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Graduate Program in Biology
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science
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Population Genetic Structure In The Pitcher Plant Flesh Fly *Fletcherimyia fletcheri*

Thesis Format: Monograph

by

John Robert O'Leary

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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Abstract

The study of population genetic structure in the pitcher plant flesh fly, *Fletcherimyia fletcheri*, is an important step in bettering our understanding of dispersal abilities, gene flow, and behavior in the species. In this paper, an extended sampling of populations across Algonquin Provincial Park was performed to elucidate an effective scale of genetic differentiation in *F. fletcheri*. Genetic differentiation between sites was compared to interceding landscape composition, and digital dispersal models were developed, testing the hypothesis that *F. fletcheri* uses aquatic tributaries for dispersal between peatlands. Data were collected on 613 specimens from 15 populations, and population differentiation was analyzed using 10 microsatellite loci. Results indicated a pattern of isolation by distance in *F. fletcheri* with the exception of select, isolated sites. Analysis of models based on landscape composition showed no clear favoring of aquatic tributaries for dispersal.

Keywords

Flesh fly, *Fletcherimyia fletcheri*, northern purple pitcher plant, *Sarracenia Purpurea*, landscape genetics, gene flow.

Co-Authorship Statement

This work was authored under the supervision of Dr. Nusha Keyghobadi. All aspects of sampling design and analysis were planned in cooperation with Dr. Keyghobadi and any publications subsequent to the completion of this work will be under co authorship with her.

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List of Abbreviations

IBD	Isolation by distance
IBB	Isolation by barrier
IBR	Isolation by resistance
F. fletcheri	*Fletcherimyia fletcheri*
UTM	Universal transverse Mercator
S. purpurea	*Sarracenia purpurea*
GPS	Geographic positioning system
GIS	Geographic information system
n	Sample size
A_R	Allelic richness
H_E	Expected heterozygosity
N_A	Allelic diversity
cfr	Cauliflower road peatland
ink	Ink lake peatland
maj	Major lake road peatland
mcc	McIntyre's clearing trail peatland
rdg	Rod and Gun peatland
rdo	Algonquin radio observatory peatland
jwl	Jewel lake peatland
dmr	David Mumbayaka river peatland
min	Minor lake peatland (Bab lake)
whl	Wolf Howl peatland
caj	Caracajou peatland
brl	Brent road into Cedar lake peatland
kwl	Karl Wilson peatland
hgn	Hogan lake peatland
prx	Proulx lake peatland
PCR	Polymerase chain reaction
TD	Touchdown
HWE	Hardy Weinberg equilibrium
LD	Linkage Disequilibrium
AMOVA	Analysis of molecular variance
F_{ST}	Fixation index
PCoA	Principal coordinates analysis
MNR	Ministry of Natural Resources
GME	Geospatial modeling environment
LCP	Least cost pathway
DEM	Slope digital elevation model
r	Mantel correlation statistic

1 Introduction

1.1 Connectivity, gene flow and genetic structure

Species typically occur in non-uniform distributions across their range, displaying spatially heterogeneous patterns of abundance at various spatial scales (Forman 1995b; Anderson et al. 2010). Spatial heterogeneity in abundance can occur naturally in an environment due to preexisting biotic or abiotic barriers to migration, settlement or survival. High levels of heterogeneity can also be brought about or increased in a landscape through anthropogenic alteration or fragmentation of previously continuous or connected habitat (Fischer and Lindenmayer 2007). Therefore, for many species, patterns of spatial variation in distribution and abundance occur within the context of a patchwork of suitable habitat separated by a matrix of less optimal or unsuitable land cover types (Forman 1995).

The amount and spatial configuration of habitat in a landscape, as well as characteristics of the intervening landscape matrix, determine the degree of isolation among populations and subpopulations of a species (Taylor et al. 1993). Landscape configuration and composition are respectively measures of the spatial arrangement and amount of habitat available to a species across a region (Forman 1995; Forman 1995b). Composition is a measure of the relative abundance of a given habitat type across a landscape, as well as the quality of habitat based on the requirements of the species in question (Wiens et al. 1986). Configuration determines the level of isolation between habitat fragments in a matrix (Taylor et al. 1993). Classically, configuration is considered to play a decreasing role in determining distribution and abundance of populations as the proportion of suitable habitat in a landscape increases. Inversely, as habitat decreases in a landscape, the effect of configuration increases (Templeton et al. 1990; Franklin and Lindenmayer 2009; Fischer and Lindenmayer 2007). Such structural attributes of a landscape interact with the dispersal ability of the species in question to determine isolation, or conversely "connectivity", among sub-populations (Taylor et al. 1993; Fahrig 2003). The degree to which an interceding landscape hampers or facilitates movement is referred to as "landscape connectivity" (Koen et al. 2012; Taylor et al. 1993).

Groups of populations can be classified into different models of spatial structure based on the degree of connectivity among the populations: (1) patchy populations, (2) isolated populations (sometimes called "island populations"), and (3) metapopulations (Templeton et al. 1990, Mayer et al. 2009). Patchy populations are characterized by high connectivity, or near panmixia among populations, and have the highest levels of movement among populations (Templeton et al. 1990). In isolated populations, populations are completely separate, with virtually no connectivity or movement among them (Frankham et al. 2002). A metapopulation consists of multiple spatially separate sub-populations connected by the dispersal of individuals between them (Hanski 1991; Forman 1995). Metapopulations show traits from both patchy and isolated population structures, they have varying degrees of connectivity between pairs of populations and, consequently, the genetic structure of each population patch is a byproduct of the genetic structure of its neighboring populations, landscape connectivity, and surrounding land cover composition (Shirk et al. 2010).

Connectivity among populations is a key determinant of local and regional abundance, as well as the degree of spatial synchrony in demographic fluctuations (Hanski 1991). Connectivity is also important in determining genetic diversity and differentiation within and among populations (Taylor et al. 1993). High connectivity leads to high rates of gene flow and is associated with lower differentiation among populations, resulting in higher genetic diversity and effective population sizes (Hanski 1991; Taylor et al. 1993; Forman 1995). High connectivity contributes to maintenance of genetic diversity overall, which is believed to be important for the persistence of species and maintenance of evolutionary potential. However, populations displaying lower connectivity may be better able to respond rapidly to local selection pressures and to become locally adapted (Slatkin 1987; Templeton et al. 1990). Connectivity, therefore, is an important attribute of populations that affects ecological and evolutionary dynamics. The quantification of connectivity, its relationship to landscape structure, and its effects on ecological population genetic patterns and processes are central research themes in the fields of landscape ecology and landscape genetics (Taylor et al. 1993).

1.2 Landscape Genetics

The field of landscape genetics was developed through the synthesis of two traditional fields: landscape ecology and population genetics (Manel et al. 2003). The field began to develop in the early 1990's with the advent of high throughput genetic fingerprinting using highly variable markers (microsatellites) and increases in processing power and the subsequent improved ability to efficiently analyze geospatial data (Holderegger and Wagner 2008). Landscape genetics is focused on understanding how landscape structure shapes population genetic structure through the effects of underlying processes, particularly gene flow and genetic drift (Storfer et al. 2007; Manel et al. 2003).

A common goal in landscape genetics research is to quantify connectivity among populations using genetic data, and relate observed patterns of connectivity to landscape structure (Holderegger and Wagner 2008). In this way, inferences about the effects of landscape attributes on gene flow can be made (Holderegger and Wagner 2008; Wu 2004).

The use of population genetic data to infer rates of dispersal and gene flow among populations provides an important approach to developing our understanding of connectivity among natural populations while avoiding some of the difficulties associated with direct field observation of movement (Hagler and Jackson 2001; Fischer and Lindenmayer 2007). In mark recapture studies on smaller insects for example, the small physical size of specimens makes the placement as well as the identification of marks difficult (Mayer et al. 2009; Krawchuk and Taylor 2003). Marks could also have significant effects on flight stability, sensory efficiency, and mating success, resulting in decreased fitness for sampled specimens (Hagler and Jackson 2001). Furthermore, direct observations of movement do not necessarily immediately equate to genetic connectivity (Slatkin 1987). Normally in mark recapture studies, tagged specimens recorded in a separate population from their release are taken as a sign of successful dispersal to a new region, although such movement does not lead to genetic connectivity if the migrant does not successfully reproduce in the new population (Slatkin 1987; Manel et al. 2003).

If a species is unable to traverse a region between two habitat fragments, this can result in a decrease or cessation of mating between populations, decreased gene flow, and an

increase in genetic drift between populations (Fischer and Lindenmayer 2007). Thus, the degree of genetic divergence between the populations can be used as an indicator of connectivity between them and, given the composition of the intervening landscape, can also be used to infer the effects of differing types of land cover on dispersal (Manel et al. 2003; Mayer et al. 2009).

Three main models are used to describe the effects of intervening landscape as an obstacle to gene flow: isolation by distance (IBD), isolation by barrier (IBB), and isolation be resistance (IBR) (Wright 1943; McRae 2006; Meirmans 2012).

Isolation by distance:

IBD is the result of the inherently limited range of dispersal of any given species, resulting in an observable trend where individuals or populations that are geographically proximate tend to also be genetically more similar than specimens that are further apart (Wright 1943). IBD is based on spatial relationships, and therefore can be analyzed independently of landscape features that may inhibit dispersal and, consequently, is usually considered a null hypothesis in landscape genetics (Meirmans 2012; Rasic 2011).

Isolation by barrier:

IBB appears when there is a clearly delineated structure or landscape feature that limits movement between regions (Schwartz et al. 2002; Cushman and McKelvey 2006). These are often individual structures such as rivers or fences, and result in a clear decrease or cessation of movement between regions on either side of the barrier resulting in a decrease or cessation of gene flow across the barrier, however gene flow in sub populations that are on the same side of the barrier remain unaffected (Forbes and Hogg 1999; Cushman and McKelvey 2006).

Isolation by resistance:

IBR is an attempt to incorporate complex landscape heterogeneity into classical isolation by distance analyses; the need for such an approach arises from the variable effect of different landcover types on a species' ability to disperse among sub-populations (McRae 2006). Most species are limited in their dispersal over certain land cover types, or prefer some landcover to others, such that rates of dispersal and gene flow between populations

will depend on the connectivity among sites and the composition and configuration of intervening landscapes (McRae 2006). IBR models are based on total accumulated resistance values, unlike IBD, which relates cost only to distance traveled. Resistance cost is based on the amount and quality of traversable landscape between sites, and takes into account distance traveled as well as dispersal resistance due to interceding landscape (McRae and Beier 2007; Graves 2012).

IBB and IBR are both a result of geographic processes, either biotic or abiotic, which can cause genetic isolation in populations. All three processes (IBD, IBR, IBB) often occur simultaneously to some extent across the range of a species, and the ability to isolate and interpret the scale of each process as it contributes to population structure is one of the main goals of landscape genetics (McGarigal and Cushman 2002; Fahrig 2003; Rasic 2011).

The understanding that landscape genetic analyses can provide about the microevolutionary processes that define genetic structure across space is highly dependent on the scale at which sampling takes place (Anderson et al. 2010). The distances among sampled populations defines the sampling grain (Wu 2004; Fortin and Dale 2005). Selecting the correct grain is important in landscape genetics studies. Species that are poor dispersers will often show increased levels of genetic isolation and greater IBD over smaller geographic ranges, thus requiring that sampling and analysis of population genetic structure be performed on a fine scale in order to detect variation among populations and individuals (Rasic and Keyghobadi 2012b; Anderson et al. 2010). Inversely, when species show a strong dispersal ability, sampling at a small scale may result in panmixia in the observed genetic structure of populations, and researchers may lose the ability to detect gene flow, broad scale barriers, or the ability to properly assess the rate and scale of gene flow among populations (Cushman and Landguth 2010).

1.3 The pitcher plant microcosm

The pitcher plant flesh fly, *Fletcherimyia fletcheri* (Aldrich 1927), is one of three dipteran insect species that develop within the fluid filled leaves of the carnivorous northern purple pitcher plant, *Sarracenia purpurea L.* (*Sarraceniaceae*) (Addicott 1974). The other

two species are the midge, *Metriocnemus knabi*, and the mosquito *Wyeomyia smithii* (Coquillett 1892; Harvey and Miller 1996; Dahlem and Naczi 2006). Northern purple pitcher plants occur exclusively in peatlands, including sphagnum bogs, poor fens and seepage swamps, all of which are areas of low nitrogen deposition (Ne'eman et al. 2006).

The first larval instar phase of *F. fletcheri* develops exclusively in the enclosed stagnant water of the fluid filled pitcher leaves; therefore, larviposition will only occur where there are living *S. purpurea* specimens (Johannsen 1935). The larvae of *F. fletcheri* use a large spiracular pit to remain afloat within the fluid filled leaves, and feed on drowned prey (Johannsen 1935). *F. fletcheri* larvae help the plant by breaking larger trapped prey into smaller, more easily dissolved and digested pieces (Johannsen 1935). The first instar larvae are actively territorial upon deposition into the leaf, and show cannibalistic tendencies towards other larvae, often killing any other larvae in the same leaf within 24 hours of deposition. The larger and more developed of the two larvae will usually win out (Rango 1999). Adult *F. fletcheri* are also dependent upon the flowers of the pitcher plant for roosting and possibly as a site for reproduction (Krawchuk and Taylor 1999). Once the larvae have approached pupation, they eat their way out of the bottom of their individual leaf, pupate, and overwinter in the peat moss at the base of their host pitcher plant (Krawchuk and Taylor 1999).

In the northern portion of its range, *F. fletcheri* is univoltine due to harsh winter conditions. This means that they only have one generation per year, with one active mating season and an extended dormant overwinter phase (Rango 1999; Krawchuk and Taylor 2003).

Pitcher plants and their inquiline inhabitants have been important in a variety of ecological and evolutionary studies. As with other microcosms, which include certain species of insect larvae in bromeliads, invertebrate colonies in pan shells, micro-arthropods in moss patches, and beetles in fungal sporocarps (Sirvastava et al. 2004), such small scale model systems are ideal in ecological studies as they provide tractability, generality, and realism (Levins 1966; Sirvastava et al. 2004). The small scale of the pitcher plant system has allowed for a number of studies in community and ecosystem ecology, including through experimental manipulation (Kneitel and Miller 2003). The

pitcher plant microcosm and its members have been the focus of papers on life history evolution, inter-specific competition for shared resources, and species succession (Bradshaw and Holzapfel 1990; Fish and Hall 1978; Hardwick and Giberson 1996). The prior use of the pitcher plant larval community to look at questions involving community ecology, ecosystem ecology, and life history evolution means that our understanding of the early life stages of the flesh fly as well as the other members of the inquiline community is strong. However, these studies have provided relatively little information on dispersal and other life history traits after the inquiline larvae pupate and reach their adult form.

