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Ecological and Evolutionary Interactions Between Song Sparrows (Melospiza melodia) and their Bloodborne Parasites

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Abstract

Local adaptation is the result of natural selection operating at a local scale, such that trade-offs in fitness across different environments result in individuals having higher fitness in their place of origin than when transported into a foreign environment. Populations may become locally adapted to features of their abiotic environment, or in the case of coevolutionary arms races between hosts and parasites, to other species comprising their biotic environment. If host populations are adapted to their local (sympatric) parasites, or conversely if parasites are adapted to their local hosts, then interactions with local parasite strains may influence the fitness consequences of host dispersal. I investigated the ecological and evolutionary impact of haematozoan parasites (genera *Plasmodium*, *Leucocytozoon*, *Haemoproteus*) on song sparrow (*Melospiza melodia*) hosts. I hypothesized that song sparrows have an advantage in defending against local parasite strains, resulting in parasite-mediated selection against natal dispersal. I predicted that condition and survivorship should correlate positively with philopatry, and that such 'home-field advantage' is mediated by enhanced ability to deal with sympatric parasites. I used genetic assignment tests to quantify natural variation in philopatry, and asked whether song sparrows of local origin showed a home-field advantage over immigrants, across multiple populations (Chapter 2). I compared population genetic structure of hosts and parasites to test for coevolution (Chapter 3). I attempted to experimentally reduce parasite load, to test if malarial parasites decrease territorial defense (Chapter 4). Finally, I used a cross-infection design to compare resistance to sympatric versus allopatric strains of *Plasmodium* (Chapter 5). Song sparrows of local origin tended to have lower parasite loads in the wild. Moreover, sparrows were less likely to become infected when experimentally inoculated with a sympatric than an allopatric *Plasmodium* lineage. Population genetic structure of song sparrows was generally not correlated to that of parasites, but I found similar genetic structuring between song sparrows and one *Plasmodium* taxon. Interactions with haematozoan parasites may influence the fitness consequences of dispersal by song sparrows. This has implications for the evolution of dispersal, patterns of biodiversity, and conservation in an era of emerging infectious disease.

Keywords: Local adaptation, host-parasite interactions, dispersal, philopatry, population genetic structure, song sparrows, avian malaria, Haemosporidia, *Plasmodium*, *Leucocytozoon*, *Haemoproteus*, cross-infection experiment.

Co-Authorship Statement

Dr. Elizabeth MacDougall-Shackleton provided guidance and funding and will be a co-author on any publications resulting from this dissertation.

A version of Chapter 5 has been submitted to *Ibis* with Dr. Elizabeth MacDougall-Shackleton as second author. Dr. MacDougall-Shackleton consulted on experimental design and statistical analyses, provided funding, and edited the manuscript.

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Chapter 1

1 Ecological and evolutionary interactions between song sparrows and avian malaria parasites

1.1 Local adaptation

Adaptation by natural selection is a key element in the theory of evolution. This process has led to the formation of millions of different species that are adapted to the most extreme habitats all over the globe (Endler 1986). An early step in the differentiation of species is the adaptation of populations of the same species to subtly different environments. This adaptation at the population level is referred to as local adaptation, whereby individuals have higher fitness in their native (local) habitat than in foreign (non local) habitats (Kawecki & Ebert 2004). For example, in a reciprocal transplant experiment across Europe using three plant species (*Trifolium pratense, Dactylis glomerata, Plantago lanceolate*), individuals replanted in their native habitat had the highest overall performance, and this performance declined with increasing distance from their native environment (Joshi *et al*. 2001). A translocation experiment with the lizard *Anolis oculatus* on the island of Dominica found that individuals translocated from a different habitat had lower fitness than control individuals that remained in their native environment (Malhotra & Thorpe 1991). Similarly, in a reciprocal transplant experiment on desert spiders (*Agelenopsis aperta*), individuals translocated into foreign enclosures had lower survival and lower body mass than spiders introduced into enclosures of their native habitat (Riechert & Hall 2000). The degree of local adaptation is expected to increase with greater habitat divergence between populations (Becker *et al*. 2006, Joshi *et*

al. 2001), and with greater genetic diversity within populations, because within-population genetic diversity affects the ability to respond to selection (Gomulkiewicz *et al*. 1999, Lenormand 2002).

To the extent that populations within a species are connected by gene flow, however, sufficient levels of gene flow can homogenize populations despite different selective environments and thus hinder the rate and acquisition of local adaptation (Endler 1986). A study on 32 populations of threespine stickleback (*Gasterosteus aculeatus*) in British Columbia found that in 31 cases, the amount of local adaptation was negatively correlated with gene flow (Hendry & Taylor 2004). Furthermore, several simulation studies revealed that even though high levels of gene flow counteract the effects of selection, intermediate levels of gene flow favour local adaptation by replenishing genetic variation (Alleaume-Benharira *et al*. 2006, Gomulkiewicz *et al*. 1999; see Garant *et al*. 2007 and Lenormand 2002 for reviews on the subject).

Different populations of the same species may encounter environments that differ in abiotic features, such as temperature, rainfall, soil nutrients, or pH. Using the bacterium *Escherichia coli*, a study found that lineages grown at 20°C for 2000 generations had a fitness decrement when grown at 40°C, suggesting a trade-off in temperature adaptation (Bennett & Lenski 2007). Similarly, lineages of *Escherichia coli* that evolved for 2000 generations at pH 5.3 had a decrease in fitness when grown at higher pH. However, lineages grown at other pH were not consistent in their fitness trade-offs and showed diverse patterns of adaptation (Hughes *et al*. 2007).

Another critical part of the environment to which populations may become locally adapted is the biotic environment - *i.e.*, interactions with other species. For example, in populations of guppies (*Poecilia reticulata*) historically subject to high predation levels from diurnal, visually-hunting predators, more cryptic colouration (lower number and size of colour patches) was favoured than in populations that had low predation intensity (Endler 1978).

Perhaps even more ecologically widespread than interactions between predators and prey are interactions between hosts and parasites (Dobson *et al*. 2008). Just as abiotic environmental features often vary spatially, the community composition and abundance of parasites frequently varies over the landscape (Poulin & Morand 2004). Thus, co-occurring assemblages of hosts and parasites may engage in regionalized host-parasite associations. Such interactions can lead to divergent selection between populations, and ultimately, to host-parasite coevolution and cospeciation (Thompson 1994). Cospeciation can be recognized when phylogenetic trees of host and parasites are topologically similar (Page 1994, 2003), implying that host and parasite lineages have tracked each other through evolutionary time. One example of cospeciation involves pocket gophers (Rodentia: Geomyidae) and their chewing lice (Phthiraptera: Trichodectidae). Pocket gophers are solitary and live in burrows, resulting in chewing lice having low dispersal and being highly host-specific. Phylogenetic analyses using protein electrophoresis and mitochondrial DNA from gophers and lice showed a high degree of parallel cladogenesis, indicative of cospeciation (Demastes *et al*. 2012, Hafner & Nadler 1988, 1990).

1.2 Host-parasite interactions

Parasitism is defined as an association between species, where one species (the parasite) depends on another (the host) for nutrition, habitat, or both, for at least a part of their life cycle, and where the association is obligatory for the parasite, and detrimental for the host (Anderson & May 1978). It is estimated that 40% of extant species are parasites (Dobson *et al*. 2008). Parasitic species do not form a monophyletic clade. Instead, this way of life appears to have evolved independently several times in many different taxa (viruses, bacteria, protozoa, fungi, plants, nematodes, arthropods, and vertebrates; Poulin & Morand 2004, Schmid-Hempel 2011).

Parasites negatively affect multiple components of host fitness, including critical traits such as reproductive success and survival. In the alpine marmot (*Marmota marmot*) heavy mite (*Echinonyssus blanchardi*) infestation was correlated with higher offspring winter mortality and delayed weaning (Arnold & Lichtenstein 1993). In Cape ground squirrels (*Xerus inauris*), ectoparasite and endoparasite removal led to higher number of offspring produced in treated females than in controls (Hillegass *et al*. 2010). In water pythons (*Liasis fuscus*), individuals with higher haematozoan (*Hepatozoon* spp.) parasite loads had lower growth rates, lower body condition, and lower recapture rates suggesting lower survival than in individuals with lower parasite loads (Madsen *et al*. 2005).

Because of the important selection pressures they can place on host species, parasites have been hypothesized to have important effects on evolutionary trajectories of host populations. Parasites have been proposed to contribute to the maintenance of genetic diversity in host species (Haldane 1949), intensify sexual selection (Hamilton & Zuk 1982), favour the persistence of sexual over asexual reproduction (Hamilton 1980,

Lively 2010), and shape patterns of speciation (Ricklefs 2010, Thornhill & Fincher 2013).

The parasite-driven-wedge hypothesis (Thornhill & Fincher 2013) proposes that coevolution with local parasites might accelerate evolutionary divergence between host populations, through favouring host behaviours that minimize the likelihood of encountering unfamiliar pathogens. Such behaviours might involve avoidance of nonlocal conspecifics (Fincher & Thornhill 2012, Kavaliers *et al*. 2014, Loehle 1995) and limited dispersal (Barber & Dingemanse 2010, Sih *et al*. 2012, Wolf & Weissing 2012). Foreign or unfamiliar parasites pose the greatest threat to host individuals due to hosts' lack of local adaptation or acquired immunity to these parasites (Beadell *et al*. 2006, Cellier-Holzem *et al*. 2010, Doolan *et al*. 2009). This 'parasite-driven wedge hypothesis' assumes that (1) localized coevolution occurs between host and parasites, and (2) hosts are locally adapted to or otherwise better able to cope with their local (sympatric) parasites. That is, host individuals are better able to avoid or control infection by sympatric than by allopatric parasite strains.

1.3 Local adaptation and coevolutionary arms races between hosts and parasites

A coevolutionary arms race is defined as the interaction between two species, where an adaptation in one creates a selection pressure on the other promoting a counter-adaptation in return (Dawkins & Krebs 1979). Localized arms races are important because they influence the strength and direction of patterns of local adaptation across the landscape (Schluter 2001, Thompson 2005). For example, in the predator-prey interaction between

garter snakes (*Thamnophis sirtalis*) and toxic newts of the genus *Taricha*, resistance levels to the newts toxin varies among populations. In areas where this selection pressure is weak ("coldspots"), due to absence of newts or low levels of toxin concentrations, garter snake resistance to toxin was low. In areas where newts are present and have high toxin levels, selection pressure is strong ("hotspots"), and snake resistance to the toxin was high (Brodie *et al*. 2002). Similarly, the coevolutionary interaction between the herbaceous plant *Lithophragma parviflorum* (Saxifragaceae) and the parasitic moth *Greya politella* varies between habitats. In some populations, the interaction between these two species is mutualistic, where the plant benefits from the pollination by the moth, and the moth benefits by laying its eggs on the plant where ultimately the larvae will feed on some of the seeds. In some other populations, however, the presence of other pollinators helps fertilize the plant, and the moth becomes purely parasitic. In populations where the plant relied on the moth for pollination, developed fruits were more likely to have moth eggs, whereas in populations where the association is mostly parasitic, plants selectively aborted fruits containing moth eggs (Thompson & Cunningham 2002).

In host-parasite systems, there are several potential outcomes of a local arms race. **Host local adaptation**, in which hosts are better able to resist sympatric than allopatric parasites, has been described in some systems. Bumblebees (*Bombus terrestris*) had lower mortality rates when experimentally infected with sympatric than with allopatric Trypanosoma parasites (*Crithidia bombi*; Imhoof & Schmid-Hempel 1998). Similarly, Canarian lizards (*Gallotia galloti*) had lower probability of becoming infected by sympatric than allopatric bloodborne parasites (*Haemogregarina* sp.; Oppliger *et al.* 1999), and *Gyrodactylus* ectoparasites had lower infectivity and virulence when infecting

sympatric than allopatric guppy (*Poecilia reticulata*) host populations (Perez-Jvostov *et al*. 2015). Because encountering unfamiliar allopatric parasites is a cost of dispersal, host local adaptation should ultimately reduce host gene flow and promote host diversification and speciation.

Conversely, some host-parasite systems display **parasite local adaptation**, the situation in which parasites are better able to infect sympatric than allopatric hosts. For example, trematode parasites (*Diplostomum phoxini*) were better at infecting sympatric than allopatric European minnows (*Phoxinus phoxinus*; Ballabeni & Ward 1993); ticks (*Ixodes uriae*) had higher success infecting sympatric than allopatric black-legged kittiwakes (*Rissa tridactyla*; McCoy *et al*. 2002); and trematodes (*Microphallus* sp.) had higher prevalence of infection when exposed to sympatric than to allopatric snails (*Potamopyrgus antipodarum*; Osnas & Lively 2004). If escape from sympatric parasites represents a benefit of dispersal, parasite local adaptation should ultimately promote host gene flow and reduce host speciation.

A last potential outcome is **no local adaptation** by either party. For example, great tits (*Parus major*) were not differentially affected by sympatric or allopatric hen fleas (*Ceratophyllus gallinae*; Dufva 1996). In the absence of host or parasite local adaptation, host-parasite interactions may neither selectively favour nor selectively disfavour host dispersal versus philopatry.

When studying coevolution and local arms races between parasites and hosts, simulations have implicated relative gene flow in determining whether hosts or parasites are ahead in the coevolutionary race - *i.e.*, whether host or parasite local adaptation is

likely to occur (Gandon & Michalakis 2002). In systems where hosts disperse farther than their parasites, hosts have an evolutionary advantage over parasites due to greater adaptive potential. Conversely, if parasites have greater gene flow than do hosts, parasite local adaptation tends to occur, providing parasites with an advantage in infecting sympatric hosts (Gandon & Michalakis 2002). Theoretical models have shown the importance of gene flow in parasite-host local adaptation in many studies (Gandon *et al*. 1996, Gandon 2002, Gandon & Michalakis 2002, Lively 1999), and the first studies to experimentally address this issue had similar findings. For example, the bacteriophage T7 showed higher measures of local adaptation to the bacterium *Escherichia coli* when its migration rate was higher (Forde *et al*. 2004). A similar study used the Gram-negative bacterium *Pseudomonas fluorescens* as the host and an associated lytic DNA phage as the parasite, and found that parasite migration increased parasite local adaptation (Morgan *et al*. 2005).

