.6678	-0.3346	-0.7741
WH	2	7	27	82	0.6660	-0.3466	0.5631	-0.3194	1.5901	1.2393	-0.3346	-0.7741
WH	2	7	28	83	-0.0040	2.9443	0.1755	-0.3194	-0.8506	-1.6750	-0.3346	-0.7741
WH	2	7	28	84	0.9640	-0.0175	0.1755	-0.3194	-0.9224	3.3721	-0.3346	-0.7741
WH	2	7	28	85	-0.8970	-1.0048	0.1755	-0.3194	-1.1019	0.8818	-0.3346	-0.7741
WH	2	7	29	86	0.2940	0.3116	2.0566	-0.3194	-0.1327	0.1690	0.2403	-0.7741
WH	2	7	29	87	1.6340	0.9698	2.0566	-0.3194	-0.1327	1.7796	0.2403	-0.7741
WH	2	7	29	88	1.4110	0.9698	2.0566	-0.3194	-0.0609	-0.8347	0.2403	-0.7741
WH	2	7	30	89	-1.6410	2.2861	1.4182	-0.3194	-0.5634	-0.3976	0.8151	-0.7741
WH	2	7	30	90	-0.8220	-0.3466	1.4182	-0.3194	-0.7787	0.2154	0.8151	-0.7741

WH	2	7	30	91	-1.2690	-0.0175	1.4182	-0.3194	-0.9941	-0.4697	0.8151	-0.7741
WH	2	7	31	92	-1.2690	0.3116	-0.1665	-0.3194	-1.2814	0.3380	0.3224	-0.7741
WH	2	7	31	93	-0.3010	0.6407	-0.1665	-0.3194	-0.3480	0.4607	0.3224	-0.7741
WH	2	7	31	94	-0.8970	-0.3466	-0.1665	-0.3194	-1.2095	-0.5625	0.3224	-0.7741

Table A3. Standardized cluster scale landscape data measured among plants per cluster (Cl) from the corresponding bog and system (Sys), where S_{pl} = patch size of the plant, A_{cl} = amount of habitat in the cluster, A_{bog} = amount of habitat in the bog (m^2), I_{pl3} = isolation of the plant (m), I_{cl} = isolation of the cluster, and I_{bog} = isolation of the bog (m).

ID	Sys	Bog	Cl	S_{pl}	A_{cl}	A_{bog}	I_{pl3}	I_{cl}	I_{bog}
Min	1	1	1	0.7030	-0.2006	-0.9471	0.9150	-0.2560	-0.2265
Min	1	1	2	-0.7379	-0.7206	-0.9471	-0.0510	-0.4180	-0.2265
Min	1	1	3	-1.0982	-1.0597	-0.9471	0.1872	-0.2560	-0.2265
Min	1	1	4	-1.2781	-0.0536	-0.9471	0.3139	-0.0131	-0.2265
Min	1	1	5	-0.1974	-0.3362	-0.9471	-0.0434	0.1489	-0.2265
RSB	1	2	6	-1.6385	-1.0032	-0.8288	0.8806	-0.0131	1.2635
RSB	1	2	7	-1.4584	-0.6188	-0.8288	-0.3739	2.1735	1.2635
RSB	1	2	8	0.7030	-0.9128	-0.8288	-1.4802	-0.5800	1.2635
SB	1	3	9	-1.2783	1.0203	-1.1773	-0.0034	-0.4990	-0.0465
SB	1	3	10	0.5229	2.5352	-1.1773	1.2431	-0.4990	-0.0465
SB	1	3	11	-0.9180	-0.3023	-1.1773	-0.3898	-1.0659	-0.0465
BB	2	4	12	-0.3777	-0.6528	0.0247	-0.6491	-0.1750	1.7962
BB	2	4	13	-0.1976	-0.7884	0.0247	-0.7709	-0.8229	1.7962
BB	2	4	14	-0.5578	0.2629	0.0247	-0.0722	1.1207	1.7962
BB	2	4	15	-1.8186	-0.0084	0.0247	-1.4987	2.9024	1.7962
BB	2	4	16	-0.1974	-0.8110	0.0247	2.3703	-0.3370	1.7962
DZ	2	5	17	-0.1976	0.1612	0.5714	-0.4374	-0.7419	-0.7639
DZ	2	5	18	-0.3777	-0.2797	0.5714	-1.3875	-0.8229	-0.7639
DZ	2	5	19	0.5229	-0.6980	0.5714	-1.9989	-1.3088	-0.7639
DZ	2	5	20	-0.9180	-0.6754	0.5714	0.2004	-1.3088	-0.7639
DZ	2	5	21	0.5229	-0.6188	0.5714	0.8911	0.0679	-0.7639
MZ	2	6	22	1.0633	-1.2067	1.8552	0.5717	-0.6609	-0.7639
MZ	2	6	23	1.4235	-0.8562	1.8552	0.5418	-0.5800	-0.7639
MZ	2	6	24	1.7837	2.1734	1.8552	1.5893	1.8496	-0.7639
MZ	2	6	25	0.1625	0.3760	1.8552	-0.8373	-0.1750	-0.7639
MZ	2	6	26	0.8833	1.3256	1.8552	0.9118	1.6066	-0.7639
WH	2	7	27	1.2434	0.5455	-0.3004	-0.1913	-0.3370	-0.7721
WH	2	7	28	1.0633	0.1612	-0.3004	0.1607	-0.3370	-0.7721
WH	2	7	29	1.2434	2.0264	-0.3004	0.4545	0.2299	-0.7721
WH	2	7	30	1.0633	1.3934	-0.3004	0.6792	0.7968	-0.7721
WH	2	7	31	0.3430	-0.1780	-0.3004	-1.7266	0.3109	-0.7721