A poor understanding of adult dispersal in an ecological model system is not ideal. One of the key benefits of studying enclosed inquiline communities and other microcosms is their easily manageable size, and the ability to isolate and analyze the effects of alterations to system parameters on species' traits, abundance and distribution (Srivastava et al. 2004). Examples of this can be seen in studies looking at the effects of nutrient availability, species diversity, and inter-specific competition on inquiline populations undergoing different treatments in *S. purpurea* (Fish and Hall 1974; Hoekman 2011). A better understanding of adult dispersal and spatial population genetic structure in pitcher plant inquilines will contribute to more complete interpretation of the results of such experimental manipulations.

Recently, pitcher plant inquilines have been used to study the effects of habitat spatial structure on dispersal and gene flow due to the ability to define discrete patches of habitat for these species at multiple nested spatial scales (Figure 1) (Millette 2012; Rasic and Keyghobadi 2012b; Zilic 2013).

Previous papers also discussed the significance of varying dispersal abilities inherent to the various adult insects native to the pitcher plant microcosm, as well as the effects that this has had on population genetic structure for each species (Rasic 2011). Results of work performed on the pitcher plant midge and mosquito from within Algonquin park showed IBD and IBB occurring at finer spatial scales in the midge, and a stronger wind assisted dispersal ability in the pitcher plant mosquito resulting in a high level of gene flow between distant populations (Rasic and Keyghobadi 2012b; Zilic 2013). This

supports a population structure where the effects of similar landscape distances decrease in species that are stronger dispersers, such as *Flectherimyia fletcheri* (Rasic and Keyghobadi 2012a).

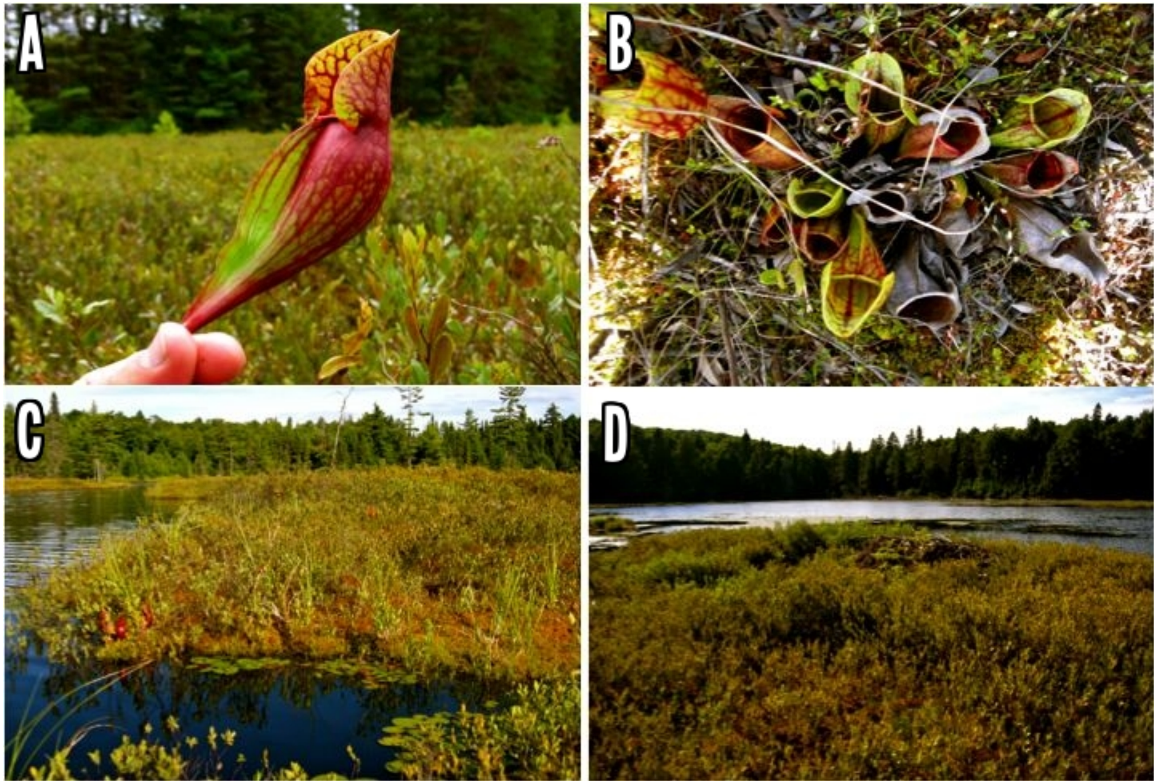

Figure 1: Habitat of *F. fletcheri* at various spatial scales. These consist of: (A) functionally isolated leaves on a single plant at the lowest scale. (B) A crown of leaves around a central flower or flowers. (C) Plants that occur in clusters due to rhizomes sprouting new flowers near original founder individuals (Parisod et al. 2005). (D) A complete peatland.

1.4 Scale of dispersal and gene flow in *F. fletcheri*

Determination of appropriate sampling scale, or distance between sites, also known as the sampling "grain", is important in landscape genetics. An improper sampling grain can have a significant impact on the conclusions drawn about effects of landscape structure on genetic differentiation (Balkenhol et al. 2009). If sampling grain is set too low, variation among sites may not be observed at all, and significant genetic differentiation may be missed in an analysis (Shirk et al. 2010). However, if the scale of sampling is too large, relevant landscape information may be overlooked, and the effect of intervening landscape on genetic differentiation of populations will be obscured (Wu 2004; Fortin and Dale 2005).

The first objective of this research was to determine the grain at which sampling of *F. fletcheri* would reveal significant genetic differentiation among populations in different peatlands, and allow for the study of effects of landscape on population genetic structure. Determining an effective sampling scale for *F. fletcheri* as well as landscape effects on connectivity is important as *F. fletcheri* is a key member of a heavily studied model system in ecology, yet we still know very little about its adult dispersal ability and population structure (Dahlem and Naczi 2006; Rasic and Keyghobadi 2009; Rasic and Keyghobadi 2012a). Previous studies using direct observation or mark recapture to infer dispersal rates in *F. fletcheri* have been unable to determine an effective sampling grain for the species, and research that did look at genetic structure recommended increases in the geographic scale of sampling in order to more effectively infer patterns of differentiation among populations (Rasic and Keyghobadi 2012a; Krawchuk and Taylor 2003).

Observational studies looking at dispersal in adult *F. fletcheri* have not been able to estimate long-range dispersal (i.e. between peatlands). Previous mark recapture experiments performed on *F. fletcheri* have shown a maximum dispersal distance of an individual of 85m, a figure that is indicative of a dispersal rate that contrasts with observations of gene flow over significantly greater distances in *F. fletcheri* using inferences from genetic population structure (Krawchuk and Taylor 2003; Rasic 2011). The dense deciduous and coniferous forests surrounding peatlands make physically

following adults beyond the borders of a peatland nearly impossible (Krawchuk and Taylor 1999). Genetic analyses of *F. fletcheri* populations to infer dispersal and gene flow found significant IBD between two systems that were roughly 25 km apart, but not at smaller spatial scales; among peatlands within-systems, or within-peatlands (Rasic and Keyghobadi 2012a). IBD was detected only at the largest spatial scale included in the previous study indicating that an expansion of geographic scale of sampling was needed in order to fully describe patterns of genetic differentiation among *F. fletcheri* populations in different peatlands (Rasic and Keyghobadi 2012a).

Previous study of population genetic structure in *F. fletcheri* indicated that an ideal sampling design for this study should have greater distances between sampled populations (i.e. peatlands) than those used in past observational studies. A sampling grain was selected that fell just below the largest scale of the previous study on *F. fletcheri* population genetic structure (20 km between two bog systems), with the aim of sampling at least 10 separate populations at that scale (Rasic and Keyghobadi 2012a). Population-based sampling was used as opposed to individual-based sampling since a population-based approach is often applied when there are clearly defined habitat patches in the study landscape, and the study is taking place on a large geographic scale (Anderson et al. 2010).

1.5 Effect of landscape on dispersal in *F. fletcheri*

The second research objective was to examine the effect of landscape structure on *F. fletcheri* gene flow using an isolation by resistance (IBR) framework. Specifically, the hypothesis that gene flow in *F. fletcheri* occurs along wetland and riparian areas, where stepping stone habitat of *S. purpurea* plants can potentially occur, was tested.

Several components of *F. fletcheri*'s life cycle are dependent on a close proximity to both pitcher plants as well as the peatlands the plants inhabit (Forsyth and Robertson 1975). As mentioned previously, larval development occurs exclusively within *S. purpurea* leaves and adult *F. fletcheri* use *S. purpurea* flowers as roosting sites (Forsyth and Robertson 1975; Krawchuk and Taylor 1999).

Also*, S. purpurea* is very effective in the development of successful novel populations from a few founder individuals (Schwaegerle and Schaal 1979; Parisod et al. 2005). While *S. purpurea* is commonly observed as large colonies inhabiting discrete peatlands, it can also occur as smaller, "satellite" populations in other areas such as along riverbanks and lake edges. Small satellite populations of *S. purpurea* are unlikely to support self-reliant populations of *F. fletcheri*, but could act as layover points for *F. fletcheri* undergoing extensive dispersal events. Since *S. purpurea* is highly dependent on waterlogged regions in order to effectively develop, the potential habitat for these "satellite" founder populations is limited to inundated areas (Gotelli and Ellison 2002). Therefore, while peatlands are geographically isolated, *F. fletcheri* in separate peatland systems may be connected by small populations of *S. purpurea* in other wetland and riparian areas, which could act as refuges for dispersing *F. fletcheri.*

Two contrasting hypotheses were tested regarding two possible mechanisms of dispersal in *F. fletcheri*. The first is that *F. fletcheri* is simply a strong disperser and that individuals can travel over the forested landscape between peatland patches of habitat effectively and maintain high levels of gene flow without selecting easier routes of egress. The second hypothesis is that individuals prefer, or require, intermediate satellite populations of *S. purpurea* which act as stepping stones while traveling between more distant, larger pitcher plant colonies that can support populations of *F. fletcheri*. This would limit effective dispersal routes for *F. fletcheri* to corridors where *S. purpurea* is capable of dispersal and establishment. The most likely locations of these satellite *S. purpurea* populations would be along aquatic and wetland systems connected to peatlands, due to the hydrochoric nature of the pitcher plant's seed dispersal (i.e. dispersed by aquatic channels) (Ellison and Parker 2002).

1.6 Research questions and hypotheses

The following questions, hypotheses and predictions were developed to allow for the analysis of theories on dispersal patterns and population genetic structure in *F. fletcheri*:

Question 1: At what spatial scale is gene flow between populations limited, such that significant genetic differentiation and isolation-by-distance is detected in *F. fletcheri*?

Hypothesis 1: At a spatial grain of 15-20 km, gene flow is sufficiently limited that there is significant differentiation among *F. fletcheri* populations in different bogs.

Prediction 1: Euclidean geographic distance and genetic differentiation between populations sampled at a grain of 15-20 km will be significantly positively correlated, and most populations will be significantly differentiated from each other.

Question 2: Are there certain habitat types or barriers in the landscape that limit gene flow by the flesh fly?

Hypothesis 2: Wetland and riparian habitats, through the provision of satellite *S. purpurea* colonies, facilitate movement and gene flow in *F. fletcheri*, while upland forests are avoided and act as a barrier to gene flow.

Prediction 2: Resistance distances between sampled peatlands that are based on use of wetlands and riparian areas as movement corridors will be more strongly correlated with measures of genetic differentiation between *F. fletcheri* populations than straight-line geographic distance.

An analysis of dispersal, gene flow, and connectivity patterns of *F. fletcheri* based on population genetic structure provides novel insight into the ecology of this important member of *S. purpurea* inquiline community.

2 Materials and methods

2.1 Collection of specimens

Samples were collected from Algonquin Provincial Park in Ontario Canada (UTM Zone 17T/18T) during the late summer season of 2012. Potential sampling sites were first identified with the aid of the Ministry of Natural Resources cataloging project kept for Algonquin Provincial Park, which records spatially referenced species sightings across townships within the park boundaries. Searches were performed for sightings of *S. purpurea*, with results ranging as far back as 1901. Also, other peatland-dependent species were cross-referenced within the cataloging project to identify locations that may have been overlooked in the initial *S. purpurea* listing but could also contain pitcher plant communities. These peatland indicator species include bog Labrador tea, *Rhododendron groenlandicum*, bog cranberry, *Vaccinium oxycoccos*, leatherleaf *Chamaedaphne calyculata*, and several genera of Sphagnum (Family: Sphagnaceae) mosses (Glaser 1992; Tiner 2002). Locations of potential sample sites were then reconsidered based on proximity to accessible road and river routes, and interpretation of aerial imagery provided by the Ministry of Natural Resources (Ontario Ministry of Natural Resources, Forest Resource inventory for Algonquin Park, 2006). Finally, sites were scouted in spring 2012 using logging road access where possible, and canoeing and hiking trails where such access was restricted. Fifteen accessible sites were located containing *S. purpurea* that were large enough to potentially provide a target sample size of forty-five *F. fletcheri* specimens (Table 1). A target number of forty-five specimens was set for sampling to ensure a sufficient sample size for inference of population genetic structure (Leberg 2002). Sites containing *S. purpurea* were visited again to collect specimens between June 24th and August 21st, 2012. Specimens were collected in their third larval instar phase. Collection of adults is more difficult due to their instinctual predator evasion mechanism in which the flies descend into the sphagnum mats and bury themselves when approached or disturbed (Krawchuk and Taylor 1999).

Sampling was standardized by walking in a spiral pattern outward from the center of the peatland to its edges, flagging areas that had previously been observed. Leaves of *S.*

purpurea were inspected for the presence of *F. fletcheri* larvae. When a larva was found, a converted plastic Pasteur pipette with a truncated tip was used to quickly collect specimens before they were able to swim to the base of the leaf and conceal themselves in detritus. Individuals at each peatland were removed from separate plants in an attempt to avoid sampling direct siblings (Forsyth and Robertson 1975). Sampling was also expanded to encompass a greater search radius in larger peatlands to avoid sampling a subsection of the peatland.