Similarly, a meta-analysis of 54 published papers on host-parasite interactions across multiple taxa (bacteria, protozoans, fungi, invertebrates, and vertebrates) confirmed the importance of relative migration and gene flow (Greischar & Koskella 2007). Higher host migration relative to that of their parasites was generally associated with host local adaptation, whereas higher parasite migration relative to that of their hosts was generally associated with parasite local adaptation. A similar meta-analysis that reviewed 29 host-parasite local adaptation studies also drew similar conclusions (Hoeksema & Forde 2008). Thus, the relative adaptive potential of parasite versus host populations can be an important determinant of whether parasites adapt to hosts or hosts

to their parasites, and ultimately of the fitness consequences of hosts dispersing away from the natal grounds and encountering novel parasites.

1.4 Fitness consequences of natal dispersal

Natal dispersal is the movement of an organism from the site of birth to the site of the first reproduction (Greenwood & Harvey 1982). Natal dispersal can result in gene flow among subpopulations, which involves several population-level benefits. These include cohesion between populations, movement of alleles in ever changing environments, and the rescue of small populations from local extinction (Ronce 2007). Conversely, natal dispersal and the resultant gene flow may involve population-level costs. In particular, dispersal may hinder the evolution of local adaptation if gene flow is high enough (Lenormand 2002). For example, *Culex pipiens* mosquitoes in Corsica (France) have very low resistance to the insecticide use in that area despite 17 years of this existing selection pressure, due to dispersal distances being higher for this mosquito than the area being treated (Raymond & Marquine 1994).

At the level of individual fitness, natal dispersal likewise involves both costs and benefits. One potential benefit to dispersing involves avoiding inbreeding and reducing resource competition, especially kin competition. For example, high population relatedness induces dispersal of offspring in common lizards (*Lacerta vivipara*), particularly offspring with bigger body size that could potentially have high colonization success elsewhere (Cote *et al*. 2007). Similar results were found in two-spotted spider mite (*Tetranychus urticae*), where levels of population density and genetic relatedness

were correlated with dispersal distances (Bitume *et al*. 2013, Bitume *et al*. 2014). Natal dispersal can also entail costs for individual fitness, including time and energy costs during dispersal itself, and increasing mortality risks (Bonte *et al*. 2012). For example, survival decreased with dispersal distance in the lesser kestrel (*Falco naumanni*; Serrano & Tella 2012), and in great reed warblers (*Acrocephalus arundinaceus*; Hansson *et al*. 2004).

Fitness consequences of dispersal may also be influenced by host or parasite local adaptation. If host populations are locally adapted to their parasites, and/or if host individuals acquire resistance to previously-encountered strains through acquired immune training, then dispersing individuals may have lower fitness in their new habitats due to the costs of encountering novel parasites. Consistent with host local adaptation, parasite loads (*Haemoproteus* spp.) were higher in immigrant than in philopatric male mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*; MacDougall-Shackleton *et al*. 2002). Similarly, in barn swallows (*Hirundo rustica*), philopatric females had lower infestation of the haematophagous louse fly than females that had immigrated from elsewhere (*Ornithomya biloba*; Saino *et al*. 2014). Conversely, the enemy release hypothesis predicts that if parasites are locally adapted to their hosts, host individuals that disperse may benefit by escaping locally adapted parasites (Keane & Crawley 2002). For example, prevalence and diversity of malarial parasites was lower among house sparrows (*Passer domesticus*) in colonized areas than in their native range, suggesting that enemy release might be one reason behind the success of this invasive species (Marzal *et al*. 2011).

1.5 Study system

My dissertation examines ecological and evolutionary interactions between hosts and parasites within the context of local adaptation and arms races, focusing on interactions between song sparrows (*Melospiza melodia*) and their bloodborne parasites.

1.5.1 Song sparrows

Song sparrows are medium-sized (20-25 g), socially monogamous, ground-foraging songbirds that are distributed across North America. Song sparrows show remarkable geographic variation in morphology, with approximately 30 subspecies currently recognized (Arcese *et al*. 2002). In eastern North America (*melodia* subspecies), song sparrows are seasonally migratory; for example, birds breeding at our long-term study site near Newboro, Ontario, Canada (44.633°N, 76.330°W) have been recaptured in Tennessee and Maryland during winter. Estimates of natal dispersal distances for this species range from as little as 300 m based on mark-recapture methods (Nice 1937) to 6.1 km based on mtDNA sequence variation (Zink & Dittmann 1993).

As in many other songbird species, birdsong varies geographically in song sparrows, and females prefer singers of local origin (e.g., Searcy *et al*. 2002). Consistent with the possibility of local adaptation, in an eastern Ontario focal population of song sparrows, males with high genetic similarity to the local population (presumably of local origin) were in better physiological condition (including lower blood-borne parasite loads) than males that were less genetically similar (presumably immigrants; Stewart & MacDougall-Shackleton 2008). However, this finding is not conclusive evidence of local adaptation by the birds to their parasites, because the reduced performance of dispersing

individuals could be due to host local adaptation, lack of acquired immunity or to intrinsic differences in quality between philopatric and dispersing individuals (e.g., Solmsen *et al*. 2011). Moreover, it is important to take a landscape approach when comparing performance of philopatric and dispersing individuals because the outcome of host-parasite interactions may not necessarily be consistent from place to place.

1.5.2 Avian haemosporidian parasites

The order Haemosporidia is a group of Apicomplexan protozoan parasites that infect amphibians, reptiles, mammals, and birds (Valkiunas 2005). These parasites have a sexual stage in a blood-sucking insect vector, and complete their asexual stage in a vertebrate host. When an infected vector bites a suitable host, the parasite invades organs such as the liver, spleen, heart or brain, potentially causing capillary blockage, haemorrhages and organ malfunction. From the organs, parasites release gametes into the bloodstream, which invade erythrocytes and cause anaemia. The lifecycle continues when a new insect (Dipteran) vector collects a blood meal from the infected vertebrate host (Valkiunas 2005).

Interactions between haemosporidian parasites and vertebrate hosts have been particularly well-studied in birds, due in part to catastrophic effects of these parasites on naïve Hawaiian avifauna (Beadell *et al*. 2006). The three most prominent genera of haemosporidia infecting birds are *Plasmodium*, *Haemoproteus* and *Leucocytozoon* (Valkiunas 2005). Collectively, these three genera infect about 70% of avian species (Atkinson & van Riper 1991). Genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*

are transmitted by mosquitoes (family Culicidae), biting midges (family Ceratopogonidae), and blackflies (family Simuliidae) respectively. The general life cycle is similar for all three genera, except that *Plasmodium* species are able to undergo asexual reproduction in the bloodstream of the host, whereas in *Haemoproteus* and *Leucocytozoon* asexual reproduction occurs only inside the host organs (Valkiunas 2005). This feature of *Plasmodium*'s life cycle makes it amenable to experimental infection studies because infectious stages can be transferred directly between vertebrate host individuals through blood transfusions (Zehtindjiev *et al*. 2008, Palinauskas *et al*. 2011, Dimitrov *et al*. 2015).

1.5.3 Techniques and approaches to studying host-parasite interactions

Despite the importance of studying host-parasite interactions, there are important limitations in traditional methods for studying them. First, observational studies attempting to relate parasite load to host fitness in the wild almost always fail to sample individuals that died as a result of the infection, and may thus underestimate the effects of parasites on host fitness. Such studies may be unable to distinguish between individuals that have avoided infection and those that have successfully withstood the acute stage of parasitemia (Zehtindjiev *et al*. 2008). Indeed, many studies failed to find negative effects of Haemosporidians on wild birds (Weatherhead & Bennett 1992, Davidar & Morton 1993, Bensch *et al.* 2007), and many studies have even found positive effects to being parasitized, for example in offspring survival (Kilpatrick *et al*. 2006), fledging success (Norte *et al*. 2009), clutch size (Sanz *et al*. 2001, Fargallo & Merino 2004), and

overwinter survival (Zylberberg *et al*. 2015). The correlational nature of these studies and the failure to sample individuals that succumbed to infection makes their findings, particularly regarding the importance of parasites as a selection factor, difficult to interpret.

Thus, an experimental approach (*i.e.*, manipulating infection status through antiparasitic drugs or experimental infection) can be more powerful in estimating the fitness effects of parasites. For example, treating female blue tits (*Cyanistes caeruleus*) with the drug Primaquine experimentally reduced intensity of infection by *Haemoproteus majoris*, and reduced prevalence of infection by *Leucocytozoon majoris*. This treatment resulted in significantly higher reproductive success and better condition in drug-treated than in control individuals, demonstrating an effect of parasites on these components of fitness (Merino *et al*. 2000). In another study in blue tits, females treated with the drug Malarone had lower *Plasmodium* spp. intensities, and displayed higher hatching success, higher provisioning rates and higher fledging success than did untreated individuals (Knowles *et al*. 2010). In house martins (*Delichon urbica*), individuals treated with Primaquine had lower levels of infection of *H. prognei*, and showed increased clutch size, and higher hatching and fledging success than control individuals (Marzal *et al*. 2005). In another study of blue tits, females treated with Primaquine had lower levels of infection by *Haemoproteus* spp., and this resulted in increased overwinter survival compared to control birds (Martinez-de la Puente *et al*. 2010).

Moreover, in the case of haematozoan (bloodborne) microparasites, traditional methods of estimating prevalence and intensity of parasitic infections (*i.e.*, scanning thin-film blood smears under a light microscope; Bennett 1970) may fail to detect low-level,

subclinical or chronic infections. Recently however, PCR based techniques have been developed to detect and sequence a portion of the cytochrome b of avian bloodborne parasites (Bensch *et al*. 2000; Hellgren *et al*. 2004). These molecular methods permit detecting very low-level infections that cannot be detected via microscopic examination of thin-film blood smears. For example, a study comparing the effectiveness of microscopy versus molecular methods in 29 different species of African rainforest birds found that PCR detected infections in 40% of birds, whereas microscopy detected infections in only 27% of birds in the same sample (Richard *et al*. 2002). Moreover, molecular methods have revealed greater diversity of haematozoan parasites than previously found through identifying morphologically distinct strains using a microscope (Bensch *et al*. 2000, Hellgren *et al*. 2004, Waldenström *et al*. 2004). This enhanced resolution facilitates comparative population genetics so that the relative levels of population genetic structuring in host versus parasite populations can be compared. Population genetic structure can be used to infer relative gene flow (e.g. Dybdahl & Lively 1996), which may be an important determinant in whether hosts become locally adapted to parasites or parasites become locally adapted to their hosts.

1.6 Thesis Objectives and Structure

The over-arching goal of my dissertation was to examine the fitness consequences of natal dispersal by song sparrows. Specifically, I investigated whether individuals of local origin have a home-field advantage over immigrants coming from other source populations, and whether this advantage is mediated by an enhanced ability to deal with local (sympatric) parasites. Such a pattern could arise by means of local adaptation at the

level of the population over many generations, or through previous exposure of host individuals to local parasite strains within an individual's lifetime. I hypothesized that interactions with parasites provide a selective advantage to philopatric over dispersing host individuals.

Support for this hypothesis would be provided by the following observations: (1) birds of local origin having enhanced survivorship and body condition, and lower parasite loads, relative to birds breeding at the same site but having immigrated from elsewhere; (2) lower levels of population genetic structuring in song sparrows than in their haematozoan parasites sampled over the same geographic scale, indicative of song sparrows having greater gene flow and greater adaptive potential (Gandon & Michalakis 2002); (3) concordant genetic structuring in song sparrows and their haematozoan parasites, indicative of coevolution between hosts and parasites; (4) experimentally demonstrating a fitness cost of haematozoan parasites by manipulating infection status; and (5) demonstrating, through experimental infection, that song sparrows are better able to resist or control infection by sympatric than allopatric haematozoan parasites. My dissertation comprises four data chapters that collectively address each of these predictions.

In Chapter 2 (Inferred philopatry and its relationship to condition, parasitism, and survivorship in song sparrows) I examined naturally-occurring variation in natal philopatry (as assessed through genetic assignment tests), parasite load, body condition, and overwinter survivorship/ return rates to determine whether song sparrows of relatively local origin had a "home-field advantage" over birds that immigrated from

somewhere else. This work was carried out at multiple sites to allow me to examine the possibility that home-field advantage may vary over the landscape.

In Chapter 3 (Comparative population genetics of song sparrows and their haemosporidian parasites) I compared levels of population genetic structure between song sparrows (based on microsatellite allele frequencies) and haematozoan parasites (based on variation in mitochondrial cytochrome b sequence). I also compared host and parasite phylogenies to assess evidence for incipient cospeciation as a possible outcome if song sparrows and their bloodborne parasites are engaged in intense coevolutionary arms races.

In Chapter 4 (Effects of haemosporidians on territorial defense by male song sparrows: an experimental approach) I used an experimental approach (*i.e.*, administration of antiparasitic drugs) to measure the effects of Haemosporidian parasites on one component of host fitness (*i.e.*, territorial defence by breeding males). Theoretical models of parasite effects on host evolution assume that parasites represent an important selection pressure, but as reviewed above, this assumption is most powerfully tested by manipulating parasite load rather than by measuring naturally-occurring variation in parasitism.

In Chapter 5 (Song sparrows *Melospiza melodia* have a home-field advantage in defending against sympatric malarial parasites) I conducted a cross-infection experiment to determine whether song sparrows have a home-field advantage in dealing with their sympatric haematozoan parasites. I captured birds from two breeding sites 437 km apart, inoculated them with *Plasmodium* cultured either from their capture site or from the other

site, and monitored infection success and infection intensity. This experimental approach is complementary to the study of naturally-occurring variation in infection and philopatry outlined in Chapter 2, but represents an important next step because it controls for differences in quality between dispersing and philopatric individuals.

1.7 Significance

Examining local adaptation and how parasites may mediate the fitness consequences of natal dispersal provides important empirical evidence that may identify a key role for infectious disease in shaping patterns of population connectivity or isolation. Thus, this study has the potential to contribute to our understanding of basic ecological and evolutionary processes such as arms races, gene flow, population connectivity, and speciation.

Moreover, haematozoan parasites represent a major concern from the perspective of conservation. The globally widespread nature of these parasites, combined with a history of host switching and ability to infect multiple host species, together with the great mobility of migratory birds, raises concerns for the fate of naïve host species encountering unfamiliar parasite strains (Beadell *et al*. 2006), particularly if avian hosts have a disadvantage in dealing with unfamiliar parasites (host local adaptation). Conversely, to the extent that parasite local adaptation occurs, this can promote the spread of invasive (host) species, through enemy release. Understanding host-parasite interactions is increasingly important from a conservation perspective in light of

anticipated range shifts and range expansions of parasites and vectors, associated with climate change (Rosenthal 2009, Tabachnick 2010).