Table A4. Standardized bog scale landscape data measured among clusters per bog from the corresponding system (Sys), where S_{cl} = patch size of the cluster, A_{bog} = amount of habitat in the bog (m^2), I_{cl3} = isolation of the cluster (m), and I_{bog} = isolation of the bog (m).

ID	Sys	Bog	S_{cl}	A_{bog}	I_{cl3}	I_{bog}
Min	1	1	-0.6673	-0.7852	-0.0492	-0.2833
RSB	1	2	-1.1747	-0.6741	-1.0502	1.1373
SB	1	3	1.4665	-1.0027	-0.7721	-0.1118
BB	2	4	-0.5637	0.1314	0.5427	1.6467
DZ	2	5	-0.5972	0.6469	1.7917	-0.7938
MZ	2	6	0.4774	1.8593	-0.8175	-0.7938
WH	2	7	1.0591	-0.1757	0.3545	-0.8012

Figure A1. Measurements of pitcher plant leaves: 1 = pitcher mouth, 2 = pitcher width, 3 = hood height, 4 = pitcher height.

Table A5. Summary statistics of the correlation between leaf measurements (1 = pitcher mouth, 2 = pitcher width, 3 = hood height, 4 = pitcher height) and potential leaf volume (mL). Potential leaf volume was measured for 249 leaves by emptying the leaf contents and filling the leaf with water to volumetric capacity. Water was then poured into a graduated cylinder and measured. Potential leaf volume was cube-root transformed for correlation analysis.

Leaf measurement	R^2	r	p-value	df
1	0.7555	0.8612	< 0.0001	246
2	0.9169	0.9575	< 0.0001	246
3	0.6838	0.8269	< 0.0001	246
4	0.0015	0.8727	< 0.0001	239

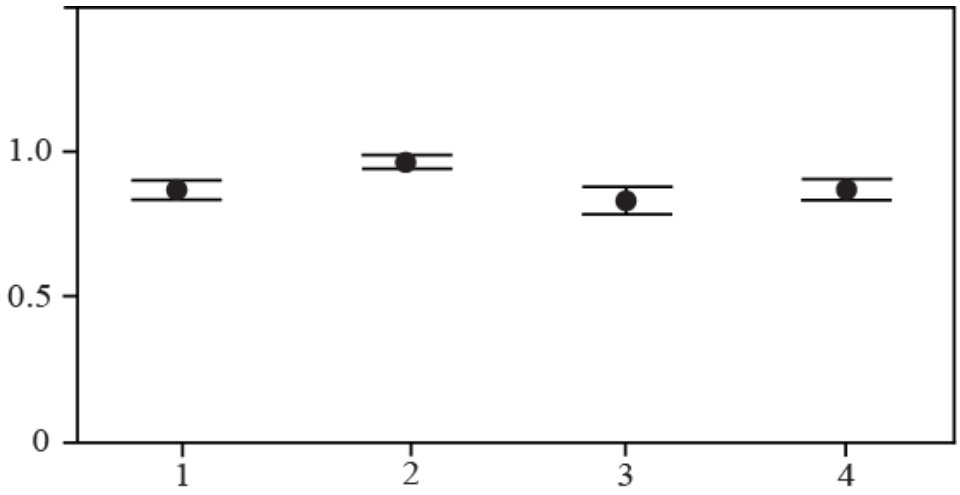

Figure A2. Plot of Pearson's correlation coefficients (*r*) for pitcher plant measurements (1 = pitcher mouth, 2 = pitcher width, 3 = hood height, 4 = pitcher height) and potential leaf volumes, where points represent correlation coefficients and error bars represent 95% confidence intervals.