Once samples had been collected, larvae were immediately placed into a separate 100% ethanol solution, and the location of each individual sample collected was recorded using a high accuracy GPS receiver accurate to 1.5 m (Trimble GeoXH geoexplorer 2005 series, Westminster CO, USA), within a minimum satellite count set to 6 to ensure 1.5 m accuracy. All samples were rinsed with 100% ethanol upon return to base camp, placed in a new 1.5 ml Eppendorf of 100% ethanol, and maintained at -20°C to minimize DNA and tissue degradation. Geographic location data were stored in Terrasync GPS software (ver. 3.21, ®Trimble Nav. Ltd. Sunnyvale CA, 2008) until transferred to GPS Pathfinder Office (ver. 4.10, ®Trimble Nav. Ltd. Sunnyvale CA, 2008) and finally converted to ArcGIS format (Ver. 10.1, ®ESRI, Redlands CA, 2012) for further analyses.

Table 1: Locations, names and abbreviated (code) names for peatlands sampled for *F. fletcheri* in Algonquin Park. Locations of bogs are given in UTM NAD 1983 coordinates, as well as decimal degrees of latitude and longitude, 'n' represents the number of larvae sampled from each peatland.

Peatland Name	Code	n	UTM coordinates			Lat/Long coord.	
			Zone	Easting	Northing	Lat	Long
Cauliflower rd.	cfr	44	17N	715711	5036313	45.44702	-78.24153
Ink lk.	ink	45	17N	674336	5057350	45.64778	-78.76267
Major lk. rd.	maj	36	17N	725557	5056558	45.62591	-78.10644
McIntyre's clearing trail	mcc	44	18N	267182	5074442	45.78427	-77.99515
Rod & Gun	rdg	45	17N	694183	5039522	45.48221	-78.51529
Algonquin radio observatory	rdo	11	17N	727516	5093672	45.95889	-78.06387
Jewel lk. bog (forestry tower)	jwl	45	17N	655201	5060603	45.68159	-79.00705
David Mumbayaka R.	dmr	45	17N	660480	5065526	45.72467	-78.93768
Minor lk. (Bab lk.)	min	39	17N	701404	5055990	45.62823	-78.41621
Wolf Howl	whl	42	18N	284284	5080235	45.57941	-78.68901
Caracajou bog (hydro line)	caj	45	18N	274008	5083288	45.86605	-77.91159
Brent rd. into cedar lk.	brl	41	17N	699316	5108583	46.10171	-78.42059
Karl Wilson	kwl	45	17N	684482	5100324	46.03162	-78.61612
Hogan lk.	hgn	44	17N	692624	5079039	45.83799	-78.51956
Proulx lk.	prx	42	17N	700702	5070423	45.75821	-78.41923

2.2 DNA extraction and microsatellite analysis

DNA was extracted from individual *F. fletcheri* larvae using the DNEasy Blood and Tissue Extraction Kit (®QIAGEN, Germantown, MD). Sample extraction was modified so that only 100µL of elution buffer was used, allowing for a higher concentration of DNA in the final solution. The quantity of DNA in extracted samples was determined before PCR analysis using a spectrophotometer (Shimadzu Biotech Bio-spec Mini DNA/RNA protein analyzer spectrophotometer, λ260, λ280, and λ230) (®Shimadzu Biotech, Manchester, UK). Samples were confirmed to have concentrations ranging from 85µg/mL to 223µg/mL of DNA in successfully extracted samples.

Larvae were genotyped at 11 microsatellite loci developed specifically for *F. fletcheri*: FF104, FF72, FF10, FF189, FF231, FF09, FF249, FF62, FF238, FF82 and FF65 (Rasic and Keyghobadi 2009). PCR amplification of the microsatellites occurred with one reaction of 6 PCR loci (FF104, FF72, FF10, FF189, FF231 and FF09), one multiplex of two loci (FF 249 and FF62), and individual amplifications of the remaining three loci (FF65, FF238 and FF82) (Rasic and Keyghobadi 2009). PCR amplifications were performed in a PTC 0200 DNA Engine Cycler (BioRad, Hercules, CA). PCR reactions occurred in a final solution of volume 20µL; each amplification contained 1x PCR Buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3 at room temperature), 3.75 mM $MgCl_2$, 0.15 mg/ml bovine serum albumin (BSA), 0.3 mM of each dNTP, 1.5 U of *AmpliTaq* DNA polymerase (Applied Biosystems, Foster City, CA), and approximately 300 ng of *F. fletcheri* DNA (Rasic and Keyghobadi 2009). Concentrations of *F. fletcheri* DNA were increased or decreased in reruns of individual specimens due to poor amplification or excessive amounts of amplified product. For each locus, one of the two primers was labeled with a fluorescent dye (6FAM, NED, PET, or VIC; Applied Biosystems, Foster City, CA) to allow visualization for PCR products in a genetic analyzer.

PCR thermal cycling profiles followed one of four protocols as follows (Rasic and Keyghobadi 2009):

(1) FF231, FF217, FF189, FF238, FF10, FF72, FF104, FF9:

Denaturation for 180s at 96°C; 2 cycles of 30s at 96°C; 30s at 60°C; 30s at 72°C; 14 touchdown (TD) cycles of 15s at 96°C; 15s at 60°C (-0.5°C each cycle); 15s at 72°C; 17 cycles of 15 at 96°C, 15s at 53°C, 15s at 72; and final elongation at 72°C for 180s.

(2) FF065:

Denaturation for 180s at 96°C; 2 cycles of 30s at 56°C, 30s at 72°C; 12 TD cycles of 15s at 96°C, 15s at 56°C (-0.5°C each cycle), 15s at 72°C; 20 cycles of 15s at 96°C, 15s at 50.5°C, 15s at 72°C; and a final elongation step of 180s at 72°C.

(3) FF249, FF62:

Denaturation for 180s at 96°C; 2 cycles of 30s at 96°C, 30s at 53°C, 30s at 72°C; 6 TD cycles of 15s at 96°C, 15s at 53°C (-0.5°C each cycle), 15s at 72°C; 25 cycles of 15s at 96°C, 15s at 50.5°C, 15s at 72°C, and a final elongation for 180s at 72°C.

(4) FF82:

Denaturation for 180s at 96°C, 30 cycles of 30s at 96°C, 30s at 50°C, 30s at 72°C, and a final elongation step of 180s at 72°C.

PCR products were sent to Michael Smith Laboratories at the University of British Columbia for fragment size analysis in an Applied Biosystems® 3730S 96 capillary DNA Analyzer, using LIZ-500 as a size standard. The multiplex PCR was diluted to 1:1 with H_2O and loaded onto a single lane of the DNA analyzer. The five remaining loci were mixed in the following proportions and loaded together in a single lane of the DNA analyzer: 5 H_2O: 1.5 FF249+FF62: 1 FF65: 1 FF238: 1 FF82.

Electropherograms generated by the DNA analyzer were processed and reviewed using Genemapper software Ver. 4.0 (®Applied Biosystems 2005) to determine microsatellite genotypes of the individual larvae.

2.3 Hardy Weinberg and Linkage Disequilibrium tests

Tests for Hardy Weinberg equilibrium (HWE) and linkage disequilibrium were run in Arlequin 3.11 (Excoffier et al. 2005).

Tests for HWE were run using an allowed level of missing data of 0.05, 1000000 steps in the Markov chain, and 100000 steps of Dememorisation, using the method for HWE test for populations using multiple alleles as outlined by Guo & Thompson (1992). This test is analogous to a Fisher's exact test, with a triangular contingency table of arbitrary size instead of a two by two contingency table (Guo and Thompson 1992). It is designed and recommended for use in populations where the number of samples in each site is low (typically, $n<50$), and there is no allelic masking, that is, the zygotic state of a diploid organism is fully know at each locus (Peakall and Smouse 2012; Guo and Thompson 1992).

2.4 Metrics of diversity

Metrics of within-population genetic diversity were measured using Genalex 6.5 (Peakall and Smouse 2012): expected heterozygosity (H_E) averaged over loci was measured for each peatland. Allelic diversity (N_A) and mean allelic richness (A_R), which is a measure of allelic diversity corrected for sample size, was also averaged over loci (Table 2). In this case N_A is defined as the number of alleles found at each loci determined by direct count and averaged over all observed locus for each site (Slatkin 1995). A_R is also a direct count of alleles at each locus then averaged over all observed loci for a site, however it is rarified to the smallest number of alleles sampled (Leberg 2002). Allelic richness was calculated in the program *allelic.richness()* from the *Hierfstat* program library in R 2.14.1, and rarified to a sample size of 11, with an allele count of 22 (the smallest sample size collected) (Goudet 2005).

2.5 AMOVA and pairwise F_{ST}

Analysis of molecular variance (AMOVA) was run using Arlequin 3.11 (Excoffier et al. 2005; Peakall and Smouse 2012). AMOVA allows for the partitioning of genetic variance among nested hierarchical levels (within-individuals, within-populations, within groups

of populations and among groups), along with estimation of variance components and F statistics (or fixation indices) and hypothesis tests for fixation indices using non-parametric permutation procedures (Excoffier et al. 1992). Fixation indices are measures of genetic differentiation at the respective level of the nested hierarchy.

In this study, there were three nested levels of structure: among-peatlands, within-peatlands, and within-individuals. Also, genetic differentiation between sampled populations was analyzed using pairwise fixation indices (pairwise F_{ST}), and tested for significance using permutation procedures. It is important to note that F_{ST} is a statistic used when trying to determine an effective estimate of θ, or the correlation of genes across individuals in a population as a measure of coancestry, and is not an estimate of F_{IS}, which is a direct measure of inbreeding within populations (Clarke and Weir 1984). Pairwise F_{ST} was used as a measure of subpopulation differentiation rather than a metric modeled after a step-wise mutation model, such as R_{ST}. A step-wise mutation model may be more theoretically accurate in describing the mechanism of genetic differentiation of microsatellite alleles, however studies of population structure in real populations have shown that a step-wise model rarely holds true (Excoffier et al 1992).

2.6 Principal Coordinates Analysis (PCoA)

A principal coordinates analysis, or multidimensional scaling, was performed on pairwise F_{ST} values to visualize relationships among peatland populations using Genalex 6.5. Classical multidimensional scaling uses an input matrix of pairwise distances and develops coordinate axes in multiple dimensions, these axes minimize the distances among points, also known as minimizing strain, and attempt to explain the maximal variation in distances in the fewest axes possible (Orlóci 1978; Peakall and Smouse 2012). The model describes proportionately less and less variance with each successive axis, and if there are no distinct groups within the input matrix, in our case pairwise F_{ST} values, the majority of the variance should be explained in the first two or three axes (Peakall and Smouse 2012). The method used for this analysis follows that outlined by Orlóci (1978) on basic multidimensional scaling.

2.7 Isolation by Distance analysis

Analysis of IBD in *F. fletcheri* was performed using standard Mantel tests of correlation and Mantel correlograms. A geographic distance matrix was computed using the Euclidean (straight-line) distance (in meters) between the geometric center of each sampled peatland and the center of all 14 other sampled peatlands. To determine the geometric centers of peatlands, Ontario Ministry of Natural Resources (MNR) land cover maps of Algonquin Park were used where possible. Peatlands that fell outside of the park boundaries (Cauliflower rd. and Brent rd. into Cedar, a.k.a. cfr and brl) were measured using images taken from the Ontario MNR provincial land cover index for southern Ontario (Table 1)(Ontario Ministry of Natural Resources, Forest Resource Index, province wide, $5m^2$ resolution, 2005). The matrix of Euclidean distances was calculated using arcGIS 10.1 and Geospatial Modeling Environment 0.7.2.1 (GME), an external program with accessory functions for spatial analysis in arcGIS 10.1 (ArcGIS Ver. 10.1, ®ESRI, Redlands, CA; GME 0.7.2.1 Spatial ecology LLC, Queensland, AUS). The command *pointdistances* was used in GME and set to measure straight-line distances from the geometric center of each peatland to the center of the other 14 sampled sites.

A second matrix of transformed pairwise fixation indices (F_{ST}) was developed using pairwise F_{ST} values calculated in Genalex 6.1 (see section 2.5) and transformed in *R* 2.14.1 using the following equation (Venables et al. 2002):

$$f = \frac{F_{ST}}{(1 - F_{ST})}$$

The linearization transformation above is recommended by Rousset (1997) for IBD analysis in two dimensional landscapes.

Significance of the Pearson product-moment correlation between pairwise geographic distance of peatlands and transformed pairwise fixation indices was determined using the Mantel test with 99999 permutations using the function *mantel()* within the ecology package *Vegan* in R 2.14.1 (Venables et al. 2002; Oksanen et al. 2010). Patterns of spatial correlation were examined using Mantel correlograms using geographic classes of 10 km.

Sturges' Rule was used to determine the most effective number of break points when developing our Mantel correlograms using the function *pmgram()* from the *Ecodist* package (Goslee and Urban 2007; Mantel 1967). Sturge's rule is as follows:

$$b = 1 + 3.3\log(n)$$

In this equation n represents the number of pairwise comparisons and b represents the correct number of breaks to be used in the correlogram.