Moreover, little is known about the diversity or prevalence of Haemosporidian parasites in Canada. To my knowledge, my dissertation research represents the first examination and survey of avian Haemosporidians in Ontario, and only the second such study in Canada (previously studied in the Swainson's thrush (*Catharus ustulatus*) in British Columbia; Svensson *et al*. 2007). Although parasite biodiversity has not traditionally been the subject of much conservation concern (Gomez & Nichols 2013), parasites can have key roles in ecosystem function as well as in host evolution.

Finally, studying host-parasite interactions between birds and their malarial parasites can advance our understanding of diseases that represent major concerns for human health. Phylogenetic studies suggest *Plasmodium* parasites in humans could be a result of a lateral transfer from birds, rodents, or primates, making the study of these parasites a valuable subject both in theory and in practice (Perkins 2014, Waters *et al*. 1991).

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Chapter 2

2 Philopatry and its Relationship to Condition, Parasitism, and Survivorship in Song Sparrows

2.1 Introduction

An adaptation is a trait that enhances the fitness of the individual that carries it (Endler 1986). Selection should thus favour the persistence of traits that improve fitness in a particular environment, such that these local adaptations increase in frequency and spread throughout the population. Therefore, populations may become adapted to local conditions, with the result that local individuals have higher fitness than individuals immigrating from elsewhere (Kawecki & Ebert 2004).

Local adaptation has been demonstrated through transplant experiments that compare fitness of local versus non-local individuals in a common environment. For example, in the Dominican lizard (*Anolis oculatus*) individuals transplanted to native environments exhibited, in two months, higher fitness than individuals transplanted to different environments (Malhotra & Thorpe 1991). Similarly, in desert spiders (*Agelenopsis aperta*) individuals transplanted to a foreign environment had slower growth and higher mortality compared to spiders that were introduced into native environment (Riechert & Hall 2000). In a study using reciprocal transplants with three plant species (*Trifolium pratense, Dactylis glomerata, Plantago lanceolata*) across Europe, the overall performance was highest for plants replanted in their native habitat, and the performance declined with distance from site of origin (Joshi *et al*. 2001). Such

findings imply a fitness cost to dispersal, and highlight the importance of local adaptation.

In highly mobile vertebrates such as birds, transplant studies of free-living individuals are not always possible. However, local adaptation can be inferred in other ways, for example through observational studies comparing dispersing versus philopatric individuals at traits related to fitness. For instance, in collared flycatchers (*Ficedula albicollis*), philopatric males (*i.e.*, those hatched on the study plot) secured better territories, attracted mates more quickly and were less likely than immigrants to remain unmated (Pärt 1994). Similarly, in lesser kestrels (*Falco naumanni*), average lifetime fledgling production decreased with natal dispersal distance (Serrano & Tella 2012). In an island population of song sparrows (*Melospiza melodia*), immigrant males were less likely to breed than resident males of the same age, and immigrant females tended to lay fewer clutches and bred later in the year than resident females (Marr *et al*. 2002). These studies suggest that philopatric individuals enjoy a fitness advantage over non-philopatric individuals.

Parasites represent an important feature of the local environment with which host individuals interact. By definition, parasites divert resources from their hosts and as a result reduce host fitness. Parasites are taxonomically diverse, with representatives from taxa such as viruses, bacteria, protozoa, fungi, plants, nematodes, arthropods, and vertebrates (e.g. Poulin & Morand 2004; Schmid-Hempel 2011). In addition to their effects on the fitness of host individuals, parasites may play an important role in shaping the evolutionary trajectories of host populations. For example, parasites have been proposed to affect the strength of sexual selection (Hamilton & Zuk 1982), influence the

persistence of sexual over asexual reproduction (Hamilton 1980, Lively 2010), and shape patterns of host biodiversity and speciation (Ricklefs 2010, Thornhill & Fincher 2013). In particular, the parasite-driven-wedge hypothesis (Thornhill & Fincher 2013) proposes that coevolution between parasite and host drives the evolution of locally adapted anti-parasitic host behaviours. That is, if hosts have an advantage in defending against their local parasites, selection may favour increased philopatry and social preference for local conspecifics, which may promote speciation (Thornhill & Fincher 2013).

Protozoan parasites belonging to the order Haemosporidia are distributed across all continents except Antarctica (Valkiunas 2005). Haemosporidia are obligate blood-borne parasites that alternate between a vertebrate host and a blood sucking invertebrate (Dipteran) vector (Perkins & Schall 2002, Perez-Tris *et al*. 2005). In birds, the most prominent haemosporidian parasites comprise the genera *Haemoproteus*, *Plasmodium* and *Leucocytozoon*. These intracellular parasites are transmitted by biting midges (family Ceratopogonidae), mosquitoes (family Culicidae), and black flies (family Simuliidae), respectively, and collectively infect about 70% of avian species (Atkinson & van Riper 1991). In their avian hosts, these haemosporidia invade organs such as the liver, spleen, heart or brain, then release gametes into the bloodstream that enter red blood cells. Haematozoan infections can cause capillary blockage, organ malfunction, and anemia, and may ultimately lead to the death of the host (Valkiunas 2005).

Even sublethal haemosporidian infections may reduce the fitness of their avian hosts. For example, experimentally treating blue tits (*Cyanistes caeruleus*) with the anti-parasitic drug Primaquine to reduce *Haemoproteus* and *Leucocytozoon* infection increases reproductive success, condition, and survival from one year to the next, thus

demonstrating that these parasites reduce reproductive success, condition and survivorship (Martinez-de la Puente *et al*. 2010, Merino *et al*. 2000). Also in blue tits, experimental treatment with the drug Malarone to reduce *Plasmodium* burden increases hatching success, offspring provisioning rates, and fledging success (Knowles *et al*. 2010). Similarly, house martins (*Delichon urbica*) that were treated with Primaquine to reduce infection by *Haemoproteus prognei* had increased clutch size, hatching and fledging success (Marzal *et al*. 2005). Thus, haematozoan parasites have measurable effects on host fitness and constitute an important part of their biotic environment.

The studies reviewed above show that Haemosporidian parasites can represent an important selection pressure on their avian hosts. Depending on the strength of selection, this could lead to local adaptation of the host to the parasite, such that host individuals of local origin have an advantage in defending against the local parasites (Greischar & Koskella 2007). Conventional wisdom predicts that the shorter generation time and larger population size of parasites relative to their hosts should allow parasites to adapt to their local hosts rather than hosts adapting to local parasites. However, recent studies (Imhoof & Schmid-Hempel 1998, Kaltz *et al*. 1999, Oppliger *et al*. 1999, Kalbe & Kurtz 2006) show that in some cases local adaptation by hosts can occur. Moreover, host individuals of local origin could also have an advantage in dealing with the local parasites even in the absence of host local adaptation. This is because prior immune experience with a particular parasite can train the adaptive immune system to control that parasite in the future. For example, in domestic canaries (*Serinus canaria*) individuals that were repeatedly exposed to a lineage of *Plasmodium relictum* (SGS1) developed resistance to the parasite, demonstrated by decreasing parasite load upon re-infection (Cellier-Holzem

et al. 2010). Thus, through local adaptation (ultimate) or inoculation (proximate) with the local parasites philopatric individuals may enjoy a relative advantage over dispersing individuals.

A growing body of evidence supports the possibility that birds of local origin may have an advantage in defending against local parasite strains. For example, in white-crowned sparrows (*Zonotrichia leucophrys*), males singing local dialects (and thus inferred to be of local origin) had lower haematozoan parasite loads than males singing foreign dialects (and thus inferred to have immigrated; MacDougall-Shackleton *et al*. 2002). In barn swallows (*Hirundo rustica*), philopatric females had lower intensity of infection by a haematophagous louse fly (Saino *et al*. 2014), suggesting that local adaptation, prior immune experience, or both, might give individuals of local origin an advantage in resisting local parasite strains. Similarly, male song sparrows with high genetic similarity to the local population (and thus inferred to be of local origin) were in better physiological condition and had lower blood-borne parasite loads relative to males that were less genetically similar (Stewart & MacDougall-Shackleton 2008).

One limitation of the above-reviewed studies is that they were conducted on a single population, thus restricting our ability to draw general conclusions about the fitness consequences of dispersal. First, source populations may differ in the abundance of parasites and vectors, making it difficult to determine whether high parasite loads are a consequence or a cause of natal dispersal. Moreover, host-parasite arms races involve cycles of adaptation and counter-adaptation, played out in parallel over the landscape, and thus whether parasites are adapted to their local hosts or hosts are adapted to their local parasites at any point in time may vary from site to site. Thus, an important next

step in assessing the fitness consequences of dispersal (particularly as regards susceptibility to parasites) is to determine the degree to which home-field advantage is general as opposed to site-specific. Another advantage of landscape-scale genetic sampling is that it permits the use of genetic assignment tests (Piry *et al*. 2004) as an alternative means to identify immigrants and birds of local origin, as opposed to inferring philopatry from song types (MacDougall-Shackleton et al. 2002) or assuming that locally common genotypes reflect high philopatry (Stewart & MacDougall-Shackleton 2008).

2.1.1 Objectives

In this study, my first objective was to determine whether song sparrows of local origin have a fitness advantage over birds that immigrated from elsewhere (hereafter "home-field advantage"). My second objective was to determine whether such home-field advantage is mediated by the ability to avoid or otherwise control infection against local strains of blood-borne parasites. My third objective was to investigate the degree to which home-field advantage varies over the landscape. I used genetic assignment tests based on neutral-locus (microsatellite) allele frequencies across multiple sites, and measured the following three proxies of fitness: body condition (comprising skeletal size and current body condition), prevalence and load of bloodborne parasites, and overwinter survivorship. If individuals of local origin have a home-field advantage, then I expected immigrants to have relatively poor body condition and potentially decreased overwinter survivorship. Moreover, if home-field advantage is mediated by familiarity with, or adaptation to, local parasite strains, immigrant individuals should be at higher risk of parasitism or be more heavily parasitized if infected. Finally, if home-field advantage is a

pattern that is consistent over the landscape, I expected immigrants to be in poorer condition than birds of local origin (e.g. lower body condition, more parasitism) regardless of their site of capture. Alternatively, if home-field advantage is site-specific, birds of local origin may outperform immigrants at some sites, but not at others (that is, site and philopatry may interact to predict condition and parasitism).

The fitness consequences of natal dispersal are important to understand because they are likely to affect gene flow, population connectivity, and ultimately speciation. Determining how interactions with parasites may influence the fitness consequences of dispersal is increasingly important due to anticipated range shifts and expansions of parasites and vectors associated with a changing climate (Rosenthal 2009, Tabachnick 2010).

2.2 Methods

2.2.1 Study Populations and Sites

I investigated the relationships between inferred philopatry, bloodborne parasites, and condition in a total of 328 song sparrows, captured over four years in twelve geographically distinct locations in southern Ontario (Table 2.1). Eleven of the capture sites were located in southeastern Ontario, and included lands owned by the Queen's University Biological Station (Sites 1, 2, 3, 4; Figure 2.1), Provincial Parks (Sites 5, 6, 7, 8, 9), and regional conservation areas (Sites 10, 11). The remaining capture site (site 12) was located in southwestern Ontario, on land owned by Western University, approximately 440 km from the other sites. Each site contained typical breeding habitat

for song sparrows - *i.e.*, old fields, forest edges, generally near lakes, streams or wetlands (Arcese *et al*. 2002).

The largest number of song sparrows (a total sample size of 141 over two breeding seasons; 43% of the 328 birds in this study) was captured at site 1 (Bracken), the breeding location for a long-term colour-banded population of song sparrows monitored by our research group since 2004.

Table 2.1 Capture sites, showing precise GPS coordinates, years sampled, and sample size by site and sex. Sites 6 and 7 (Silver Lake and Sharbot Lake) were subsequently pooled due to close geographic distance and the low number of individuals captured at each site.

Site #	Site name	Coordinates	Years sampled	# Birds sampled	# Males	# Females	# Sex unknown
1	Bracken	44.633°N, 76.330°W	2009, 2010	141	104	36	1
2	Station	44.567°N, 76.324°W	2009, 2010	34	29	5	0
3	HP property	44.475°N, 76.430°W	2010	21	21	0	0
4	TresGrids	44.521°N, 76.385°W	2009	7	6	1	0
5	Murphys Point	44.781°N, 76.236°W	2010	20	19	1	0
6	Silver Lake	44.830°N, 76.579°W	2010	11	10	1	0
7	Sharbot Lake	44.783°N, 76.715°W	2010	4	4	0	0
8	Frontenac	44.508°N, 76.543°W	2010	21	20	1	0
9	Charleston Lake	44.501°N, 76.035°W	2010	19	17	2	0
10	Little Cataraqui	44.289°N, 76.511°W	2009	11	6	5	0
11	Lemoine Point	44.226°N, 76.612°W	2009	20	17	3	0
12	London	43.008°N, 81.291°W	2011, 2012	19	15	4	0
Total				328	268	59	1