Table A6. Pearson correlation coefficients indicating the correlation between predictor variables used in plant scale models (significance levels $^{*}p < 0.05$, $^{**} < 0.01$, $^{***} < 0.001$).

Variable	I_{lf}	I_{pl}	I_{cl}	I_{bog}	A_{pl}	A_{cl}	A_{bog}
S_{lf}	0.425^{***}	0.030	-0.044	-0.154	0.030	0.157	0.302^{**}
I_{lf}		0.138	-0.030	-0.102	-0.009	0.191	0.285^{**}
I_{pl}			-0.091	-0.125	-0.004	0.078	-0.067
I_{cl}				0.276^{**}	-0.060	0.343^{***}	0.090
I_{bog}					-0.315^{**}	-0.294^{**}	-0.334^{***}
A_{pl}						0.224^{*}	0.296^{**}

Table A7. Pearson correlation coefficients indicating the correlation between predictor variables used in cluster scale models (significance levels $^{*}p < 0.05$, $^{**} < 0.01$, $^{***} < 0.001$).

Variable	I_{pl3}	I_{cl}	I_{bog}	A_{cl}	A_{bog}
S_{pl}	0.240	-0.092	-0.518^{**}	0.390^{*}	0.482^{**}
I_{pl3}		0.098	-0.074	0.301	0.077
I_{cl}			0.272	0.338	0.105
I_{bog}				-0.307	-0.326
A_{cl}					0.048

Table A8. Pearson correlation coefficients indicating the correlation between predictor variables used in bog scale models (significance levels $^{*}p < 0.05$, $^{**} < 0.01$, $^{***} < 0.001$).

Variable	I_{cl3}	I_{bog}	A_{bog}
S_{cl}	-0.209	-0.499	0.014
I_{cl3}		-0.209	0.194
I_{bog}			-0.344

Katie Millette – Curriculum Vitae

Education

2010 – 2012 M.Sc. Biology – Western University
2005 – 2010 B.Sc. H. Biology – University of Windsor

Awards and Scholarships

2012 Dr. Irene Uchida Fellowship in Life Sciences – Western University
Student travel award – Western University
2011– 2012 Natural Sciences and Engineering Research Council of Canada Canadian Graduate Scholarship (NSERC – CGSM)
Ontario Graduate Scholarship (declined)
2010– 2011 Ontario Graduate Scholarship

Refereed Contributions

Millette KL, Xu S, Witt JDS, Cristescu ME. 2011. Pleistocene-driven diversification in freshwater zooplankton: Genetic patterns of refugial isolation and postglacial recolonization in *Leptodora kindtii* (Crustacea, Cladocera). *Limnology and Oceanography* 56:1725–1736.

Conference Presentations

2012 *Evolution 2012 – 1st Joint Congress on Evolutionary Biology*, Ottawa, ON (oral presentation).
CIEE Landscape Genetics Symposium, University of Toronto, ON (oral presentation).
9th Annual Earth Day Colloquium, Western University, ON (oral presentation).
2010 *6th Annual Early Career Scientist Symposium: Experimental Evolution*, University of Michigan, MI (poster presentation).
23rd Annual Ontario Biology Day Colloquium, York University, ON (oral presentation).
2009 *22nd Annual Ontario Biology Day Colloquium*, University of Windsor, ON (oral presentation).

Research Experience

2012 Graduate research assistant – Dr. N. Keyghobadi lab, Western University
2009 – 2010 Research assistant – Dr. M. Cristescu lab, Great Lakes Institute for Environmental Research, University of Windsor
2008 – 2010 Research assistant – Dr. J. Ciborowski lab, University of Windsor

Teaching Experience

2011, 2012W Introductory Biology – Western University
2011F Molecular Ecology – Western University