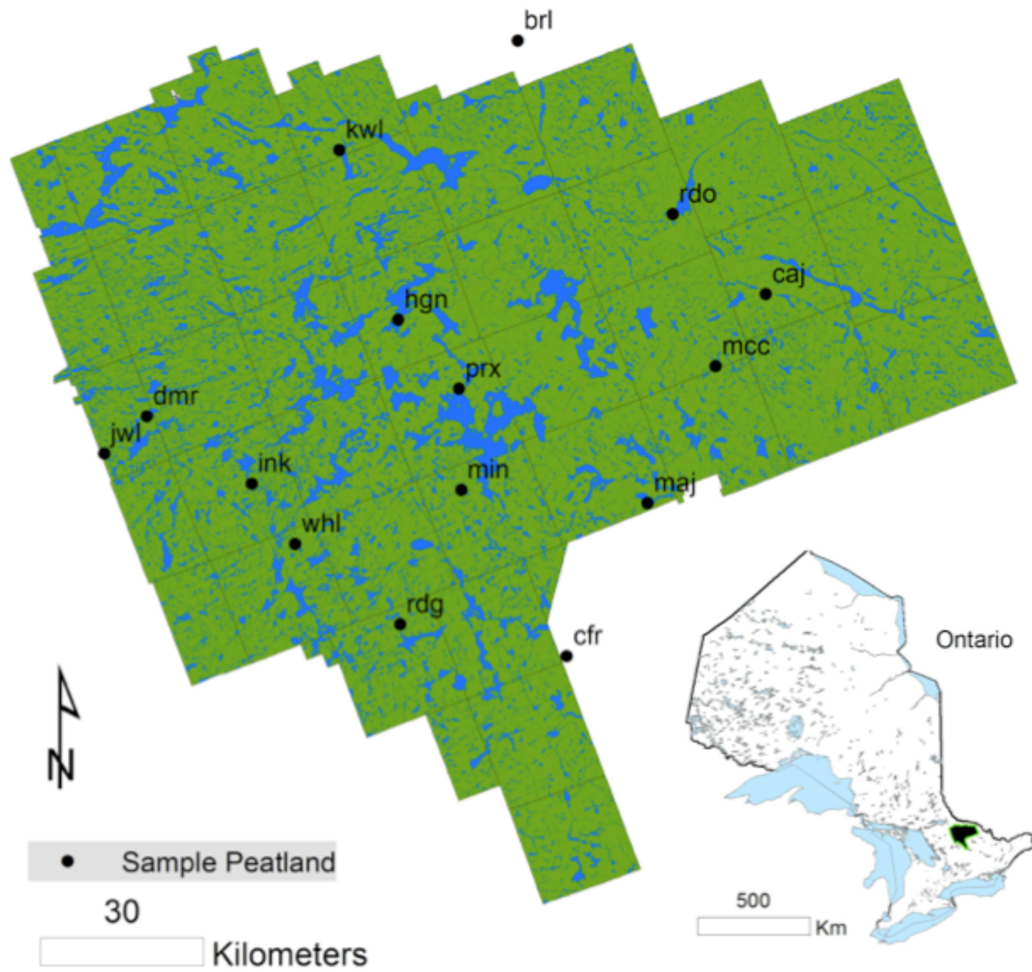

Figure 2: Map of Algonquin Provincial Park (Ontario, Canada) showing all fifteen sampled peatland sites. Samples were collected from mid to late summer of 2012, scouting was performed in the early spring of the same summer.

2.8 Isolation by resistance

To test the hypothesis that *F. fletcheri* uses riparian and wetland areas as dispersal corridors between populations, a "resistance surface" was built in ArcGIS that would reflect this hypothesis, and least cost paths (LCP) between sites were calculated. A resistance surface is a digitized matrix representing a landscape and the permeability of the landscape to animal movement (McRae 2006). The matrix is composed of a continuous series of spatially referenced pixels, and each pixel is given a resistance value or "cost" for the organism to cross it (Koen et al. 2012). Different landcover classes are assigned different costs to traverse; the easier the landscape is to move through for a given species, the lower the associated cost for that landcover type on the resistance surface (McRae 2006; Koen et al. 2012). Cost values can be assigned from independent movement data (e.g. telemetry or mark recapture), based on expert opinion, or based on a specific hypothesis to be tested about movement through the landscape (as in this study). The total cost of moving across a series of pixels within the resistance surface represents the cost of migration for an animal as it moves across the landscape. A least cost pathway is the route between two sites over a resistance surface (in our case, two peatlands) that would have the lowest total accumulated cost (sum of resistance values for each pixel traversed) for an individual, and is a byproduct of both distance traveled and difficulty of interceding terrain (Etherington and Holland 2013).

Land cover maps supplied by the Ontario Ministry of Natural Resources were used to create the resistance surface (MNR forest resource index, individual vector format, Algonquin Park, 2005). Wetland, river and open water land covers were considered to be conductive to *F. fletcheri* movement and merged into a single vector layer. Vector layers vary from raster datasets as they are not defined as organized, linear collection of pixels, but rather are spatially oriented geometric figures, consisting of series of polygons, lines and points, with no data on interceding space outside those polygons unless specifically attributed and amended (Peuquet 1984). Vector layers for each land class were supplied separately by the MNR. Vector maps were used instead of available raster formatted aerial MNR forest resource indices. This was done to maintain connectivity between sites; vector format ensured that small aquatic tributaries were maintained as complete

corridors, rather than being corrupted by overshadowing of terrestrial land cover, as often occurs in raster maps developed using aerial photography containing overlapping land cover classes (Heywood et al. 2006). The merged vector layer was then converted to a raster layer using arcGIS's *vector to raster* toolbox, and was given a resolution of $5m^2$. A raster grid represents an actual landscape artificially through an equally sized repeating tessellation (tessellations are repeated connections using a specific geometric shape; in this case, squares). This tessellated surface creates a matrix of data points, where each individual square is given a value or values that can represent a specific component of the landscape that is being isolated and analyzed (in this case, land cover type). Nearest neighbor conversion of vector to raster was used to maintain connectivity of raster polylines. In the newly created raster, two resistance values were used to create a binary pass/impasse resistance matrix. Terrestrial ecosystems, such as grasslands, upland forest, and any other landcover type that fell outside of wetland or aquatic environment were considered impassable and were graded as significantly more difficult to traverse than wetland, river, and open water. Landcover hypothesized to be non-passable was given a resistance value 50 times greater than landcover considered passable (resistance value of 50 for non-passable habitat; and 1 for passable habitat). Therefore to test the LCP hypothesis, a binary raster surface with strong contrast between passable and impassable land was developed. This binary comparison of landcover types represents the most direct test of the hypothesis that *F. fletcheri* movement is limited to wetland and aquatic areas. A strong contrast in resistance of passable and impassable landcover means that least cost pathways will strongly follow riparian and wetland corridors, and only allow for very small "jumps" over non-passable landcover before resistance cost is heavily affected by non ideal landcover traverses. Biologically, this reflects a hypothesis of very strong reliance on wetland, riparian, and water covered areas for movement, and a strong tendency to avoid movement over other landcover types for *F. fletcheri*.

After the initial resistance surface was developed, the 13 sites that were located within the park boundaries (which had sufficient data on landscape composition within the resistance surface) were overlaid the sites CFR and BRL were removed. An LCP project in arcGIS 10.1 was then created using the *project manager* toolset, which consisted of an automated set of inputs to calculate LCPs between each individual peatland and the other

12 sampled peatlands (Appendix 1). Slope Digital Elevation Models (DEMs) were used to allow for inherent data on directionality in watersheds, but were normalized to 0 in keeping with the airborne nature of *F. fletcheri*, which, it was hypothesized, should not be affected by direction of water flow. Least cost pathways were allowed with diagonal connectivity using Manhattan distances instead of Pythagorean connections and eight neighbor joining of cells to avoid overestimations of weighted distance values due to a "staircase" effect (Heywood et al. 2006).

The significance of partial correlation between pairwise LCP distance and transformed pairwise F_{ST}, controlling for Euclidean geographic distance, was tested using a partial Mantel test in R 2.14.1 using the package *mantel.partial()* from the *Vegan* library (Venables et al. 2002; Oksanen et al. 2010). A partial Mantel functions similarly to a standard Mantel. However, in addition to the initial two compared variables, the partial Mantel also includes a third matrix of data points. This third matrix is used as an explanatory variable (geographic distance), to better isolate the effect of a specific independent variable (land cover type) on the dependent variable (genetic variation, represented in this instance by transformed F_{ST}). A partial correlogram was developed using the package *pmgram()* from the *Ecodist* library (Mantel 1967; Venables et al. 2002; Goslee and Urban 2007; Legendre and Fortin 2010) This partial Mantel analysis is also referred to as an analysis of isolation by resistance in further components of this paper (IBR analysis) (McRae 2006; Guillot and Rousset 2013).

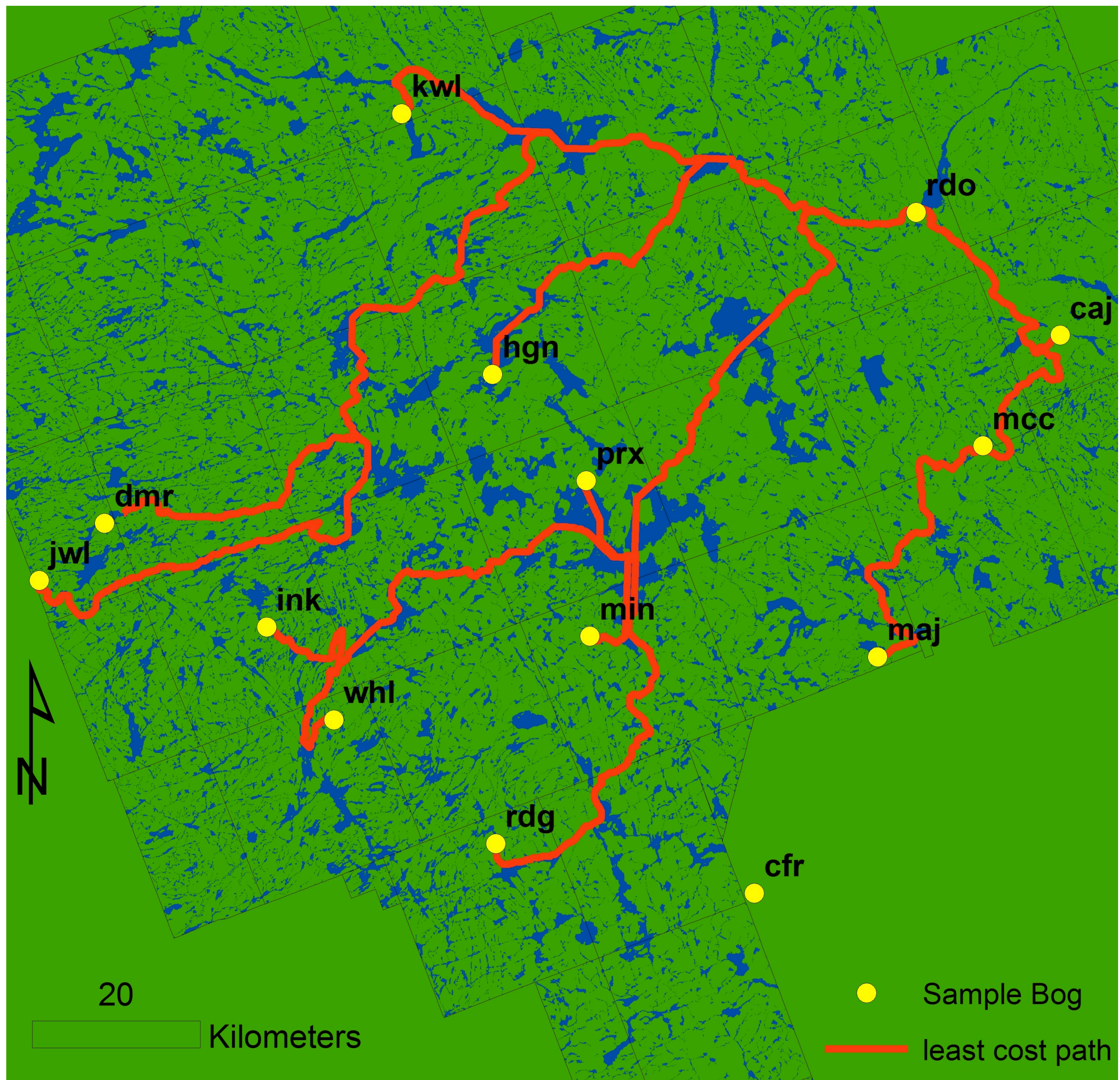

Figure 3: Least-cost paths between Caracajou peatland and each of the twelve other sampled peatlands in the park. Paths represent routes of least "resistance" or "cost", assuming that the resistance provided by aquatic, riparian and wetland landcover is two percent that provided by other land cover classes. Note that site cfr is not included in the development of least cost path analyses. Cfr is one of two sites (along with brl) that fell outside of the park boundaries. As a result, landcover information was not available for cfr and its surrounding region, and a least cost path could not be developed for it.

3 Results

3.1 Microsatellite variability

DNA was extracted from 641 individual larvae, from which the alleles of selected microsatellite loci were successfully amplified for 613 specimens. Specimens that failed to amplify initially were re-run, and, if they failed a second time, were removed from further analysis. Any individual that had more than two absent loci was also removed from analysis. Out of the 613 specimens for which microsatellites successfully amplified, four individuals were missing alleles at one locus, and one individual was missing alleles at two loci.

The total number of alleles observed at each locus ranged from two to twelve within each peatland, and all eleven loci were polymorphic across all fifteen peatlands sampled. From this it was determined that there was a moderate amount of microsatellite genetic diversity within the 15 separate sites, with mean allelic diversity (N_A) ranging from 3.40 (Algonquin radio observatory, rdo) to 6.00 (Brent lake road, brl) and an expected heterozygosity (H_E) of between 0.431 (Minor Lake, min) and 0.569 (Brent Lake road, brl) (Table 1). Allelic richness (A_R) ranged from 3.20 to 4.36 (Table 2). Therefore, A_R did remove some of the variation seen in the initial measure of N_A that was caused in part by varied sample size across sites (Table 1).

Locus FF104 was not in Hardy Weinberg equilibrium (HWE) in twelve of fifteen populations sampled, with observed heterozygosity being significantly lower than expected heterozygosity. After this observation, FF104 was excluded from any further analyses, which were then based on the ten remaining loci. The removal of FF104 is in keeping with the findings of Rasic & Keyghobadi (2012a), who found that there was excess homozygosity observed for this locus and also removed it form further analysis. Of the remaining loci, 8 sites were found to be out of HWE across the 150 run at an unadjusted alpha of 0.05. Bonferroni correction was then used to create a multiple comparison adjusted alpha, after which all loci at all sites were found to be in Hardy Weinberg Equilibrium (Wright 1992). Linkage disequilibrium was also tested between all pairs of sites for each of the 11 loci. Of the tested pairs, 70 locus pairs were found to be in

linkage disequilibrium based on 990 pairwise comparisons, with no two loci being consistently (more than twice) linked in separate sample sites (each site contained 66 pairwise comparisons).

Table 2: metrics of genetic diversity for *F. fletcheri* in 15 sampled peatlands. N_A represents mean allelic diversity averaged across 10 loci; A_R represents allelic richness, which is the mean allelic diversity over all loci adjusted for sample size; H_E represents expected heterozygosity.