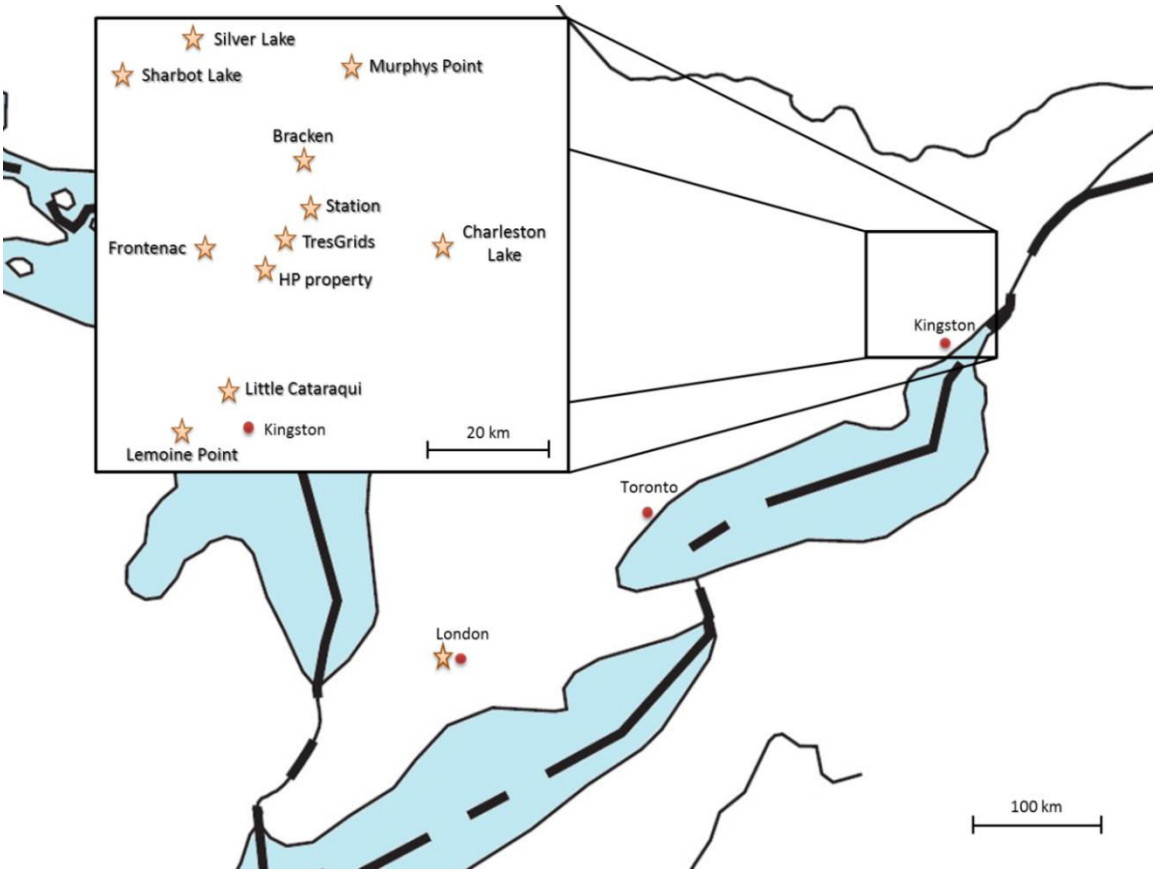

Figure 2.1 Map showing the location of the 12 sampling sites across Southern Ontario. Blood samples from these locations were used to determine the prevalence of Haemosporidian blood parasites in this region.

2.2.2 Field Techniques

I captured adult song sparrows during four sampling periods: April, May, and July of 2009 and 2010; October 2011; and July, August, and September 2012 (Table 2.1). Birds were lured into mist nets using playback of conspecific song or captured in seed-baited Potter traps. Mist nets were monitored continuously and Potter traps were checked at least once per hour. Because some sites were sampled across more than one year, some birds were captured more than once. In these cases I used only the data collected on first capture.

Upon capture, I collected approximately 20 µL of blood from each song sparrow via brachial venipuncture, for genetic analysis and analysis of infection status and parasite load. Briefly, I placed a drop of freshly-collected blood onto a clean glass microscope slide, then used a second slide to gently pull the blood along the first slide, creating a thin-film blood smear (Bennett 1970). Slides were air-dried, fixed by immersion in 100% methanol for 1 min within 24 hours of collection, and stored at room temperature. The remainder of the blood sample was blotted onto high wet-strength filter paper saturated with 0.5 M EDTA (pH 8.0) as a preservative, allowed to air-dry, and stored at room temperature prior to genetic analysis.

For each bird, mass was measured to the nearest 0.1 g using a spring scale, and right tarsus length was measured to the nearest 0.1 mm using dial calipers. Subcutaneous furcular fat (located between pectoral muscles) was scored using a 5-point scale (Gosler 1996) ranging from zero (no visible fat) through 5 (bulging fat deposits protruding beyond the furculum). Most birds were captured during the breeding season, during which time males can be identified by the presence of a cloacal protuberance (CP;

Wolfson 1952). I measured CP length from abdomen to tip (excluding feathers) to the nearest 0.1 mm using dial calipers. For all birds captured, if CP was not clearly visible I used molecular techniques to confirm sex (in the case of apparent females captured during the breeding season) or to determine sex (in the case of birds captured outside the breeding season) following Griffiths *et al.* (1998). The sex of one bird captured at Bracken outside the breeding season remained undetermined due to failure of molecular techniques.

I collected all the above morphological measurements from birds captured at sites 2 through 12, whereas at site 1 measurements were taken by two other members of the research team. While I cannot exclude the possibility of among-observer differences in absolute measurements, all three researchers used the same technique, morphological landmarks and scoring criteria. Variation among analyses in degrees of freedom reflect the fact that not all measurements were collected from all birds. Birds captured for the first time were banded with a uniquely numbered aluminum leg band, and birds at site 1 were also given an individually unique combination of coloured plastic leg bands for field identification. Nineteen birds from site 12 (London) were retained for use in an infectivity trial (Chapter 5) and all others were released at the site of capture.

Consistent and high capture effort each year at site 1 (Bracken) allowed me to assess overwinter survivorship at this site for the winters of 2009-2010 and 2010-2011. For each adult song sparrow captured in 2009 or 2010 at site 1, I recorded whether or not it was re-captured the following spring. I used these overwinter return data to infer survivorship: birds re-captured the following spring had clearly survived the winter, and birds not re-captured were assumed to have died.

2.2.3 Bloodborne parasite analyses

I used a combination of microscopic analysis of thin-film blood smears, plus PCR-based molecular methods, to assess the prevalence and intensity of bloodborne parasite infections. Thin-film blood smears were stained using Wright-Giemsa stain, following the protocol from HARLECO® Hemacolor® Stain Set. I examined each slide under a light microscope, using a 100X objective, until 10,000 erythrocytes had been screened. I recorded the total number of haematozoan parasites present within this sample of 10,000 erythrocytes. I categorized parasites to genus following Valkiunas (2005) whenever possible. However, genera *Plasmodium* and *Haemoproteus* are morphologically similar (Valkiunas 2005), and confidently differentiating them under the microscope was not possible. Thus, to be conservative, I grouped observations of these parasites together as *Plasmodium/Haemoproteus* (PH).

As a complement to microscopic analysis of thin-film blood smears, I used molecular methods to detect haematozoan infection and to assign any infections to genus. I extracted DNA (comprising both avian and blood-borne parasite DNA; Bensch *et al.* 2000) from field-collected blood blots using an ammonium acetate-based protocol (Bruford *et al.* 1998). I used a two-stage, nested polymerase chain reaction (PCR) approach (Hellgren *et al.* 2004, Waldenström *et al.* 2004) to amplify a 480 bp portion (excluding primers) of cytochrome b from haemosporidian parasites of genera *Haemoproteus*, *Plasmodium* and *Leucocytozoon* (Fig. 2.2).

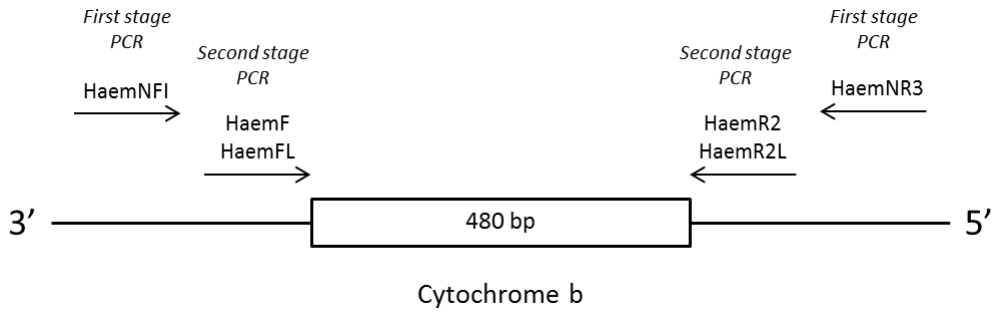

Figure 2.2. Haemosporidian cytochrome b sequence of interest that was amplified through PCR. First-stage primers HaemNFI and HaemNR3 amplify a 571 bp fragment (excluding primers). Second-stage primers HaemF and HaemR2 amplify *Plasmodium* and *Haemoproteus* spp., and primers HaemFL and HaemR2L amplify *Leucocytozoon* spp., both resulting in a fragment of 480 bp (excluding primers).

First-stage PCR used primers HaemNFI and HaemNR3 (Hellgren *et al*. 2004) to amplify a 617 bp fragment (including primers) of cytochrome b. PCR was conducted in a total volume of 25 µL and included approximately 50 ng of genomic DNA, 1.25 mM of each nucleotide, 1.5 mM $MgCl_2$, 1x PCR buffer (1 mM Tris-HCL pH 9.0; 5 mM KCL; 0.1% Triton X100; 0.2 mg/ml BSA), 0.6 mM of each primer and 0.5 U Taq DNA polymerase (Fisher Scientific). Thermal cycling conditions included an initial denaturing step of 3 min at 94 ºC; then 20 cycles of 30 sec at 94 °C, 30 sec at 50 °C, and 45 sec at 72 °C; then a final step of 10 min at 72 °C.

Second-stage PCR used 2 µL of the first-stage PCR product as a template for each of two separate amplifications: one using primers HaemF and HaemR2 (Bensch *et al*. 2000) to amplify a portion of cytochrome b from *Haemoproteus* and *Plasmodium*, and

the other using primers HaemFL and HaemR2L (Hellgren *et al*. 2004) to amplify a portion of cytochrome b from *Leucocytozoon*. PCR conditions (recipes and thermal parameters) were otherwise the same as for the first-stage PCR reaction described above. Both final products were 480 bp long (excluding primers).

I ran second-stage PCR products at 120 volts for 90 minutes through a 2% agarose gel containing ethidium bromide, and visualized bands on an ultraviolet transilluminator. Each gel included a 100-10K bp ladder (Kplus DNA ladder, GeneDirex) and two negative controls, one for each stage of PCR. The first-stage control was a first-stage PCR reaction using 1 uL of water instead of template DNA, and the second-stage control consisted of a second-stage PCR with 2 uL of first-stage control PCR product as the template. For all positive amplifications (bands of expected product size) I excised the band from the gel, purified it using a DNA purification kit (Gel/PCR DNA Extraction Kit, FroggaBio), and sent it for sequencing (using primer HaemF or HaemFL as appropriate) from the 5' end on an Applied Biosystems 3730 DNA Analyzer at the London Regional Genomics Centre. I used BioEdit version 7.1.3.0. (Hall 1999) to trim the resultant sequences to 476 bp to make them all the same length, then used Basic Local Alignment Search Tool (BLAST) to categorize them as belonging to genus *Plasmodium*, *Haemoproteus* or *Leucocytozoon*.

I combined findings from both microscopy and molecular methods to determine the infection status of individual song sparrows. That is, an individual was classified as 'infected' if one or more parasites were observed during microscopic examination of 10,000 erythrocytes, or if a positive amplification of haematozoan DNA was observed.

2.2.4 Inferring philopatry

I used genetic assignment testing (Piry *et al*. 2004) to estimate the likelihood that an individual had originated from the site in which it was captured. I genotyped all birds at 17 microsatellite loci: Mme 1, Mme 2, Mme 7 (sex-linked) and Mme 12 (Jeffery *et al*. 2001); Escμ1 (Hanotte *et al*. 1994); Pdoμ5 (Griffith *et al*. 1999); SOSP 1, SOSP 2, SOSP 4, SOSP 5, SOSP 7, SOSP 13, SOSP 14 (Sardell *et al*. 2010); SOSP 3, SOSP 9 (Dr. Lukas Keller, pers. comm. to Dr. Beth MacDougall-Shackleton); Zole B03, and Zole C02 (Poesel *et al*. 2009). One primer at each locus was labelled with fluorescent dye (Life Technologies).

Each PCR reaction was conducted in a total volume of 10 μL and included 10 mM Tris-HCl, 50mM KCl, 0.1% Triton X-100, 0.2 mg/mL BSA, 2.5mM $MgCl_2$, 0.2 mM of each dNTP, 0.1-0.4 mM of each primer, 0.5 U *Taq* polymerase (Fisher Scientific) and approximately 25 ng of genomic DNA. Cycling conditions included an initial step of either 180 s (SOSP 1, 2, 3, 4, 5, 7, 9, 13, 14, Zole B03, C02, Mme 1, 12) or 270 s (Mme 2, 7, Escμ1, Pdoμ5) at 94°C, followed by 28 cycles of either 30 s at 94°C, 90s at the annealing temperature, and 60 s at 72°C (SOSP 1, 2, 3, 4, 5, 7, 9, 13, 14, Zole B03, C02, Mme 1, 12); or of 30 s at 94°C, 40 s at the annealing temperature, and 40s at 72°C (Mme 2, 7, Escμ 1, Pdoμ 5). Annealing temperatures were 57°C for SOSP 2, 3, 4, 9, 13, 14, Mme 1, 12, Zole B03, and C02; 55°C for SOSP 1, 5, and 7; and dropped from 52°C to 48°C using a touchdown protocol for Mme 2, 7, Escμ 1 and Pdoμ 5. All reactions had a final step of 270 s at 72°C.

PCR products were analyzed for fragment size through capillary electrophoresis on Applied Biosystems 3130 and 3730 Genetic Analyzers, according to the

manufacturer's protocol. For each locus, I scored alleles manually using the software GENE MAPPER (Applied Biosystems) to visualize peaks with reference to LIZ size standard. Locus Mme 7 (Jeffery *et al*. 2001) is sex-linked so females were coded as hemizygous at this locus.

I tested for null alleles using Dempster's Expectation Maximum method (Dempster *et al.* 1977) implemented in GENEPOP ON THE WEB (http://genepop.curtin.edu.au/; Rousset 2008). Similarly, deviations from Hardy-Weinberg equilibrium were tested across the remaining 16 loci, separately for each capture site, using GENEPOP ON THE WEB. Markov chain parameters included a de-memorization number of 1000, 100 batches, and 1000 iterations per batch. Linkage disequilibrium was tested on the 16 loci using GENEPOP ON THE WEB, implementing the Markov chain parameters outlined above. Linkage disequilibrium was investigated separately at each site.

To estimate each individual's inferred philopatry (L_home), that is, the likelihood that it was born at the site in which it was captured as a breeding adult, I used the software GENECLASS2 (Piry *et al*. 2004). Specifically, I used the L_home algorithm to detect first-generation migrants (Paetkau *et al*. 2004). This option computes for each individual the probability that it was born at the site in which it is sampled, without assuming that all potential source populations have been sampled (Piry *et al*. 2004). Values of L_home can range between 0 and 1, with higher values interpreted as more likely to have been born at the site at which an individual is sampled (*i.e.*, more philopatric). I used the criterion of Rannala & Mountain (1997) to estimate L_home; and conducted Monte-Carlo simulations over 1000 individuals. This criterion compares each

individual allele frequencies to the allele frequencies across all populations and as a result generates a likelihood that the individual genotype originates from the population in which it was sampled.

2.2.5 Statistical analysis

To reduce the dimensionality of morphological measurements, I conducted principal components analysis (PCA) on a correlation matrix conducted in SPSS version 21 (SPSS, Inc.). Variables entered into the PCA included mass, tarsus length, fat score, and date-corrected cloacal protuberance (CP) length. Specifically, I calculated the residuals of a simple linear regression between CP and capture date, because CP length increases throughout the breeding season ($R^2_{205} = 0.449$, $p < 0.001$). Male and female song sparrows differ somewhat in size and mass (Arcese *et al*. 2002), and only males have a CP, thus only males were included in the morphological PCA. Moreover, because some of the above morphological measures vary seasonally, I calculated body condition only for males captured during April and May. Missing data were handled by list-wise deletion resulting in a final sample size of 180 males caught during the breeding season for which full data were available.

Principal components with eigenvalues greater than 'one' were retained for analysis. A Varimax rotation of the extracted principal components was also calculated, but since it did not qualitatively affect the results (data not shown), I report only the results from the un-rotated components.

To investigate the association between inferred philopatry (L_home) and measures of morphology and condition, I constructed multiple regressions with the retained principal components as dependent variables. For each principal component, I first constructed an initial model with independent variables consisting of L_home, capture site (coded as a factor), the interaction between L_home and capture site, capture date, and year of capture (coded as a factor). I then used model selection to sequentially eliminate non-significant predictors ($p > 0.05$), beginning with the least significant. However, because I was specifically interested in the L_home × capture site interaction, I retained the terms L_home, capture site and their interaction in the final model regardless of significance.

To determine whether inferred philopatry (L_home) predicted the likelihood of being parasitized, I used a generalized linear model with a binary logistic error distribution. The dependent variable was the individual's infection status (parasitized or unparasitized), as determined by microscopy or molecular methods. Independent variables in the initial model included L_home, capture site, the interaction between L_home and capture site, capture date, year of capture, and sex. To improve parsimony, I then sequentially eliminated non-significant predictors as described above. Because I was specifically interested in the L_home × capture site interaction, I retained the terms L_home, capture site and their interaction in the final model regardless of significance.

To determine whether inferred philopatry (L_home) predicts the intensity of infection (parasite load) among the subset of birds that were parasitized, I used a generalized linear model with a negative binomial error distribution, because parasite load was not normally distributed. The dependent variable was total parasite load (*i.e.*,

combined count of parasites of all three genera per 10,000 erythrocytes). Song sparrows that scored positive for parasitism using molecular methods but with no parasites observed in microscopic examination of 10,000 erythrocytes (n = 109) were included in this analysis and assigned a parasite load of zero. Independent variables in the initial model included L_home, capture site, the interaction between L_home and capture site, capture date, year of capture, and sex. As outlined above, I sequentially eliminated non-significant predictors but retained the terms L_home, capture site and their interaction in the final model regardless of significance.

To determine whether inferred philopatry (L_home) predicted apparent overwinter survivorship (*i.e.*, recapture the following spring), I used a generalized linear model with a binary logistic distribution for each of winters 2009-2010 and 2010-2011. The dependent variable was apparent survivorship, and independent variables in the initial model were L_home, date of capture, and sex. To improve parsimony, I sequentially removed nonsignificant predictors, beginning with the least significant, except that L_home was retained in the final model regardless of significance. This analysis was restricted to adult birds captured at the Bracken field site, during spring and summer (April-July).

Non-significant terms that did not remain in the final models are reported with the statistic values they had before leaving the model. All statistical analyses were performed with SPSS version 21 (SPSS, Inc.) and all tests were two-tailed.

2.3 Results

2.3.1 Inferring philopatry

I found heterozygote deficits consistent with non-amplifying (null) alleles in four of the 17 loci: Sosp 5, Mme 7, Mme 12, and Zole B03. Locus Zole B03 showed a null allele frequency higher than 10% in most (8/11) of the capture sites and was excluded from further analysis. The other three loci (Sosp 5, Mme 7, Mme 12) showed a null allele frequency higher than 10% in only four sites (Sosp 5), or in only one site (Mme 7, Mme 12). Values of L_home (see below) derived from the 16-locus dataset were correlated to those derived from the 13-locus dataset that excluded all four questionable loci (Pearson's $r_{325} = 0.942$, $p < 0.001$). Because Sosp 5, Mme 7 and Mme 12 did not consistently show appreciable frequencies of null alleles, in order to make use of maximum genetic information I retained these loci in subsequent analyses, for a total of 16 loci examined.

Following Bonferroni correction for multiple tests (11 sites x 16 loci, $\alpha = 0.05/176$), no significant heterozygote excesses were observed but significant heterozygote deficits were found at some loci at some capture sites. A total of 8 locus/site combinations (Bracken – Sosp3, Sosp5, Sosp14, Mme7, Escu1, Sosp9, ZoleC02; Station – Sosp5), affecting 7 loci, showed a heterozygote deficit. To determine whether inclusion or exclusion of questionable loci affects estimates of inferred philopatry, I performed genetic assignment by calculating L_home (see below) with and without these 7 loci. I coded these questionable loci as missing data at the sites at which heterozygote deficits were observed (2 sites), for a total of 8 locus/site combinations, and compare it with the L_home values derived from the full 16-locus dataset. Values of L_home calculated by

excluding questionable locus-site combinations were highly correlated with those derived from the full 16-locus dataset ($r_{325} = 0.840$, $P < 0.001$). Thus, the inclusion or exclusion of questionable loci appears to have negligible effects on calculations of L_home. In the interest of making maximum use of the genotypes available, I retained all 16 loci for subsequent analysis.

After applying the Bonferroni correction for multiple tests (120 pairwise combinations x 11 sites, $\alpha = 0.05/1320$), I found evidence of linkage disequilibrium between two pairs of loci (Sosp 5 and Mme 12; Sosp 7 and Sosp 14) at site 1, and between one pair of loci (Sosp 3 and Mme 2) at site 2. Because no pairs of loci were consistently in linkage disequilibrium across multiple sites, I do not expect these loci to be physically linked. Thus I retained all 16 loci for subsequent analysis.

2.3.2 Morphological Measures and Body Condition

Principal Component Analysis (PCA) conducted on morphological measures identified two principal components with eigenvalues greater than 1, that together explained 66.5% of the variance (Table 2.2). The first component (PC1) had high positive loadings for mass and tarsus, and was interpreted as reflecting skeletal size. PC2 had high positive loadings for mass and fat, and was interpreted as reflecting body condition.

Table 2.2. Factor loadings for principal component analysis of four morphological measurements collected from 180 adult male song sparrows, captured in April-May of 2009 and 2010. CP length was residual-corrected for capture date. PC1 and PC2 were retained for subsequent analysis.

	Component			
	1	2	3	4
Mass (g)	0.767	0.46	0.038	-0.445
Tarsus (mm)	0.859	-0.236	-0.089	0.446
Fat score	-0.08	0.763	0.579	0.276
Residual CP	0.132	-0.68	0.707	-0.146
Cumulative % variance explained	33.77	66.56	87.66	100.000
Eigenvalue	1.351	1.311	0.844	0.494

2.3.3 Philopatry and Morphological Measures

Observed values of L_home ranged from 0 to 1 (mean = 0.590; S.E.= 0.019). Predictors of male PC1, interpreted as skeletal size, are summarized in Table 2.3. I found no main effect of philopatry (L_home) on PC1. However, I observed a significant main effect of site and a near-significant interaction between L_home and site. Thus, skeletal size varies across the capture sites in this study, and moreover, the relationship between philopatry and skeletal size may also vary from site to site (Fig. 2.3).

Predictors of male PC2, interpreted as body condition, are summarized in Table 2.4. PC2 varied significantly among sites, but I found no main effect of inferred philopatry (L_home) on PC2 (current body condition), nor was there a significant interaction between L_home and site.

Table 2.3. Reduced model of a linear multiple regression for predictors of PC1 (mass and tarsus) for 180 adult male song sparrows. L_home, site of capture, and L_home x site of capture were retained in the model regardless of significance.

Predictor	*df*	*Wald* χ^2	*p*
L_home	1,171	0.017	0.898
site of capture	7,171	17.201	0.016
L_home x Site	7,171	12.698	0.080
overall model	15,171	64.365	<0.001

Eliminated variables	*df*	*Wald* χ^2	*p*
date of capture	1,164	0.112	0.737
year of capture	1,176	1.298	0.255

Table 2.4. Reduced model of a linear multiple regression for predictors of PC2 (mass and fat) for 180 adult male song sparrows captured during April and May of 2009 and 2010. L_home, site of capture, and L_home x site of capture were retained in the model regardless of significance.

Predictor	*df*	*Wald* χ^2	*p*
L_home	1,171	1.857	0.173
Site of capture	7,171	35.234	<0.001
L_home x Site	7,171	5.439	0.607
overall model	15,171	135.909	<0.001

Eliminated variables	*df*	*Wald* χ^2	*p*
date of capture	1,169	0.303	0.582
year of capture	1,170	1.701	0.192

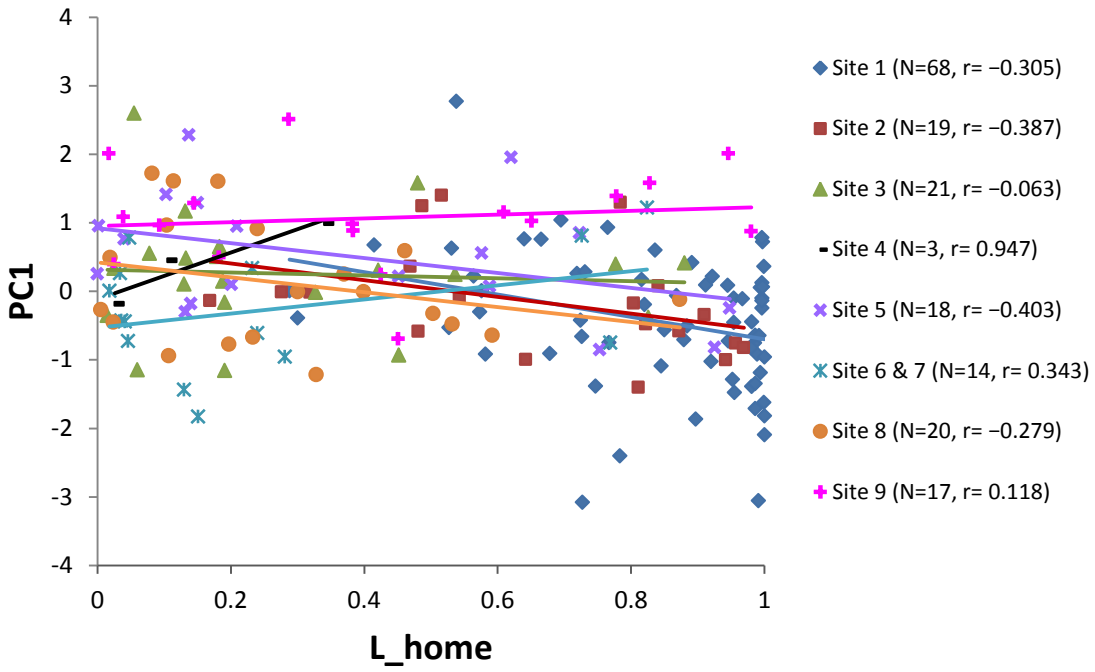

Figure 2.3. Relationship between inferred philopatry values (L_home) and PC1 (skeletal size) for 180 male song sparrows caught at different sites throughout southeastern Ontario. Each line represents the line-of-best-fit for one capture site. No consistent relationship between philopatry and skeletal size was observed across sites.

2.3.4 Philopatry and Parasitism

The three most commonly observed parasite genera were *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* (Table 2.5). Microscopy detected one case of infection with the extracellular parasite nematode microfilariae, but this parasite was omitted from subsequent analyses and the individual was counted as uninfected. A total of 17% of birds examined through microscopy showed evidence of haemosporidian infection, whereas 24% of birds screened via PCR were infected (Table 2.6). However, a combination approach using both microscopy and PCR to identify infected birds yielded the highest prevalence of infection (33.2%). Using this combined approach, I found infection prevalence to range from a minimum of 0% at Lemoine Point to 52.6% at Charleston Lake.

The most commonly observed parasite genus was *Leucocytozoon* (detected in 65 of 328 birds, or 19.8%) with *Plasmodium* slightly less commonly observed (detected in 43 of 328 birds, or 13.1%), and *Haemoproteus* detected in 4 of 238 birds (1.2%; Table 2.6). The total prevalence of mixed infections (*Plasmodium*/*Haemoproteus* and *Leucocytozoon*) was 4.9%.

For the subset of 109 song sparrows classified as infected, microscopic analysis of parasite load (parasites per 10,000 erythrocytes) revealed loads ranging from zero (*i.e.*, individuals with infections detected through PCR but no parasites observed in thin-film blood smear) through approximately 700 (estimation based on 70 parasites seen in 1000 erythrocytes). Two birds had extremely high loads of *Plasmodium*/*Haemoproteus* (210 and 700 parasites per 10,000 erythrocytes), which are greater than other observed parasite loads (all < 22 parasites per 10,000 erythrocytes). Because these two individuals had

most likely been infected only recently and thus remained in the acute phase of infection (Valkiunas 2005) I excluded them from subsequent analyses of parasite load, following the recommendation of Valkiunas (2005) to consider only chronic-stage infections when using parasite load to infer performance. However, including these two individuals in the analyses does not qualitatively alter results (data not shown). *Leucocytozoon* infections ranged in intensity (parasite load) from 0 through 35 parasites per 10,000 erythrocytes.