Peatland	**N_A(±SE)**	**A_R**	**H_E(±SE)**
min	4.50(0.86)	3.36	0.43(0.06)
cfr	4.60(0.40)	3.36	0.46(0.06)
maj	3.90(0.55)	3.21	0.46(0.05)
ink	5.40(0.78)	3.71	0.50(0.05)
whl	4.90(0.88)	3.65	0.53(0.05)
brl	6.00(0.83)	4.36	0.56(0.06)
kwl	5.30(0.97)	3.67	0.51(0.06)
mcc	5.00(0.93)	3.76	0.54(0.06)
rdg	4.90(0.71)	3.80	0.51(0.05)
jwl	4.60(0.70)	3.51	0.51(0.05)
dmr	5.20(0.81)	3.72	0.54(0.04)
rdo	3.40(0.56)	3.20	0.48(0.07)
caj	4.50(0.65)	3.54	0.47(0.05)
hgn	5.40(0.82)	3.80	0.54(0.04)
prx	5.10(0.84)	3.73	0.52(0.04)

3.2 AMOVA

Globally, three percent of microsatellite variance could be attributed to genetic differentiation among populations in different peatlands (Table 4). Four percent could be attributed to within-population variance, and the remaining ninety three percent was attributed to within-individual variance (Table 4). Global F_{ST} was estimated at 0.027 and significantly greater than zero ($p<0.001$). F_{ST} was used for further analyses as levels of expected heterozygosity within sites was not overly inflated (>0.9) and therefore should not arbitrarily skew F_{ST} results through compression (Meirmans and Hedrick 2011).

Pairwise F_{ST} values showed significant genetic differentiation between most pairs of sampled populations at $\alpha = 0.05$ (Table 3). Notably however, several populations were not significantly differentiated from Hogan Bog (hgn), a large centrally located peatland in the park (Table 1, Table 4, Figure 2). Conversely, Algonquin radio observatory (rdo) had the highest differentiation from other populations, with its lowest pairwise F_{ST} being higher than that between any other two sites. (Table 3).

Table 3: Matrix of pairwise F_{ST} values for populations of *F. fletcheri* from different peatlands across Algonquin Provincial Park. * indicates F_{ST} is not significantly greater than zero; p>0.05.

	min	cfr	maj	ink	whl	brl	kwl	mcc	rdg	jwl	dmr	rdo	caj	hgn
cfr	0.027													
maj	0.034	0.036												
ink	0.053	0.029	0.024											
whl	0.055	0.029	0.037	0.019										
brl	0.054	0.035	0.043	0.027	0.029									
kwl	0.041	0.016	0.037	0.020	0.013	0.025								
mcc	0.049	0.020	0.050	0.027	0.020	0.037	0.018							
rdg	0.049	0.013	0.041	0.006*	0.014	0.027	0.014	0.004*						
jwl	0.030	0.009	0.019	0.010	0.002*	0.020	0.004*	0.012	0.008					
dmr	0.058	0.038	0.025	0.011	0.007*	0.032	0.020	0.034	0.024	0.009				
rdo	0.169	0.122	0.092	0.077	0.080	0.103	0.119	0.080	0.085	0.085	0.073			
caj	0.041	0.027	0.027	0.034	0.022	0.044	0.031	0.038	0.028	0.015	0.033	0.108		
hgn	0.043	0.019	0.041	0.012	0.011	0.026	0.006*	0.005*	0.001*	0.003*	0.019	0.101	0.019	
prx	0.052	0.014	0.034	0.015	0.016	0.032	0.012	0.019	0.011	0.004*	0.019	0.098	0.014	0.004*

Table 4: AMOVA table for 15 *F. fletcheri* populations across Algonquin Provincial Park based on genotypes at 10 microsatellite loci. Source indicates the nested scale that is being analyzed. df represents degrees of freedom, SS is the sum of squares, MS the mean squares, Est. Var. is the estimated variance for each calculation, and % indicates the total percentage of molecular variance that can be attributed to each scale of population organization.

Source	df	SS	MS	Est. Var.	%
Among Pops	14	119.85	8.56	0.07	3%
Within-Pops	598	1598.35	2.67	0.11	4%
Within-Indiv.	613	1503.87	2.45	2.45	93%
Total	1225	3222.08		2.64	100%

3.3 Principal Coordinates Analysis

PCoA results indicated that populations in Algonquin radio observatory, Major lake, and Minor lake peatlands (rdo, maj, and min), were highly differentiated from the other sample populations, as well as from each other. The first three principal axes accounted for 76.51% of variance seen in pairwise F_{ST} between populations, with 64.24% described by only the first two principal axes (Figure 4).

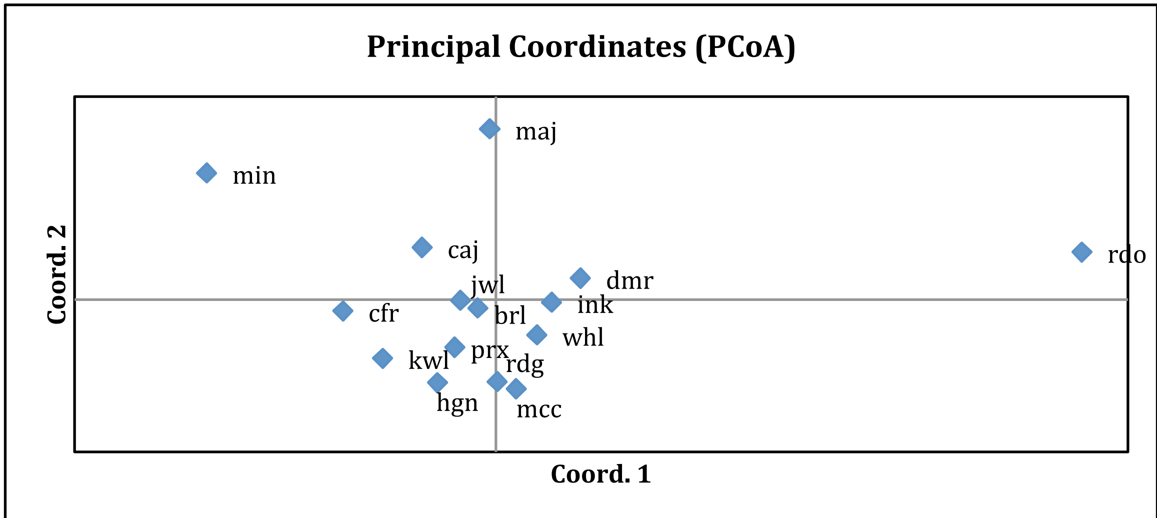

Figure 4: Principal coordinates analysis of genetic differentiation for all 15 sampled populations of *F. fletcheri* sampled based on pairwise genetic distance matrix (F_{ST}) between peatlands. The first two principal coordinate axes are shown. The unit of measure for each axis are not shown because PCoA uses eigenvectors and eigenvalues derived from the original dataset that do not have specific units of measure.

3.4 Isolation by distance

A scatter plot of transformed fixation indices (transformed F_{ST}) and geographic distance for all sites sampled as well as a Mantel correlogram showed no clear pattern of spatial correlation, indicating a lack of IBD (Figure 5, Figure 7). The overall Mantel statistic r across all 15 sites was 0.0543, with a p-value of 0.378. This is also indicative of a lack of correlation between genetic diversity and simple Euclidean distance, and is non-supportive of a genetic structure that is undergoing IBD given the scale sampled and molecular markers used. However, upon examination of the principal coordinates analysis for possible outliers that could be interfering with the correlation results, Algonquin radio observatory, Minor lake, and Major lake rd. (rdo, min, and maj) sites were removed from the Mantel analyses, and the overall Mantel correlation statistic increased to 0.49 (r), with a significance of 0.0006 (Figure 6).

A second correlogram calculated after the removal of outlier sites was indicative of IBD across the remaining 12 sites, with genetic correlation staring high in spatially close sites, and decreasing as the distance between sites increases (Figure 8). It is also important to note that the initial distance class was shown to be insignificant as it represented sites that were within 10.5 km, and not enough paired sample peatlands fell into this category to provide a significant result (Table 6).

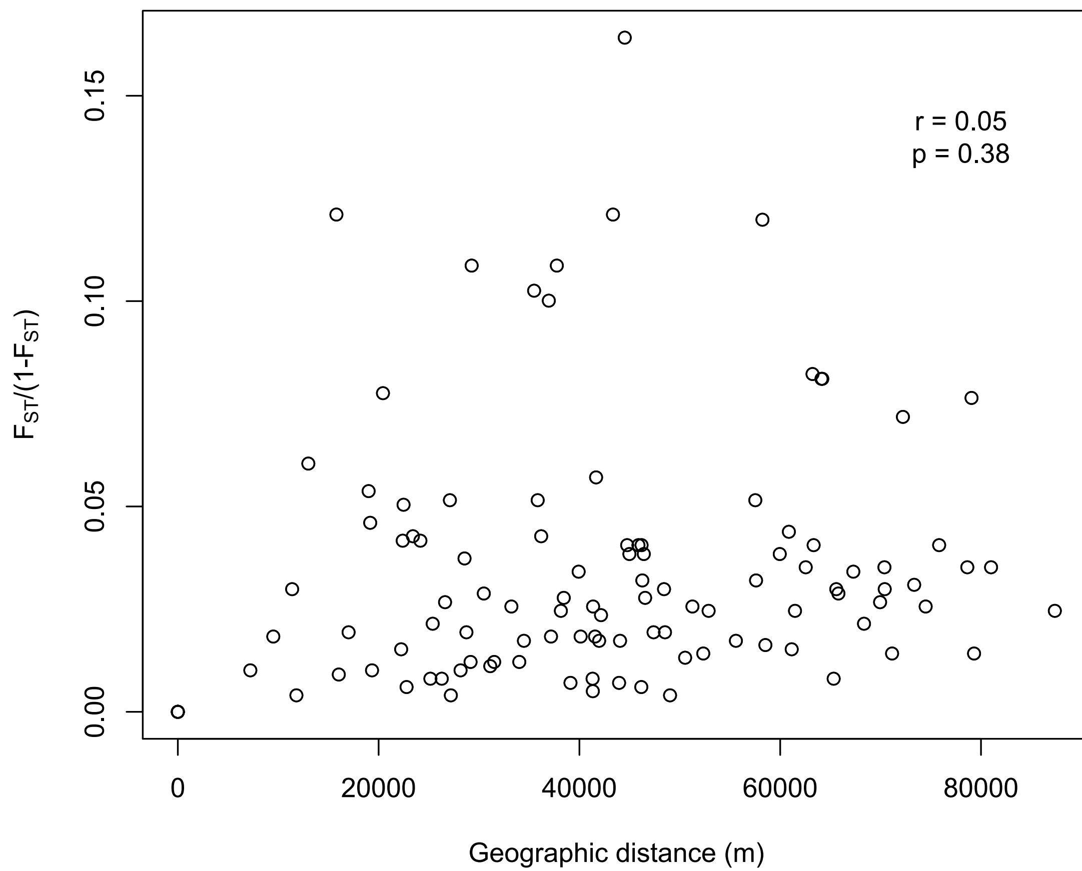

Figure 5: Scatter plot of transformed pairwise fixation indices ($F_{ST}/(1-F_{ST})$) relative to geographic distance for all 15 sampled peatland sites across Algonquin Provincial Park with Mantel Pearson product-moment correlation value (r) and corresponding p-value (p) included.

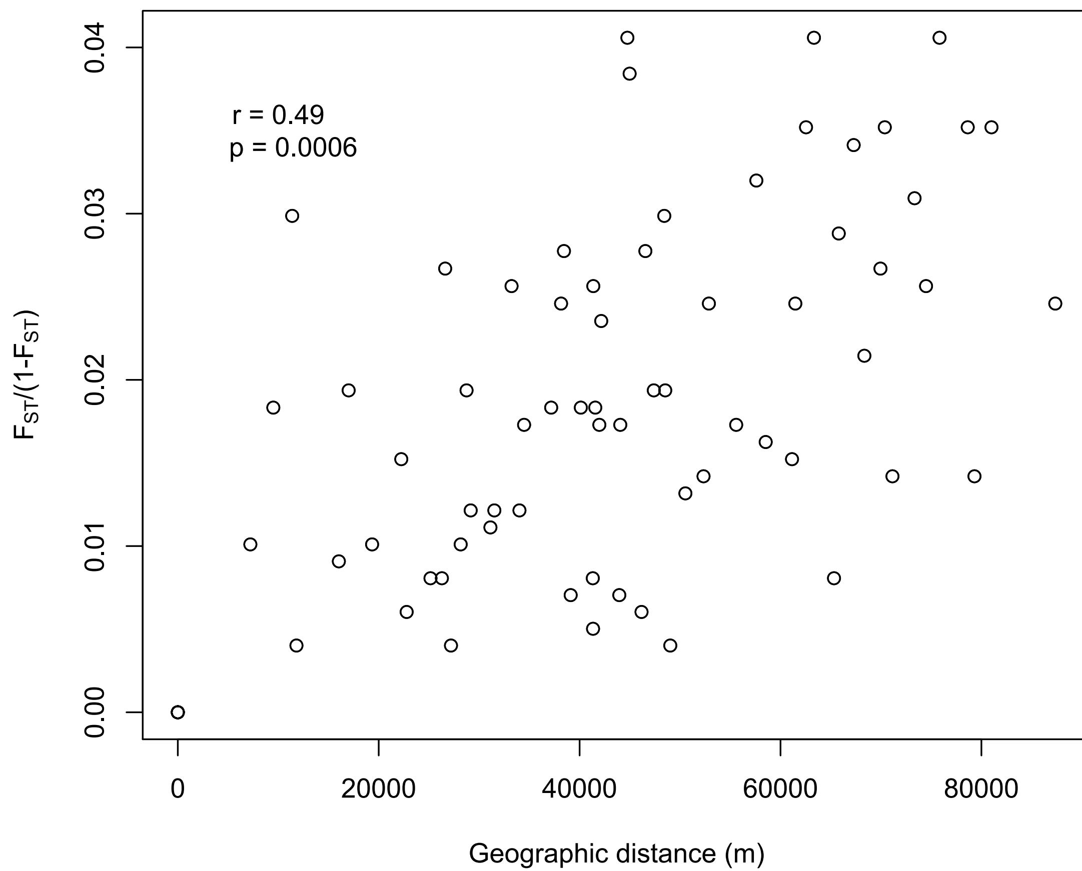

Figure 6: Scatter plot of transformed pairwise fixation indices ($F_{ST}/(1-F_{ST})$) relative to geographic distance for 12 sampled peatland sites across Algonquin Provincial Park after removal of outlier sites identified in PCoA analysis. With Mantel Pearson product-moment correlation result (r) and corresponding p-value (p) included.

Table 5: Correlogram values for transformed pairwise F_{ST} over 8 distance classes for 15 *F. fletcheri* populations across Algonquin Park *r* is the value of the Mantel correlation, and (*p*), the associated p value (significance) for each distance class.