The likelihood of being parasitized by any combination of haematozoa did not vary with inferred philopatry (Table 2.7a). There was no main effect of site of capture, and there was no significant interaction between L_home and site. In investigating the prevalence of specific parasites, I found that inferred philopatry did not predict the likelihood of being parasitized by *Plasmodium* (Table 2.7b). However, *Plasmodium* prevalence did vary with the year of capture (Table 2.7b). Site and the interaction between L_home and site were not significant predictors of *Plasmodium* prevalence (Table 2.7b). Similarly, inferred philopatry did not predict the likelihood of being infected by *Leucocytozoon* (Table 2.7c). However, *Leucocytozoon* prevalence varied with sex, with males being significantly more parasitized than females. There was no main effect of site of capture, nor was there a significant interaction between L_home and site, in predicting *Leucocytozoon* prevalence (Table 2.7c). I observed a near-significant trend for birds with higher L_home values to have lower parasite loads of all haematozoa combined (Fig. 2.4; Table 2.8). Parasite load did not significantly vary with site of capture, nor was parasite load predicted by the interaction between L_home and site (Table 2.8).

Table 2.5. Prevalence (% of birds infected) of the three different genera of Haemosporidian found in song sparrows captured throughout southern Ontario: *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*. For *Plasmodium* and *Haemoproteus*, values reported represent only estimations from PCR amplification. Values reported for *Leucocytozoon* represent both microscopy and PCR amplification. Mixed infections comprised of two parasite genera were scored as positive for each parasite genus detected.

Site #	Site	Number of birds screened	Number infected with *Plasmodium*	Number infected with *Haemoproteus*	Number infected with *Leucocytozoon*
1	Bracken	141	19 (13.5%)	2 (1.4%)	32 (22.7%)
2	Station	34	1 (2.9%)	0	3 (8.8%)
3	HP property	21	3 (14.3%)	0	8 (38.1%)
4	TresGrids	7	2 (28.6%)	0	0
5	Murphys Point	20	1 (5%)	0	3 (15%)
6 & 7	Silver-Sharbot	15	4 (26.7%)	0	1 (6.7%)
8	Frontenac	21	4 (19%)	0	8 (38.1%)
9	Charleston Lake	19	4 (21%)	2 (10.5%)	5 (26.3%)
10	Little Cataraqui	11	0	0	3 (27.3%)
11	Lemoine Point	20	0	0	0
12	London	19	5 (26.3%)	0	2 (10.5%)
Total		328	43 (13.1%)	4 (1.2%)	65 (19.8%)

Table 2.6. Haemosporidian prevalence in song sparrows (number of individuals infected by one or more Haemosporidia of any genus in a scan of 10,000 erythrocytes) among the different capture sites, as determined by microscopy, PCR amplification, and both methods combined.

Site #	Site name	Number of birds screened	Prevalence determined by blood smears	Prevalence determined by PCR amplification	Prevalence determined by both methods
1	Bracken	141	26 (18.4%)	35 (24.8%)	50 (35.5%)
2	Station	34	2 (2.9%)	4 (14.7%)	6 (17.6%)
3	HP property	21	5 (23.8%)	9 (42.8%)	10 (47.6%)
4	TresGrids	7	1 (14.3%)	2 (28.6%)	2 (28.6%)
5	Murphys Point	20	2 (10%)	3 (15%)	4 (20%)
6 & 7	Silver-Sharbot Lake	15	2 (13.3%)	5 (33.3%)	6 (40%)
8	Frontenac	21	6 (28.6%)	6 (28.6%)	9 (42.8%)
9	Charleston Lake	19	5 (26.3%)	8 (42.1%)	10 (52.6%)
10	Little Cataraqui	11	3 (27.3%)	0	3 (27.3%)
11	Lemoine Point	20	0	0	0
12	London	19	3 (15.8%)	6 (31.6%)	9 (47.4%)
Total		328	55 (16.8%)	79 (24.1%)	109 (33.2%)

Table 2.7. Reduced model of a logistic multiple regression for predictors of the likelihood of being parasitized by (a) all haematozoa combined, (b) *Plasmodium* spp., and (c) *Leucocytozoon* spp., for 327 song sparrows caught in 2009, 2010, 2011, and 2012. L_home, site of capture, and L_home x site of capture were retained in the model regardless of significance.

	a)			b)			c)		
Predictor	*df*	*Wald* χ^2	*p*	*df*	*Wald* χ^2	*p*	*df*	*Wald* χ^2	*p*
L_home	1,315	<0.001	1	1,314	<0.001	1	1,313	<0.001	0.994
Site of capture	10,315	8.08	0.621	9,314	9.449	0.397	10,313	7.029	0.723
L_home x Site	10,315	5.362	0.866	10,314	8.225	0.607	10,313	4.487	0.923
year of capture	-	-	-	2,314	7.141	0.028	-	-	-
sex	-	-	-	-	-	-	1,313	5.956	0.015
overall model	21,315	31.274	0.069	23,314	54.431	<0.001	22,313	64.093	<0.001
Eliminated variables	*df*	*Wald* χ^2	*p*	*df*	*Wald* χ^2	*p*	*df*	*Wald* χ^2	*p*
date of capture	1,311	0.054	0.816	1,313	1.025	0.311	1,313	2.783	0.095
year of capture	2,312	0.743	0.69	-	-	-	2,311	0.594	0.743
sex	1,313	1.255	0.263	1,311	0.047	0.829	-	-	-

Table 2.8. Reduced model of a negative binomial multiple regression for predictors of intensity of infection for all haematozoa combined, for the subset of 109 song sparrows classified as infected (as determined by microscopy or molecular methods). L_home, site of capture, and L_home x site of capture were retained in the model regardless of significance.

Predictor	*df*	*Wald* χ^2	*p*
L_home	1,96	3.789	0.052
Site of capture	7,96	6.949	0.434
L_home x Site	7,96	5.414	0.610
overall model	19,96	26.929	0.106

Eliminated variables	*df*	*Wald* χ^2	*p*
date of capture	1,94	1.594	0.207
year of capture	1,94	0.090	0.764
sex	1,95	1.746	0.186

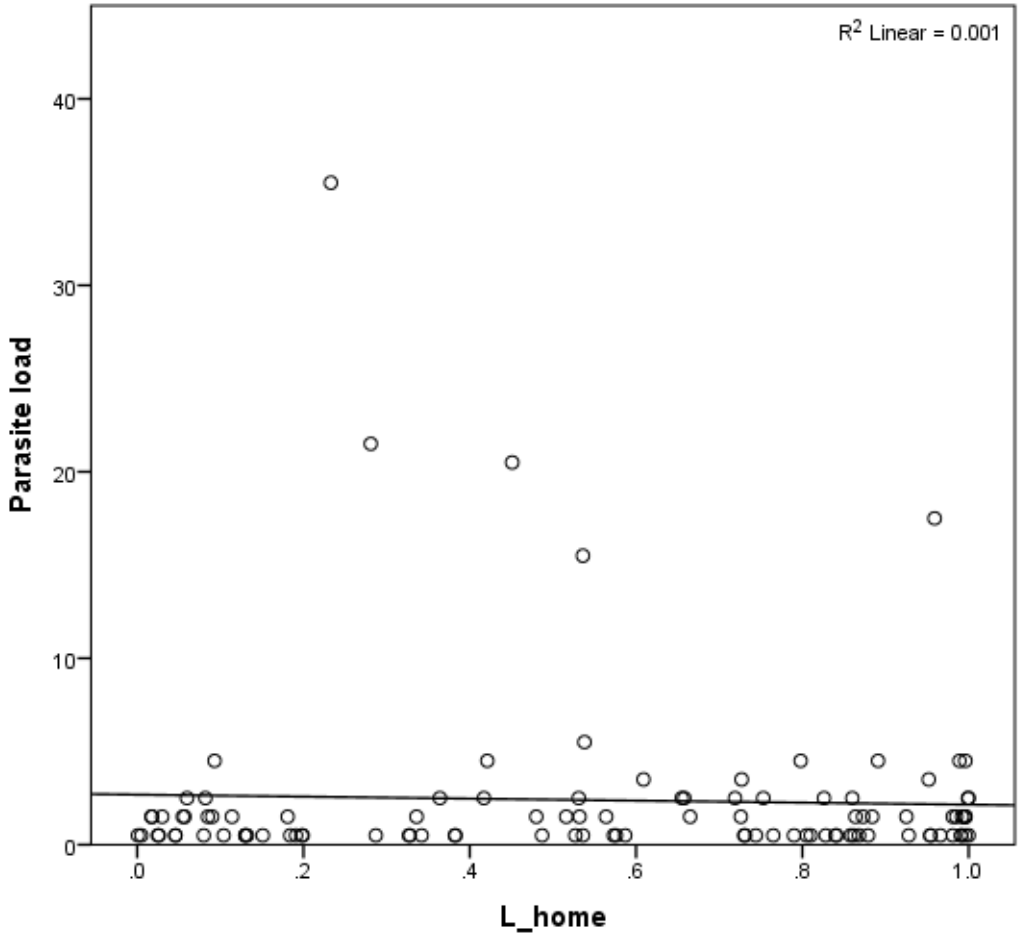

Figure 2.4. Combined parasite load (all haematozoa combined, per 10,000 cells examined) as a function of inferred philopatry (L_home). Birds with higher inferred philopatry tended to have lower parasite load counts.

2.3.5 Morphological Measures and Parasitism

For the subset of males classified as 'parasitized', overall parasite load (all haematozoa combined) did not correlate with PC1 (r_{68} = -0.118, p= 0.330) or PC2 (r_{68} = -0.087, p= 0.473).

2.3.6 Philopatry and Survivorship

Apparent survivorship of song sparrows at Bracken from 2009 to 2010 was not predicted by L_home. However, apparent survivorship was predicted by date of capture, such that birds captured earlier in the spring were more likely to return in 2010 (Fig 2.5). Predictors of apparent survival from 2009 to 2010 are summarized in Table 2.9a. Apparent survivorship of song sparrows at Bracken from 2010 to 2011 was again not predicted by L_home. Predictors of apparent survival from 2010 to 2011 are summarized in Table 2.9b.

Table 2.9. Reduced model of a multiple regression for predictors of apparent survivorship from 2009 to 2010 (a), and from 2010 to 2011 (b), for 69 song sparrows caught in Bracken in 2009. The variable L_home was retained in this model regardless of its significance.

a)				b)		
Predictor	*df*	*Wald* χ^2	*p*	*df*	*Wald* χ^2	*p*
L_home	1,66	0.023	0.88	1,90	0.004	0.947
date of capture	1,66	14.46	<0.001			
overall model	2,66	39.866	<0.001	1,90	0.004	0.947
Eliminated variables	*df*	*Wald* χ^2	*p*	*df*	*Wald* χ^2	*p*
date of capture				1,88	0.502	0.479
sex	1,65	0.167	0.682	1,87	0.014	0.905

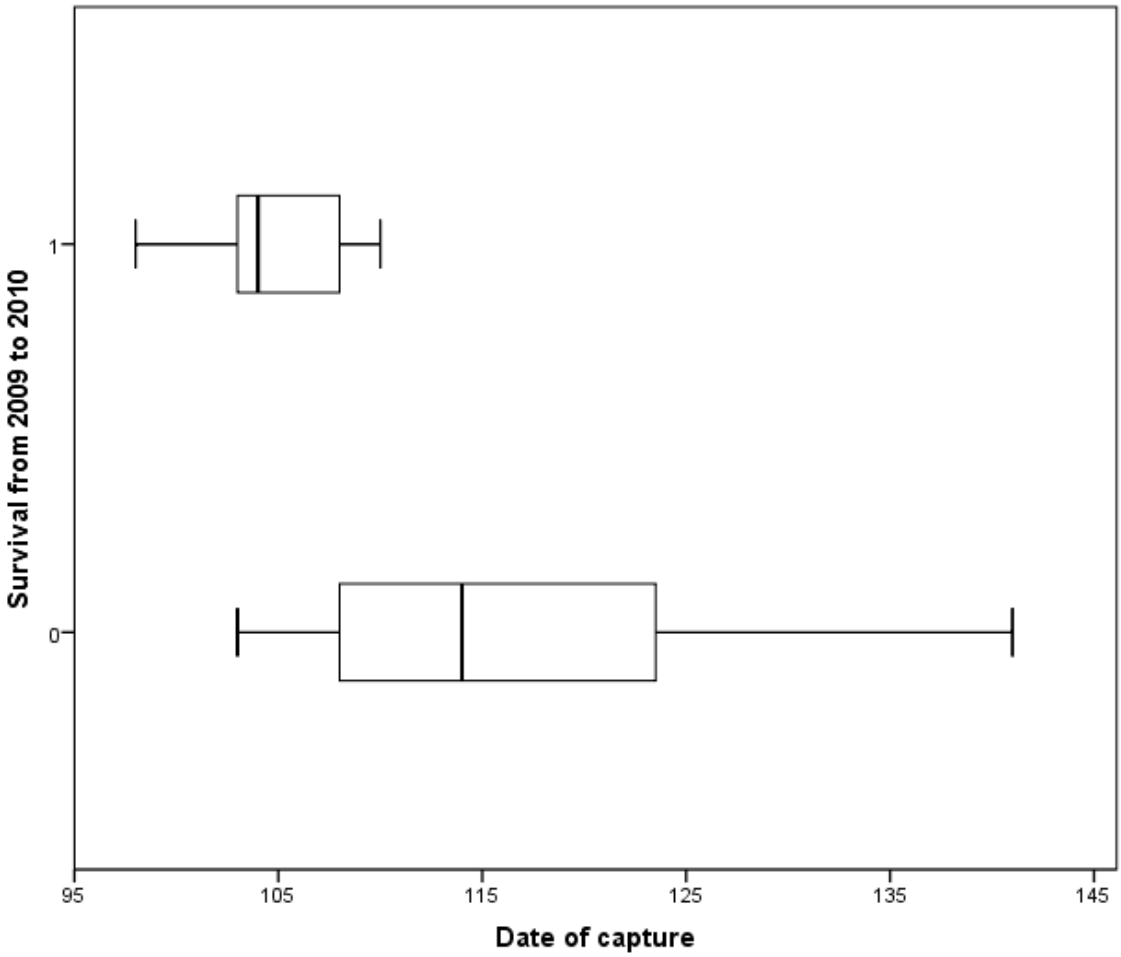

Figure 2.5. Survival from 2009 to 2010 as a function of capture date, for song sparrows caught between April and May 2009. The date of capture is represented by a continuous count of days from April 8 (98) to May 21 (141). Birds caught earlier in the spring were more likely to survive from 2009 to 2010. Box includes data between the 25th and 75th percentile, midline indicates the median, and error bars represent 95% confidence intervals.