Distance class (median, m)	**n in group**	**r**	**P**
5008.37	2	0.71	0.26
15025.12	9	-0.12	0.74
25041.87	18	0.11	0.61
35058.62	16	-0.11	0.63
45075.37	24	0.05	0.79
55092.12	10	0.01	0.97
65108.87	14	-0.13	0.63
75125.62	10	-0.06	0.87

Table 6: Correlogram values for transformed pairwise F_{ST} over 7 distance classes for populations of *F. fletcheri* in Algonquin Provincial Park across 12 peatland sites after the removal of rdo, min, and maj populations.

Distance class (median, m)	n in group	r	p
5723.86	3	0.03	0.97
17171.57	6	0.89	0.02
28619.28	11	0.60	0.04
40067.00	16	0.00	0.99
51514.71	10	0.21	0.51
62962.42	10	-0.58	0.05
74410.14	8	-0.80	0.02

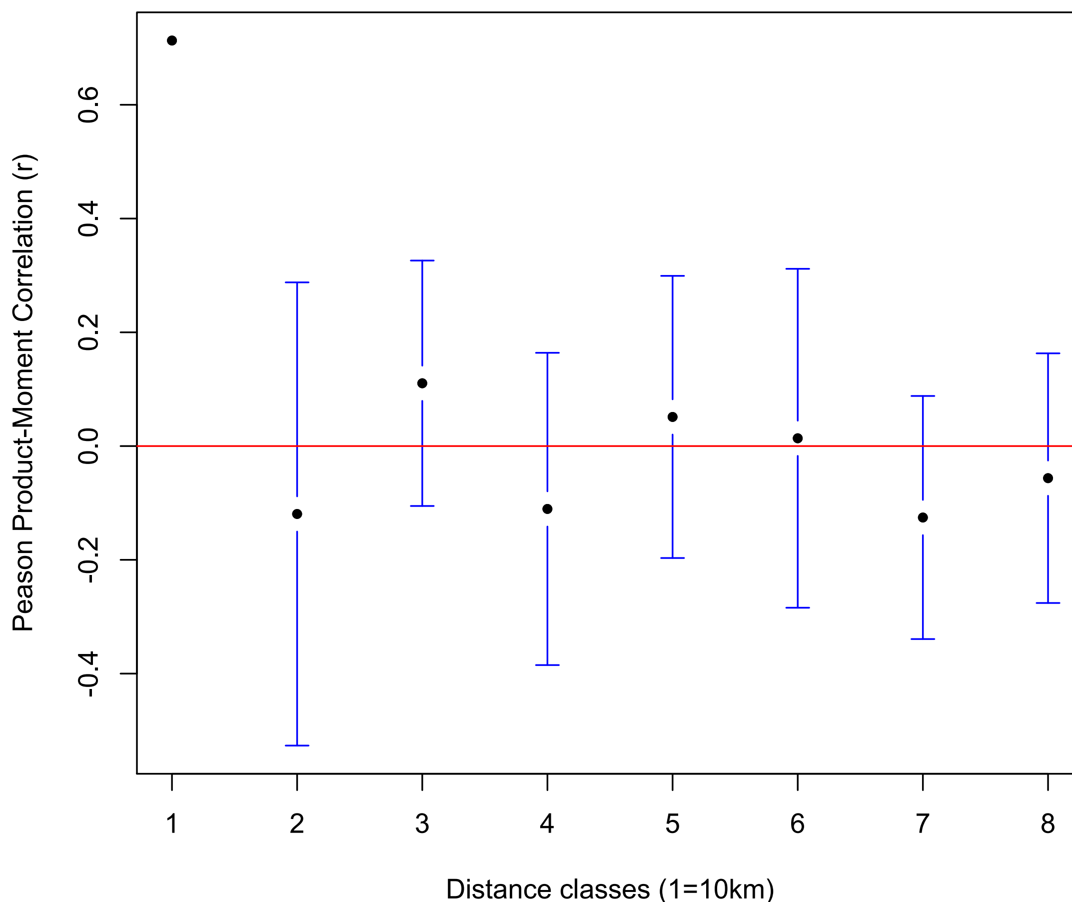

Figure 7: Mantel Correlogram of Pearson-Product Moment correlation (r) over 10 km distance classes for all 15 sampled sites. Sites that fell within each 10 km distance class were separated and individual Mantel tests were run using transformed F_{ST} versus geographic distance. No significant trend in genetic correlation relative to distance that would indicate IBD is shown. Error bars indicate 95% significance level. Distance class 1 (5 km median) shows no error bars due to the use of only 2 sites with 1 corresponding distance between the (therefore no variance in correlation can be calculated for that class).

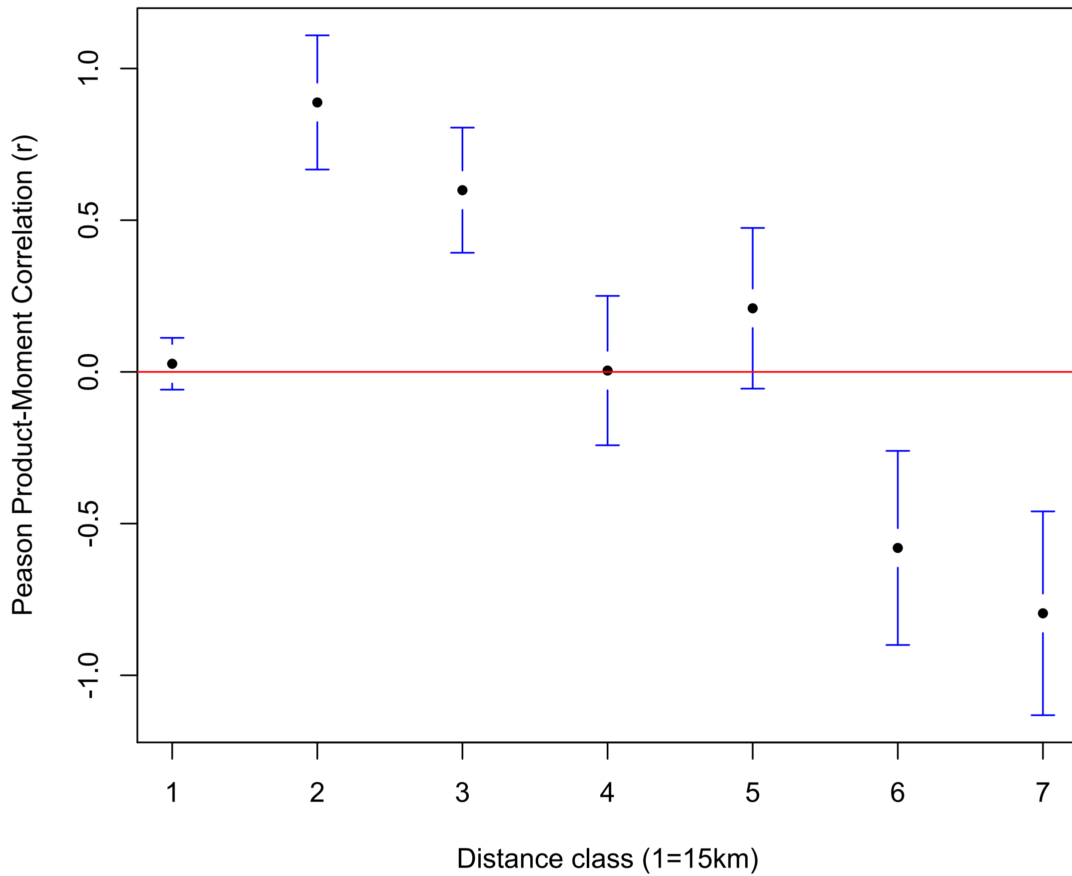

Figure 8: Mantel correlogram of Pearson Product Moment Correlation (r) versus geographic distance. Distance classes of 15 km were used for *F. fletcheri* populations across Algonquin Park after the removal of PCoA outlier sample sites. Distance classes have been increased to 15 km in accordance with Sturge's Rule for implementing break points.

3.5 Isolation by Resistance (IBR)

No correlation was found between LCP distance and transformed F_{ST} after controlling for Euclidean distance for all 15 sampled populations (r= -0.28, p=0.99). After removing Algonquin radio observatory, Minor lake, and Major lake rd. (rdo, min and maj), there was still no significant correlation between LCP distance and transformed F_{ST}, controlling for Euclidean distance (r= - 0.18, p= 0.91). These general results did not change when both LCP and Euclidean distances were log transformed, or when Nei's standard genetic distances were used in place of transformed F_{ST} (results not shown). Correlograms also showed no pattern of spatial correlation in transformed F_{ST} values based on LCP resistance cost classes (Table 8, Table 9, Figure 9, Figure 10, Figure 11, Figure 12).

Table 7: Matrix of pairwise "resistance cost" least cost path values between peatlands from which *F. fletcheri* were sampled.

	kwl	jwl	dmr	ink	hgn	prx	min	whl	rdg	maj	mcc	caj	rdo
kwl	0												
jwl	101954	0											
dmr	57198	124812	0										
ink	77463	43249	100128	0									
hgn	47668	68796	53907	44116	0								
prx	90829	71795	60741	44613	27005	0							
min	98316	83735	62542	56557	43303	18085	0						
whl	96315	62651	65399	17882	62687	45216	57136	0					
rdg	121186	90806	92022	43224	87566	47884	37242	32639	0				
maj	116398	112095	80628	84918	86678	46383	46168	85616	77659	0			
mcc	90449	131795	35806	104617	87151	66196	67987	105422	97472	31847	0		
caj	81104	148417	130047	123448	77818	84642	86442	123886	115923	52823	18756	0	
rdo	56962	124067	105642	99117	53670	60610	62309	99965	91770	80479	33751	24145	0

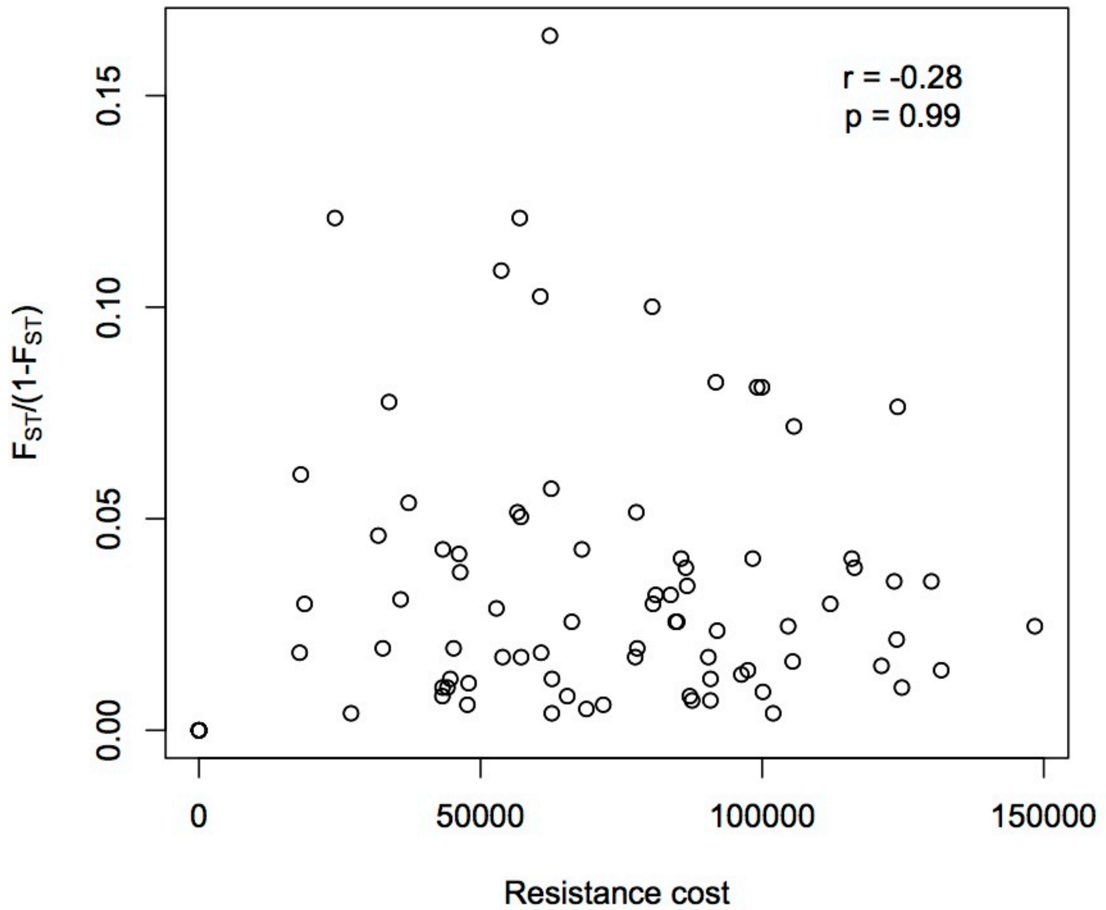

Figure 9: Scatter plot of transformed pairwise fixation indices ($F_{ST}/(1-F_{ST})$) relative to resistance cost for 13 sampled peatland sites within Algonquin Provincial Park with a partial Mantel Pearson product-moment correlation with geographic distance as an explanatory variable result (r), and corresponding significance value (p).

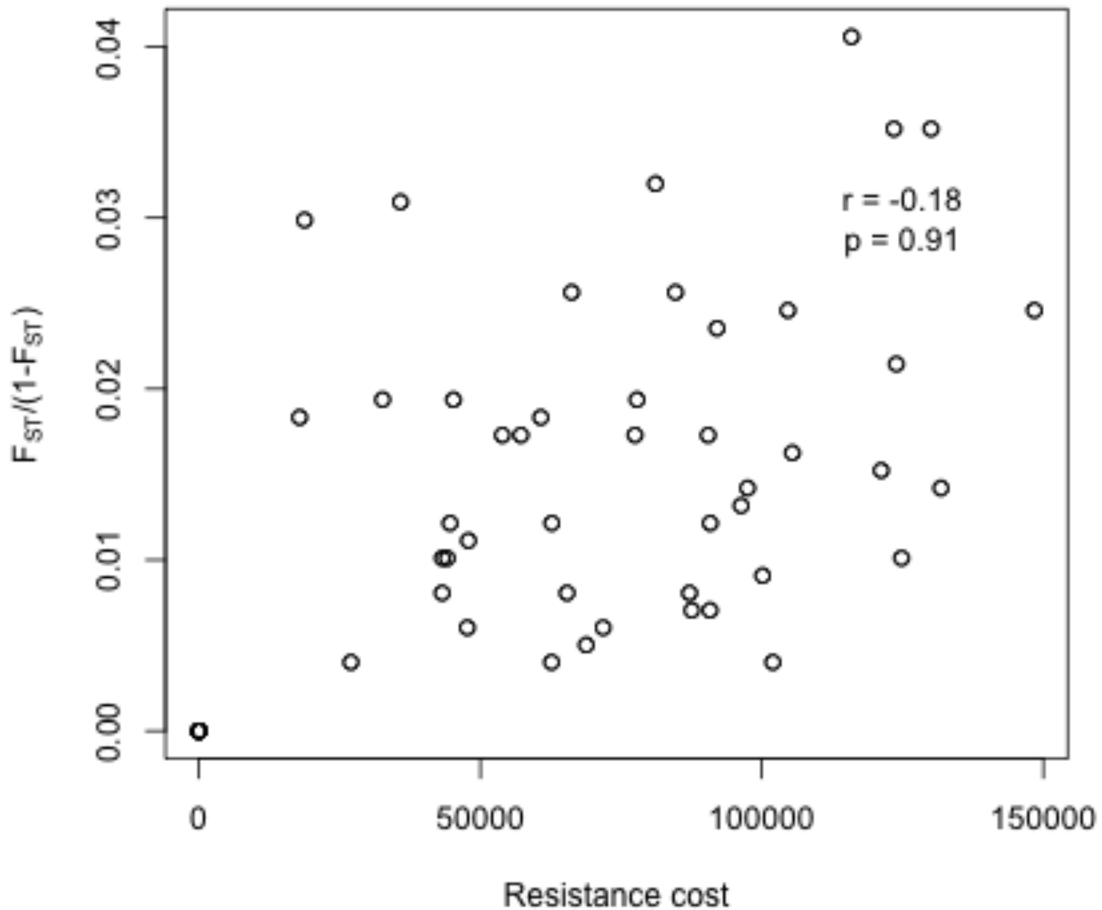

Figure 10: Scatter plot of transformed pairwise fixation indices ($F_{ST}/(1-F_{ST})$) relative to resistance cost for 10 sampled peatland sites within Algonquin Provincial Park with a partial Mantel Pearson product-moment correlation with geographic distance as an explanatory variable result (r), and corresponding significance value (p). Note that though the scatter plot shows a general positive correlation between resistance cost and transformed fixation, the associated partial Mantel result is not significant. This is because the relationship is largely due to the distances between paired sites, and when geographic distance is compensated for in analysis (Figure 12), there is no visible association of genetic similarity and landscape resistance.

Table 8: Correlogram values for transformed pairwise F_{ST} for 13 *F. fletcheri* populations over 7 distance classes, based on LCP resistance costs between peatland sites, based on total calculated LCP cost resistance.

Range median (resistance cost)	n in group	r	p
18648	2	-0.23	0.66
37296	8	-0.45	0.26
55944	13	0.22	0.37
74591	15	-0.33	0.29
93239	19	0.15	0.53
111887	10	0.06	0.90
130535	9	0.15	0.55

Table 9: Correlogram values for transformed pairwise F_{ST} for 10 *F. fletcheri* populations (rdo, min & maj excluded) over 6 distance classes, based on total calculated LCP cost resistance.

Range median (resistance cost)	**n in group**	**r**	**p**
21756	2	-1.62	0.02
43512	5	-0.10	0.76
65268	10	0.14	0.63
87023	8	-0.10	0.76
108779	12	0.46	0.09
130535	6	-0.65	0.10

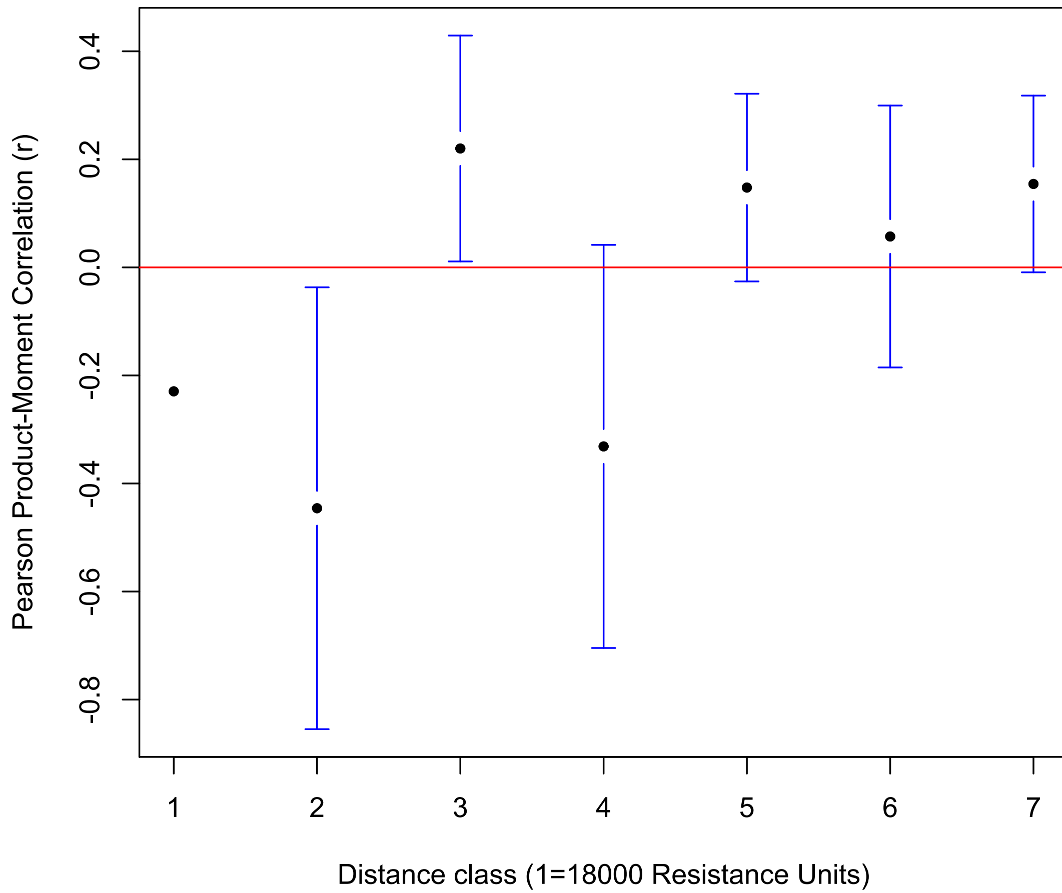

Figure 11: Partial Mantel Correlogram for all sites, comparing transformed fixation indices (Transformed F_{ST}) versus cost resistance, while controlling for Euclidean distance by using geographic distance as an explanatory variable.

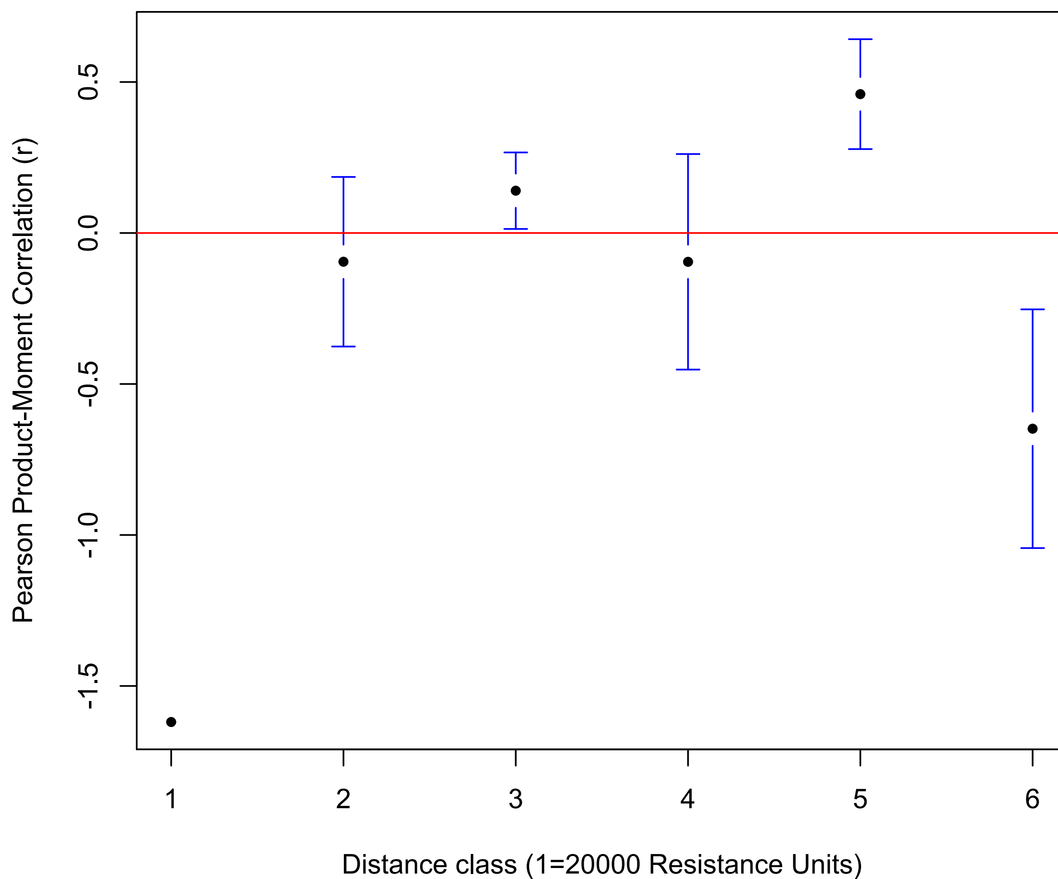

Figure 12: Partial Mantel correlogram for *F. fletcheri* populations across Algonquin Provincial park comparing transformed F_{ST} versus cost resistance while controlling for Euclidean distance as an explanatory variable. after the removal of outlier sites rdo, maj, min as determined by Principal Coordinates analysis.

4 Discussion

4.1 Within-Site Variation

Among populations of *F. fletcheri* sampled across Algonquin Provincial Park, levels of genetic variation were comparable to those previously reported for populations of this species from the same region (Rasic and Keyghobadi 2012a). All observed microsatellite loci were polymorphic, with 2 to 12 different observed alleles at each locus across all sampled peatlands. The number of alleles observed at each locus across the entire data set was slightly higher than that reported by Rasic and Keyghobadi (2012a), as would be expected given that populations were sampled at a larger spatial scale and included previously unsampled populations over a greater area in this study (Fortin and Dale 2005; Anderson et al. 2010).

Two populations that were sampled in this study, Wolf Howl and Minor lakes, were also sampled by Rasic and Keyghobadi (2012a) in 2008 and 2009. Within-population metrics of genetic diversity for these populations were very similar in the two studies. Wolf Howl Bog continued to have a fairly high expected heterozygosity (H_E) of 0.53±0.05 relative to 0.51±0.04 during the previous study, and Minor lake had an H_E of 0.43±0.06 compared to 0.46±0.01 in the previous study (Rasic and Keyghobadi 2012a). Consistent levels of expected heterozygosity across sampling years indicate that there is little temporal effect on the partitioning of genetic diversity across populations in the flesh fly in this region, and suggest stable populations within peatland sites (Allendorf 1986; Rasic and Keyghobadi 2012a).

A global AMOVA partitioned genetic diversity in *F. fletcheri* among three levels: within-individuals, within-sites, and among-sites. In this analysis, 93 and 4 percent of total genetic variation in the population was ascribed to within-individuals and within-sites, respectively (Table 4). Such results are typical of microsatellite loci, which are highly variable due to the high rates of slipped-strand mutation associated with tandem sequence repeats (Weber and Wong 1993; Slatkin 1995). As a result, the vast majority of genetic diversity at microsatellite loci occurs within-individuals (Slatkin 1995; Meirmans and Hedrick 2011).

4.2 Among site variation

The global AMOVA analysis ascribed 3 percent of total genetic variability among populations, and indicated a global fixation index (F_{ST}) of 0.027, which was significantly greater than zero. This result is indicative of significant genetic differentiation across the sampled region (Table 4)(Guillot and Rousset 2013).

Pairwise F_{ST} values between populations in different peatlands showed that there was significant genetic differentiation in the majority of sampled populations (Table 3). However, there were exceptions to this overall trend, most notably Hogan Lake peatland (hgn), which was not significantly differentiated from five of the fourteen other populations: kwl, mcc, rdg, jwl and prx. Furthermore, although the remainder of pairwise F_{ST} estimates involving Hogan lake were significantly greater then zero, the estimates of pairwise F_{ST} were low relative to those among other pairs of sites (Table 3). Therefore, Hogan Lake demonstrates a markedly lower level of genetic differentiation than all other populations. Hogan Lake is located centrally within the sampled region, and is also the largest peatland site sampled in terms of area (Figure 2). The central location and large size of this habitat patch could result in a marked increase in movement and gene flow to and/or from this site, which would lead to lower levels of differentiation from other populations. Given the large size of the Hogan lake peatland, it likely supports a relatively large population of *F. fletcheri* that could act as a source of immigrants to other populations. Therefore, it may represent an important source population within the network of *F. fletcheri* populations in Algonquin Park.

In contrast to Hogan lake, three sampled populations showed distinctively high levels of genetic differentiation: Major lake road bog (maj), Minor lake bog (min), and Algonquin radio observatory (rdo). These sites had some of the highest estimated pairwise values and the PCoA analysis also showed them to be highly differentiated from all other sampled populations (Figure 4, Table 3). The high levels of genetic isolation observed for these populations could be the result of three possible processes or factors. The first possibility is small sample size. A small sample size can skew calculations of population genetic indices, and artificially inflate indices of genetic differentiation such as F_{ST} values (Willing et al. 2012). While sample size could certainly have contributed to the high

levels of differentiation observed for Algonquin radio observatory (rdo), where only 11 individuals were sampled, it is unlikely to explain the high levels of differentiation seen for populations in Minor lake road and Major lake peatlands (min and maj). In these latter peatlands, 36 and 39 individuals were sampled, respectively. These sample sizes are comparable to those for all other populations in this study (with the exception of Algonquin radio observatory), are above typically recommended sample sizes for population genetic analyses, and are were therefore unlikely to result in spuriously high estimates of differentiation (Kalinowski 2005; Willing et al. 2012).