2.4 Discussion

This study examined whether song sparrows inferred by genetic assignment tests to be of local origin have a home-field advantage (*i.e.*, better body condition, lower prevalence and intensity of haematozoan parasites, and greater overwinter survivorship) relative to individuals inferred to have immigrated from outside the area in which they bred. I found some evidence for home-field advantage, in that inferred philopatry tended to correlate with lower parasite loads. This relationship is consistent with hosts being locally adapted to native parasite strains but was not statistically significant for the populations examined here. Further, inferred philopatry did not predict parasite prevalence, body condition or (at the main study site) apparent overwinter survivorship. I also examined variation among sites in order to determine whether home-field advantage is general to all sites or whether it varies from site to site. I found a near-significant interaction between philopatry and site for PC1 (skeletal size) suggesting that the relationship between philopatry and skeletal size may vary over the landscape. Taken together, these findings suggest that song sparrows of local origin could have a home-field advantage in the ability to control infection by haematozoan parasites, but that such advantage is weak or difficult to isolate from other variables in the field, and does not extend to all traits likely to affect fitness. Moreover, the relationship between philopatry and performance may differ from site to site.

2.4.1 Inferred philopatry and morphological measurements

In contrast to what I would expect if dispersing individuals are inherently lower-quality than philopatric individuals and less able to compete for territories near their natal site, I

found no main effect of inferred philopatry (L_home) on skeletal size (PC1). This finding is in contrast to findings from barn swallows in which dispersing individuals are structurally smaller than philopatric individuals (Saino et al 2014). However, there was a near-significant interaction between site and inferred philopatry in predicting PC1. Thus, at some sites (e.g. Station) song sparrows of immigrant origin were larger than relatively local individuals, but at other sites (e.g. Silver and Sharbot Lake) the reverse pattern was observed. Skeletal size has been linked to measures of fitness and competitive ability in many vertebrate animals (e.g. subantarctic fur seals (*Arctocephalus tropicalis*), Beauplet & Guinet 2007; mountain white-crowned sparrows, MacDougall-Shackleton et al. 2002). Assuming that high values of PC1 reflect competitive ability, the observed interaction between site and philopatry in predicting PC1 may reflect differences in habitat quality across sites. Specifically, nestlings born in high-quality sites may grow faster and attain larger body size and also be more likely to successfully compete for a breeding territory near their natal site. Conversely, nestlings born at low-quality sites may be smaller but still able to obtain a territory near their natal site if competition for such territories is less intense. If so, philopatry may be positively related to PC1 at high-quality sites but negatively related to PC1 at poor-quality sites. Comparing quality of the capture sites examined here is beyond the scope of this study, but may represent an important consideration for future research. Heterogeneity of relationships between philopatry and body size also suggests that the fitness consequences of philopatry versus dispersal may vary across the landscape. Such variation may help to maintain heritable variation in these birds tendency towards philopatry.

Unexpectedly, PC1 (skeletal size) varied significantly across sites. This variation may be due in part to inter-observer differences in measurement technique (different researchers conducting measurements at different sites). Variation among sites in habitat quality may also contribute to the observed variation in PC1, because larger males may be better able to obtain breeding territories in high-quality habitats (e.g. Balbontin & Ferrer 2008).

In contrast to my prediction with respect to body condition and philopatry, I did not find a significant effect of philopatry on PC2. This is in contrast to previous findings from my main study population (Stewart & MacDougall-Shackleton 2008) as well as to findings from other taxa (e.g. in African striped mice (*Rhabdomys pumilio*), immigrant males weighed less than philopatric males (Solmsen *et al*. 2011). In contrast to my prediction that the relationship between philopatry and body condition may vary over the landscape, I also found no significant interaction between philopatry and PC2. However, I found a main effect of site of capture on PC2. As previously noted, this among-site variation in body condition may reflect inter-observer differences in measurement technique, and/or differences in habitat quality between sites. Overall, my findings with respect to PC2 suggest that birds of local origin may not experience home-field advantage in body condition, and/or that individuals in better body condition are not necessarily more likely to breed near their natal area.

2.4.2 Inferred philopatry and parasite prevalence

Molecular methods showed a higher prevalence of haemosporidian infections than microscopy, however, this difference is not surprising since molecular methods of detecting infections are considered to be more sensitive than microscopy (e.g. Richard *et al*. 2002). Similarly, methodological differences in scanning for different genera of parasites may reflect the fact that the most commonly observed parasite genus was *Leucocytozoon*, followed by *Plasmodium* and *Haemoproteus*: *Leucocytozoon* infections could be detected through both microscopy and molecular methods, but because I could not confidently distinguish *Plasmodium* from *Haemoproteus* under the microscope, I calculated the prevalence of these two genera using PCR amplification only. Additionaly, the 4.9% prevalence estimate of mixed infections (*Plasmodium*/*Haemoproteus* and *Leucocytozoon*) may underestimate the true rate of mixed infections, since *Plasmodium* and *Haemoproteus* infections were not visually distinct from one another.

In contrast to my prediction if philopatric individuals have a general home-field advantage in avoiding infection by parasites from their natal area, I found no main effect of inferred philopatry (L_home) on the likelihood of being parasitized by one or more haematozoa. Moreover, I found no significant interaction between L_home and site of capture in predicting parasite prevalence. Complementary analyses examining the prevalence of each parasite genus separately showed the same result.

Whereas I found no relationship between philopatry and naturally-occurring parasite prevalence, other studies have used experimental infection trials to examine host susceptibility to sympatric versus allopatric parasite strains. This approach represents an important next step in examining the relationship between philopatry and susceptibility to

parasites, because it allows researchers to rule out potential effects of variation in quality between philopatric and dispersing individuals. For example, Canarian lizards (*Gallotia galloti*) are less likely to become infected when experimentally exposed to sympatric than allopatric bloodborne parasites (*Haemogregarine* spp.), a pattern that indicates local adaptation by hosts to these parasites (Oppliger *et al.* 1999). On the other hand, trematode parasites (*Microphallus* sp.) have higher infection success on sympatric than allopatric populations of snails (*Potamopyrgus antipodarum*), indicating local adaptation by parasites to their hosts (Lively & Dybdahl 2000). Similarly, within a single collection site, trematodes had higher prevalence (infection success) on common than on rare host genotypes (Lively & Dybdahl 2000). Thus, experimental infection studies suggest that host susceptibility to local versus nonlocal parasites may vary from system to system.

2.4.3 Inferred philopatry and parasite load

I found a near-significant main effect of philopatry on overall parasite load (*i.e.*, parasite loads of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* combined), such that among the subset of birds that were infected with haematozoa, relatively philopatric individuals tended to have lower parasite loads. In contrast to my expectation that the highly local nature of host-parasite arms races as well as among-site differences in parasite abundance might cause the relationship between philopatry and parasite load to vary from site to site, I found no significant interaction between inferred philopatry and site of capture in predicting parasite load. This finding suggests that the relationship between philopatry and parasite load is relatively general over the landscape and does not vary from site to site.

Whereas I found a negative relationship between philopatry and naturally-occurring variation in parasite load, other studies have shown variation across systems in whether hosts are adapted to their local parasites or parasites to their local hosts. My findings are similar to those of an experimental study in which parasitic eye flukes (*Diplostomum pseudospathaceum*) infecting three-spined sticklebacks (*Gasterosteus aculeatus*) achieved lower parasite loads on sympatric than allopatric hosts (Kalbe & Kultz 2006). On the other hand, Daphnia (*D. magna*) populations have greater parasite loads when exposed to sympatric than allopatric cytoplasmic parasites (*Pleistophora intestinalis*; Ebert 1994). Furthermore, a study on frogs (*Xenopus laevis*) and their flatworm parasites (*Protopolystoma* spp.) showed a significant interaction between combinations of hosts and parasites (Jackson & Tinsley 2005). In that study, frogs were infected with flatworms in 33 pairwise combinations of sympatric and allopatric interactions. In some cases, sympatric combinations of host and parasite were associated with high infection success (more worms), but for other combinations, allopatric pairings were associated with high infection success (Jackson & Tinsley 2005). Collectively, these findings suggest that the relationship between philopatry and parasitism may vary across host-parasite systems, and that this relationship may even vary between sites within a particular system.

It is noteworthy that I found a near-significant effect of inferred philopatry on parasite load, but not on parasite prevalence. Other studies have found comparable results, *i.e.*, different patterns observed for parasite load versus parasite prevalence. For example, similar to my findings, male mountain white-crowned sparrows of local origin had lower parasite loads than immigrant males, but philopatric and immigrant males did

not differ in parasite prevalence (MacDougall-Shackleton *et al*. 2002). Conversely, in black-legged kittiwakes (*Rissa tridactyla*) sympatric host indiviudals had a higher prevalence of parasitic ticks (*Ixodes uriae*) but did not have greater intensity of infection (McCoy *et al*. 2002). Also in mountain white-crowned sparrows, haematozoan parasite prevalence and load were predicted by two different measures of genetic diversity (MacDougall-Shackleton *et al*. 2005), suggesting that host ability to avoid parasitic infection may be distinct from the ability to control infection once it has occurred.

2.4.4 Inferred philopatry and survival

Despite the observed near-significant relationship between philopatry and parasite load, inferred philopatry was not significantly associated with overwinter return rates at my long-term study site. Interpreting return rates as survivorship is a reasonable assumption due to high adult philopatry in this population, combined with sustained capture and re-sighting effort at the study site (MacDougall-Shackleton *et al*. 2009). The lack of a relationship between philopatry and survivorship is in contrast to my original prediction, and to empirical findings on the fitness correlates of dispersal in some other wild bird species. For example, in lesser kestrels (*Falco naumanni*), survival decreased significantly with dispersal distance in the year after their first breeding season (Serrano & Tella 2012). Similarly, male great reed warblers (*Acrocephalus arundinaceus*) that dispersed long distances had lower probability of survival between years relative to philopatric males (Hansson *et al*. 2004). In contrast, some studies have reported survival advantages of dispersal. For example, in house sparrows (*Passer domesticus*) dispersers of both sexes had higher survival at the site of establishment than did philopatric birds

that had stayed on the same site of establishment (Altwegg *et al*. 2000). Similarly, in lesser snow goose (*Anser caerulescens caerulescens*) dispersing goslings had significantly higher first year survival than goslings that stayed at their native habitats (Cooch *et al*. 1993). In light of site-specific relationships between philopatry and other potential correlates of fitness like skeletal size, the lack of relationship between philopatry and apparent survivorship that I observed at a single site should be interpreted cautiously. At other sites, birds of local origin may have either advantages or disadvantages in terms of overwinter survivorship.

I found that birds caught earlier in the season in 2009 were more likely to survive the winter and be re-sighted or re-captured in 2010. At the Bracken field site, every effort is made to capture birds as soon as possible after their return from spring migration, thus capture date is a reasonable proxy for return date. Birds that are first captured earlier in the season have presumably arrived to the breeding grounds from spring migration earlier and may be in better condition (e.g. Kokko 1999) and/or obtain higher quality territories. This may in turn increase their likelihood of surviving the winter. However, capture dates in 2010 and 2011 did not predict overwinter return the following springs, suggesting that the pattern observed in 2009-2010 may not occur every year.

In conclusion, I found some evidence for general patterns of home-field advantage across multiple sites in song sparrows, in that birds of local origin had lower bloodborne parasite loads and that capture site did not interact with inferred philopatry in predicting parasite load. However, the main effect of philopatry on parasite load was weak (R^2=0.001) and fell short of statistical significance; moreover, philopatry did not predict parasite prevalence. I also found some evidence that different capture sites varied

in the relationship between philopatry and skeletal size (PC1). Specifically, at some sites more philopatric individuals were larger, while at other sites the reverse pattern was found. This pattern is consistent with site-specific home-field advantage and/or with differences among sites in the degree to which dispersing and philopatric birds differ in quality. However, this relationship was also near-significant.

Importantly, the finding that song sparrows of local origin tend to have lower parasite loads is not in itself conclusive evidence that birds are genetically adapted to their local parasites. First, philopatric individuals may benefit from familiarity with the local environment (e.g. Pärt 1994), for example through previous experience with appropriate places to find food, water or sites for nesting and roosting and this experience may allow them to better control parasitic infections. As well, if dispersal is condition-dependent, then philopatric individuals may simply be intrinsically in better condition than birds that disperse further (e.g. Saino *et al*. 2014) and thus better able to control parasitic infections. However, it should be noted that philopatric birds did not differ in PC2, interpreted as body condition, so this explanation seems unlikely. While my findings do not provide conclusive evidence that birds are adapted to their local parasites, they are generally consistent with previous findings in this study system (Stewart & MacDougall-Shackleton 2008). Thus, this work sets the stage for deeper analyses and future manipulative experimentation to continue to shed light on the subject of local adaptation and the fitness consequences of dispersal, particularly in the context of host-parasite interactions.

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Chapter 3

3 Comparative Population Genetics of Song Sparrows and their Haemosporidian Parasites

3.1 Introduction

The world harbours millions of different species of parasites, defined as organisms that divert resources from a host to support their own growth and reproduction. This general similarity in ecology does not, however, reflect close evolutionary relationships or a single evolutionary origin. Parasitic lifestyles evolved many times in many different taxa, including in viruses, bacteria, protozoa, fungi, plants, nematodes, and vertebrates (Poulin & Morand 2004; Schmid-Hempel 2011). In addition to being taxonomically widespread, parasites are also geographically widespread (e.g. Morand & Krasnov 2010; Atkinson & van Riper 1991) and are becoming increasingly so, due to range expansions associated with climate change (Rosenthal 2009, Tabachnick 2010) and other anthropogenic causes (Colwell *et al.* 2011).

By definition, parasites harm the fitness of host individuals, for example through reducing survivorship (Coltman *et al.* 1999, Martinez-de la Puente *et al*. 2010), reproductive success (Merino *et al*. 2000, Marzal *et al*. 2005, Knowles *et al.* 2010), or the expression of sexually selected traits (Hamilton & Zuk 1982). Thus, parasites can place important selective forces on host populations, and may thus influence host evolution. For instance, coevolution with parasites may help to explain the persistence of sexual reproduction in host species, because sexual recombination permits generating new

combinations of alleles and provides a fitness advantage when selection is frequency-dependent (Hamilton 1980; Lively 2010). Parasites have also been hypothesized to play a major role in sexual selection, for example if host females choose mates based on sexually selected traits that reflect health status (Hamilton & Zuk 1982). Finally, parasites have been proposed to influence patterns of host speciation and diversity. If parasites that have coevolved with one host population are pathogenic to a sister population, this may prevent secondary sympatry and slow speciation (Ricklefs 2010).