A second factor or process that could explain the high genetic isolation of Minor and Major bogs is consistently small population size and/or the occurrence of previous, or recurrent, genetic bottlenecks resulting from sudden and severe reductions in population size (Richards and Leberg 1996). Small population size or bottlenecks occurring in the two populations would result in high levels of genetic drift and lead to both decreased genetic variation and high levels of differentiation from other populations (Richards and Leberg 1996). Both of these populations have low levels of allelic diversity relative to other populations. The allelic richness, A_R, for Minor and Major was 3.36 and 3.21 respectively, while the values for all other sites, excluding Algonquin radio observatory, ranged from 3.36 to 4.36; likewise, expected heterozygosity, H_E, for Minor and Major was 0.43 and 0.46 respectively, while values for all other sites, excluding Algonquin radio observatory, ranged from 0.46 to 0.56. Therefore, excluding Algonquin radio observatory, these two populations rank as the lowest with respect to genetic diversity. This supports the possibility that these populations have experienced higher levels of genetic drift. While I did not estimate population sizes in my study, anecdotal observations during sample collection did not indicate unusually low numbers of pitcher plants or small populations of *F. fletcheri* in these sites. Rather, the slightly smaller sample sizes obtained in these sites (36 and 39, relative to 41-45 in most other sites), was the result of seasonal timing of sampling; these were some of the earliest sites sampled in the year, which resulted in sampling of younger larvae with decreased mass, making effective DNA extraction more difficult, and resulting in a lower success rate in PCR amplification. On the other hand, the occurrence of bottlenecks in these populations is not consistent with the observation that levels of genetic diversity in Minor bog did not

change between 2008/2009 and 2012, nor with previous results of Rasic and Keyghobadi (2012a) which failed to detect evidence of genetic bottlenecks in *F. fletcheri*. Long-term monitoring and quantification of population sizes over time in these sites would be necessary to more rigorously test the hypotheses of small population sizes or bottlenecks.

Interestingly, the small sample size from Algonquin radio observatory (rdo) appeared to be a result of a small population size within a poor quality habitat. The entire site was covered in the search for samples, but only 11 individuals were found. Despite being a peatland of significant size, the sphagnum mats appeared not to be ideal for *S. purpurea* growth, as they were overgrown with grasses, and the *S. purpurea* plants that were present showed stunted development and often had closed leaves (Figure 13). Algonquin radio observatory is located within the flood plane of the Petawawa River, an extensive river system that undergoes massive swell cycles each spring. This site could be experiencing regular flooding, which could alter the hydrology and chemistry of the peatland, resulting in poorer growing conditions for *S. purpurea*. Therefore, *F. fletcheri* population size is likely consistently small in this peatland, and the high genetic differentiation observed for this population actually could be due to the combination of small sample size and high genetic drift within a small population.

Finally, a third explanation for the high genetic differentiation of Minor and Major bog populations is that the populations are in fact highly isolated from the remainder of the *F. fletcheri* populations in the region, and that there is some barrier to movement into or out of these sites. This would result in low levels of gene flow into and out of these sites, which would also be consistent with their high levels of genetic differentiation and low levels of genetic diversity. While my IBR analyses did not indicate a significant effect of the landscape on patterns of genetic differentiation among populations of *F. fletcheri*, I only considered the role of aquatic and wetland areas as potentially facilitating gene flow among populations. Other aspects of the landscape not incorporated in my IBR analyses, such as topography or wind direction, could be acting as a barrier to movement into the Minor and Major populations. Landscape genetic analyses that incorporate additional landscape variables could shed light on possible barriers isolating these populations (Koen et al. 2012). Also, further field observation of adult *F. fletcheri* specimens could help to better quantify the effects of landscape variables on *F. fletcheri* migration.

The outlier sites seen in this study pose interesting questions about effectively analyzing spatial genetic structure of populations . Results indicated that across the majority of sites studied, population genetic structure was consistent with isolation by distance and a gradient of genetic differentiation across the study area. The high differentiation seen in a few outlier sites, which was accompanied depression of allelic diversity and heterozygosity, masked this pattern of spatial autocorrelation when all populations were analyzed as a group. This could be taken as a caution for future population genetic studies that preliminary descriptive analyses should never be overlooked when attempting to describe and explain patterns in spatial population structure. This result also suggests caution in the selection of populations and samples in both observational and experimental population genetic studies, and underlines the importance of replication across many populations (Balkenhol et al. 2009).

4.3 Spatial scale of genetic structure

Based on a previous population genetic study in *F. fletcheri* (Rasic and Keyghobadi 2012a), a sampling design was developed for this study based on a distance of ~15 km between all sampled sites. Results of the Mantel test for IBD after removal of outlier sites found in PCoA analysis confirmed that this is indeed an appropriate spatial scale at which one can consistently observe genetic differentiation among populations of *F. fletcheri*, and at which one can observe a pattern of isolation by distance (IBD). These results also indicated that genetic differentiation among populations becomes consistently significant at a range of roughly 18 kilometers. Specifically, Mantel correlograms showed development of significant genetic differentiation at distances >17.1 km (Figure 8). Therefore, the use of a sampling design in keeping with these distances is recommended for further studies of population genetics and landscape genetics in *F. fletcheri*.

Proper spatial scale of sampling is an important component of project design in population and landscape genetic studies that are focused on describing and explaining patterns of gene flow and connectivity. If sampling scale is too large, then genetic drift or historical population structure becomes the main process determining levels of genetic differentiation among populations, and inferences about contemporary gene flow cannot be made (Anderson et al. 2010). Conversely, if the sampling scale is too small, there may

be such high levels of gene flow among populations that that one cannot observe any differentiation or spatial genetic structure among them (Anderson et al. 2010).

4.4 Isolation by Resistance

Having confirmed that the spatial scale of sampling in the study was appropriate for detecting genetic differentiation and IBD among populations, the landscape genetic hypothesis that gene flow among *F. fletcheri* populations is facilitated along wetland and aquatic areas was then tested. This hypothesis was not supported, regardless of whether highly differentiated populations identified as outliers in the PCoA (Minor bog, Major bog, and Algonquin radio observatory) were included or excluded in the analysis (Figure 9, Figure 10). While this suggests that *F. fletcheri* gene flow does not, in fact, occur along aquatic channels, it is important to consider some limitations of this analysis. This study intentionally used a strongly binary cost surface parameterization, in which all other land cover in the landscape was given a resistance 50 times greater than that of aquatic and wetland habitats. A binary surface with a high level of contrast in resistance values was used to ensure that least cost paths (LCP) would follow strictly the selected habitats (aquatic and wetland) in determining the optimal routes of movement between populations (Koen et al. 2012), yet could still allow small jumps in regions where the raster maps (which were originally converted from vector format) had become severed due to ineffective vector to raster conversion (a distance of one, possibly two pixels). Furthermore, computational limitations meant that only one cost surface parameterization could be tested in this study. Therefore, a cost surface with lower contrast in resistance values, or with a more complex non-binary structure, could still be more representative of movement and gene flow patterns in the flesh fly than IBD alone, and should be further developed and tested in future studies. Further effective dispersal model parameterization and development will be dependent upon two factors, greater computational efficiency, and a stronger understanding of dispersal behavior in adult *F. fletcheri* in regards to landcover preference.

Alternative methods for measuring connectivity across a resistance surface, other than LCP, should also be considered in future studies. LCP analysis is designed to determine the optimal route of movement through a landscape (Holderegger and Wagner 2008).

LCP identifies the single most effective connection between two locations, rather than considering multiple possible routes, even though the latter may be more realistic from an ecological standpoint (McRae 2006). This limitation of LCP analysis for describing ecological or genetic connectivity has been criticized on the grounds that it assumes the organism has an *a priori* knowledge of the landscape being traversed, and will follow the single, most optimal route (McRae and Beier 2007). Recent developments in the field of landscape genetics, particularly those based on the use of electrical circuit theory to quantify movement of animals through a heterogeneous landscape, attempt to address this issue (Wu 2004; McRae 2006). Such circuit theory analyses therefore represent a logical next step in testing landscape genetic analyses in *F. fletcheri.* It should be kept in mind, however, that circuit analyses have their own limitations (Theobald et al. 2012). Because multiple possible movement routes are taken into consideration in circuit analyses, problems of edge effects in landscape resistance calculations can become amplified. Thus, spurious barriers or bottlenecks to movement are more likely to be created at the edges of the study area. In the case of this study, the proximity of certain sampled sites to the park borders, beyond which land cover data were not available, could have created biased circuit analysis results through artificial inflation of isolation at these sites (Hardy 2003; Wu 2004, Koen et al. 2012).

Another factor that could contribute to non-significant IBR results is that the scale at which IBD is apparent among the sampled populations may not be the same spatial scale at which IBR can be detected using LCP analyses (McRae 2006). IBD becomes apparent as the scale of sampling is increased enough that gene flow between the more distant sampled populations is noticeably lower than gene flow between closer populations (Wright 1943). However, in order to observe IBR effects using LCP analyses, a scale is needed where genetic differentiation is present, but not so great that multiple equally effective routes between sites are available, making any individual path of movement unlikely to explain the level of genetic differentiation between a pair of sites (Cushman and Landguth 2010; Koen et al. 2012). Separating the effects of IBR from those of IBD can become increasingly difficult as dispersal distance and landscape complexity increase (McRae 2006). This is a further reason for considering alternatives to LCP analysis, such as circuit theory based methods, in testing landscape genetic hypotheses.

While this study did not find evidence that *F. fletcheri* gene flow is strongly constrained along aquatic and wetland areas, the ability to analyze only one resistance surface and using LCP methods exclusively, means that this hypothesis should not yet be discarded. Further studies using other more complex forms of landscape parameterization and alternative methods of quantifying connectivity across the landscape are needed to better understand patterns of connectivity and gene flow in *F. fletcheri*. A field experiment looking at the effects of the removal of flower heads from host *S. purpurea* plants along a dispersal corridor may serve as an effective, complementary test of the use of this host plant as layover sites during longer movements between peatlands.

Figure 13: A representative photo of Radio observatory (rdo) peatland surface, the largest pitcher leaf seen in the image has an opening of 2-3cm. Note the high levels of grasses, small size and unopened state of many of the *S. purpurea* specimen's leaves.

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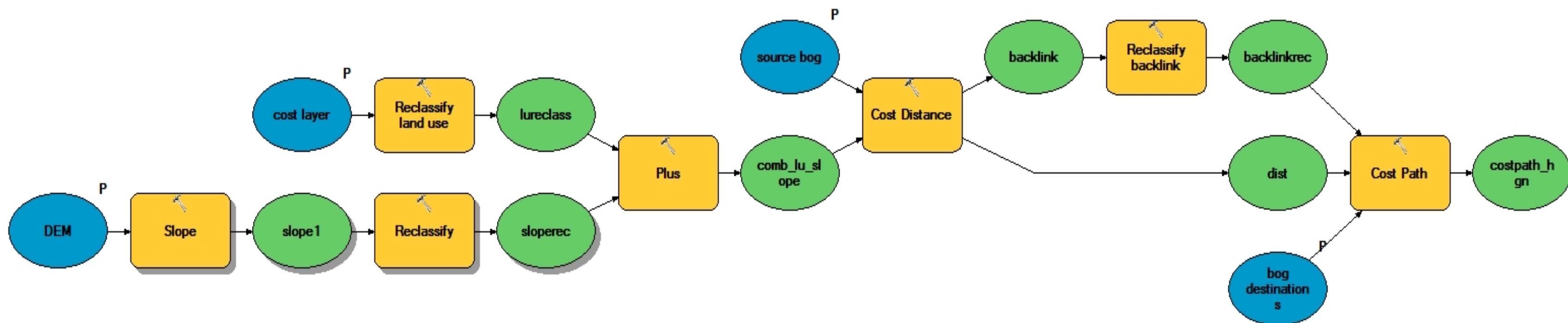
Appendix

Appendix 1: Model developed in arcGIS to produce individual Least Cost Pathways for *F. fletcheri* dispersal along riparian habitats within Algonquin Park. Blue circles represent input parameters, yellow boxes represent functions run by the program to alter those parameters, and green boxes represent the output files of those functions. Some functions require multiple inputs as well as conversion parameters. This model was run individually (in batch) for all LCP values in the resistance matrix.



Appendix 2: p-values for the exact test of Hardy Weinberg Equilibrium (HWE) for each locus in each sampled site within and near Algonquin Provincial Park. * Indicate significant tests before Bonferroni correction.

Peatland	Loci									
	FF09	**FF10**	**FF189**	**FF231**	**FF72**	**FF238**	**FF249**	**FF62**	**FF65**	**FF82**
min	0.4787	0.8418	0.2458	1.0000	0.1731	1.0000	1.0000	1.0000	0.7551	1.0000
cfr	1.0000	0.2270	1.0000	1.0000	0.7298	0.7232	1.0000	1.0000	1.0000	0.9824
maj	*0.0473	0.8800	1.0000	0.9622	1.0000	1.0000	0.9762	0.1249	0.2648	0.8424
ink	1.0000	0.4522	0.6926	1.0000	0.1913	1.0000	1.0000	0.5163	1.0000	1.0000
whl	1.0000	0.4525	0.6930	1.0000	0.1967	1.0000	1.0000	0.5176	1.0000	1.0000
brl	1.0000	1.0000	1.0000	0.1567	*0.0080	1.0000	*0.0417	0.5913	0.0567	0.1088
kwl	0.7464	*0.0282	0.6693	1.0000	0.4816	1.0000	1.0000	1.0000	1.0000	1.0000
mcc	0.3524	1.0000	0.1236	1.0000	*0.0225	0.2658	1.0000	1.0000	1.0000	0.2641
rdg	1.0000	1.0000	1.0000	1.0000	0.9287	1.0000	1.0000	1.0000	1.0000	0.0559
jwl	0.8381	0.8466	0.5706	1.0000	1.0000	0.2054	0.6235	1.0000	1.0000	0.3700
dmr	1.0000	1.0000	1.0000	0.5990	*0.0211	*0.0186	1.0000	0.1965	0.0656	0.0692
rdo	0.3691	1.0000	1.0000	0.7649	1.0000	0.6897	1.0000	1.0000	0.3747	0.8611
caj	1.0000	0.7733	0.8152	1.0000	*0.0004	1.0000	1.0000	0.7340	1.0000	0.8025
hgn	1.0000	0.7711	1.0000	0.9789	0.7402	1.0000	1.0000	0.8586	1.0000	1.0000
prx	1.0000	0.4781	0.4405	0.8216	0.9699	0.2454	0.1850	0.6214	1.0000	0.8733

Curriculum Vitae for John Robert O'Leary

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