Just as parasites represent an important component of their host's selective environment and thus influence patterns of host evolution, so too can hosts influence the evolution of their parasites. Coevolution between species that share an intimate historical association can result in evolutionary arms races (Thompson 1994). In the case of antagonistic coevolution between hosts and their parasites, selection on parasites favours improved abilities to evade the host's defense mechanisms and infect the host, while selection on the host favours improved detection of and resistance to the parasite. In some cases, host diversification can result in speciation by the parasites, a process known as cospeciation. Whether or not cospeciation occurs depends largely on the parasite's degree of host specificity, and whether the host-parasite association has been preserved through evolutionary time (Thompson 1994).

Cospeciation can be recognized when the topologies of both parasite and host phylogenies are concordant to a greater degree than expected by chance (Hafner & Nadler 1990). For example, primates and their simian foamy virus (subfamily *Spumaretrovirinae*) show congruent phylogenies, as measured both by branching order and by divergence time (Switzer *et al*. 2005), suggesting that hosts and parasites in this

system have cospeciated. Conversely, lack of concordance between host and parasite phylogenies may reflect historical host switches by parasites, or lack of host-specificity. Within avian families, host switches appear to be common in parasite lineages from *Plasmodium* and *Haemoproteus* (Ricklefs & Fallon 2002, Ricklefs *et al*. 2004). Similarly, host-generalist parasites interact with multiple species of host, decreasing the likelihood that phylogeny of the parasite will mirror that of any given host species. For instance, chewing lice (Phthiraptera: family Philopteridae) show no signs of cospeciation with their penguin hosts (order Sphenisciformes), presumably due to lack of host specificity at this taxonomic scale (Banks *et al.* 2006).

The studies reviewed above examine coevolution at and above the level of species, but this pattern can also be viewed at the population or incipient-species level. The parasite-driven-wedge model of parapatric speciation (Thornhill & Fincher 2013) proposes that local arms races between parasites and their hosts should tend to promote host philopatry, together with avoidance of nonlocal conspecifics, because avoidance minimizes contact with novel parasites. Novel parasites may be important to avoid if host populations are not locally adapted to them, or if host individuals do not have previous immune experience with such parasites. For example, the parasite *Plasmodium relictum* (lineage GRW4) caused the extinction of many endemic bird species after the human-mediated introduction of the parasite infected naïve hosts in the Hawaii archipelago (Beadell *et al*. 2006). By promoting host philopatry and assortative mating by population of origin, parasites may thus promote genetic differentiation and ultimately, speciation of host populations. This hypothesis requires that both hosts and parasites are genetically structured over the landscape, and predicts concordant divergence of host and parasite

populations (evidence of coevolution). Thus, an important first step in evaluating the parasite-driven-wedge hypothesis is to compare patterns of genetic differentiation in hosts and their parasites (Thornhill & Fincher 2013).

The parasite-driven-wedge hypothesis implicitly assumes that hosts are better able to withstand or control infection by familiar (sympatric) than by nonfamiliar (allopatric) parasites (Thornhill & Fincher 2013) - *i.e.*, that hosts are adapted to their local parasites, or that parasites maladapted to the non-local hosts. However, several empirical studies have found the opposite pattern. For example, parasitic trematodes (*Microphallus* sp.) were better at infecting sympatric than allopatric host snails (*Potamopyrgus antipodarum*; Lively *et al*. 2004). Rates of migration and average dispersal distance in hosts relative to parasites play an important role in determining whether hosts adapt to their local parasites, or parasites to their local hosts (Gandon & Michalakis 2002). In systems where hosts disperse farther than parasites, hosts are predicted to be better able to resist sympatric than allopatric parasites, whereas if parasites disperse farther than hosts, parasites should be better able to infect sympatric than allopatric hosts (Gandon & Michalakis 2002; Hoeksema & Forde 2008). Thus, determining whether gene flow is greater for hosts or parasites in a given system is helpful in testing a major prediction from the parasite-driven-wedge hypothesis.

Bloodborne parasites have been widely used as models for studying the effects of parasites on wild vertebrate populations, especially birds (Fallon *et al*. 2005, Knowles *et al.* 2010, Martinez de la Puente *et al.* 2010). The protozoan order Haemosporidia (Phylum Apicomplexa) has been particularly well studied due to its high prevalence in most avian species and global distribution (Atkinson & van Riper 1991, Valkiunas 2005).

The most common genera of Haemosporidians affecting birds are *Plasmodium*, *Haemoproteus* and *Leucocytozoon*, collectively infecting about 70% of avian species (Atkinson & van Riper 1991). These parasites have a life cycle consisting of a sexual stage in a biting insect vector (order Diptera) and an asexual stage inside organs and red blood cells of the vertebrate host (Valkiunas 2005). Soon after entering the vertebrate host, the parasite multiplies and maintains high concentration in the bloodstream (e.g. up to 7% of red blood cells infected in song sparrows, see Chapter 2) for up to several weeks (Valkiunas 2005). This 'acute stage' is the period in which host mortality is most likely, due to invasion, enlargement and inflammation of organs including the liver, spleen, lungs, heart, and brain. The acute stage may also be characterised by capillary blockage, haemorrhaging, tissue necrosis, and anaemia (Valkiunas 2005). If the host individual survives the acute stage infection, the parasite remains at low concentration in the bloodstream (known as chronic-stage infection) for many years or even the host's entire lifetime (Valkiunas 2005).

Until recently, traditional microscopic analysis of thin-film blood smears (e.g. Bennett 1970) had identified approximately 200 morphospecies of haemosporidians infecting birds (Valkiunas 2005). In recent years, the development of PCR-based protocols to amplify haemosporidian mtDNA (Bensch *et al*. 2000, Hellgren *et al*. 2004, Waldenström *et al*. 2004) has revealed a much greater diversity of haemosporidian parasites (Bensch *et al*. 2000). Species concepts and delineations for haemosporidian parasites are controversial, but some authors suggest that there may be as many distinct parasitic lineages as species of birds (Bensch *et al.* 2004). For the most part, the genus *Plasmodium* is the most host-generalist of the three genera, followed by *Haemoproteus*,

and *Leucocytozoon* being the most host-specialist. However, within each parasite genus, some lineages are host-specialists, relying on one or two host species and others are host-generalists (Bensch *et al*. 2009).

I examined genetic structuring in a host-parasite system involving song sparrows (*Melospiza melodia*) and the haematozoan parasites *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*. Song sparrows are distributed across all of North America and show substantial geographic variation in morphology, with approximately 30 subspecies currently recognized (Arcese *et al*. 2002). In Ontario, song sparrows (*M. m. melodia* subspecies) are seasonally migratory, and natal dispersal distances for this species range from estimates of 300 m based on mark-recapture methods for a population in Ohio (Nice 1937) to 6.1 km based on mtDNA sequence variation across their continental range (Zink & Dittmann 1993). Dispersal distances and gene flow for bloodborne parasites are less well established and could reflect several different scenarios (Table 3.1). If Haemosporidian dispersal is accomplished primarily by dispersal of the vertebrate host, parasite gene flow and genetic structuring may be comparable to that of the host. If Haemosporidian dispersal relies mainly on dispersal of insect vectors, parasite populations may experience lower levels of gene flow and show more genetic structuring than host populations (because mobility and dispersal of vectors is generally lower than that of avian hosts). Finally, if haematozoan dispersal is facilitated by seasonal migration of their avian hosts, parasite gene flow may be greater and genetic structuring lower than that of their hosts (because seasonal migration is generally longer-distance than natal dispersal). All these possibilities are not mutually exclusive.

Table 3.1. Potential evolutionary outcomes for host (song sparrow) and parasites (Haemosporidian), relative to parasite mode of dispersal.

Parasite mode of dispersal	Parasite gene flow	Parasite genetic structuring	Evolutionary outcome
By host dispersal	Similar to host	Similar to host	There will be no evolutionary advantage for host or parasite
By vector dispersal	Lower than host	Higher than host	Host will have an evolutionary advantage
By host migration	Higher than host	Lower than host	Parasites will have an evolutionary advantage

3.1.1 Objectives

In this study, my primary objectives were to characterize population genetic structure of song sparrows throughout eastern and southwestern Ontario, and to compare this to the species- and community-level genetic structure of their major bloodborne parasites (genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*) over the same geographic scale. Previous research on a focal population of song sparrows breeding in eastern Ontario provides some suggestive evidence that these birds might be adapted to the local parasite fauna, such that host individuals are better able to defend against local (sympatric) than nonlocal (allopatric) parasites. Specifically, males with locally common microsatellite genotypes (and thus presumably of local origin) were found to have lower measures of physiological stress, including lower haematozoan parasite loads, relative to males with less common genotypes (Stewart & MacDougall-Shackleton 2008). Whether this pattern reflects an evolutionary adaptation by song sparrow hosts to the local bloodborne parasites, as opposed to another explanation (see chapter 2), remains to be determined. However, if song sparrows are indeed adapted to their local parasites I should expect to

see higher gene flow (and hence lower population genetic structuring) among song sparrow populations, relative to their parasites at the same geographic scale (Gandon & Michalakis 2002). Moreover, I should also expect to see a concordant genetic structure between song sparrow populations and their parasite communities, indicative of a localized host-parasite assemblage that could lead to coevolution. I predicted that hosts in this system have lower genetic structure (higher gene flow, consistent with greater dispersal ability) than their parasites. I also predicted a concordant pattern between host (F_{ST}) and parasite (Φ_{ST}) phylogenies when comparing their genetic distance from different sites of capture; and moreover, that pairwise genetic distance among hosts should be positively correlated with pairwise genetic distance among parasites.

As a secondary objective, I tested whether parasite haplotype frequencies are stable over time, or whether they vary from year to year. If parasite haplotype frequencies are stable over time, specific parasite lineages may impose strong selection pressure on hosts, potentially facilitating their coevolution.

3.2 Methods

3.2.1 Field work

During April, May, and July 2009; April, May and July 2010; October 2011; and July, August, and September 2012, I captured a total of 344 song sparrows in 12 different locations in southern Ontario (Table 3.2, Fig. 3.1). I decided to group two of the sites, Silver and Sharbot Lake, due to their proximity (10 km apart) and low number of individuals captured at each site. Field sites included Provincial Parks, conservation

areas, and land owned by the Queen's University Biological Station (QUBS) in southeastern Ontario, and land owned by Western University in southwestern Ontario. Capture sites were a mixture of old fields, wetlands, and shorelines of rivers and lakes, and are presumed to represent suitable breeding habitat for song sparrows.

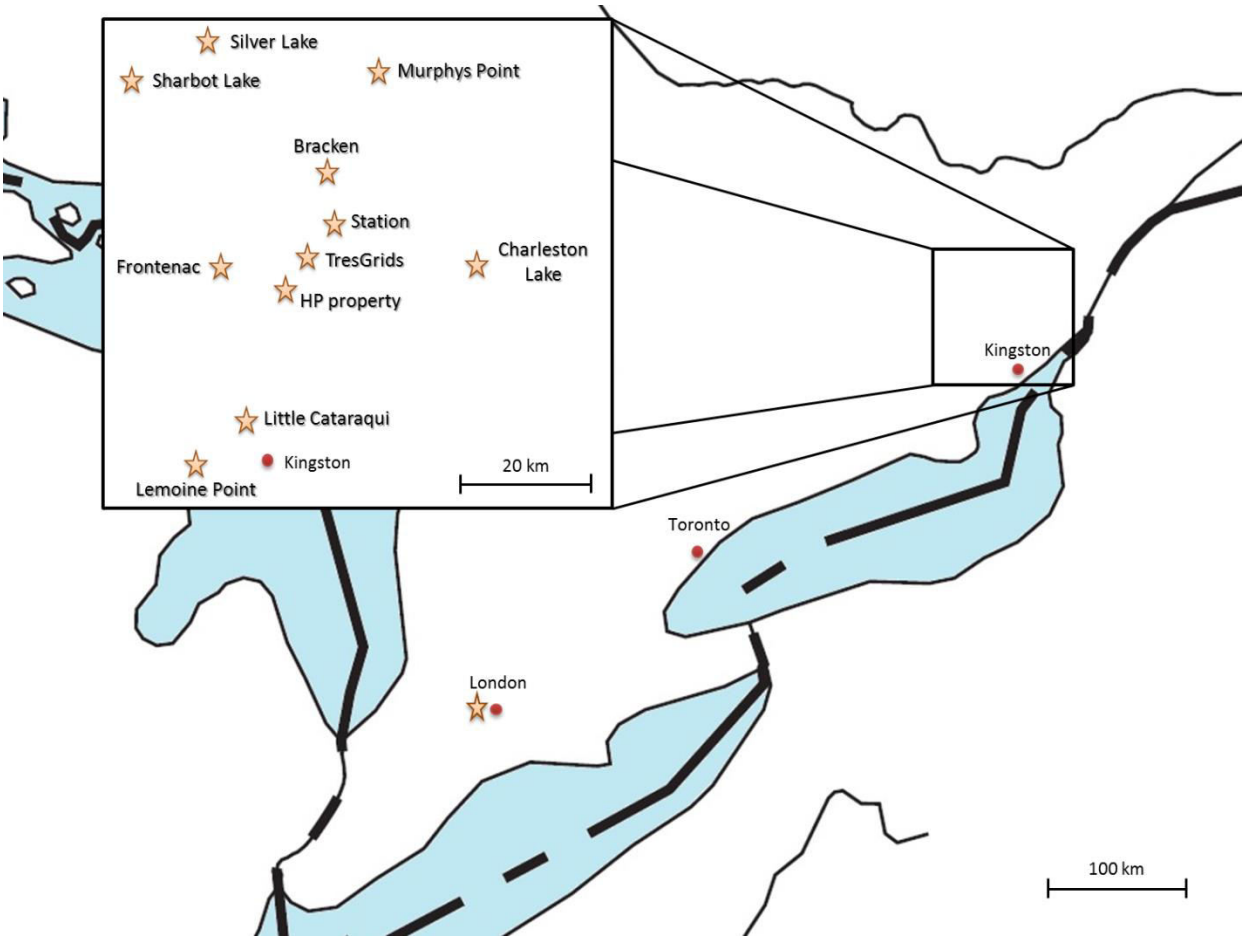

Figure 3.1. Location of 12 sampling sites across Southern Ontario where blood samples from song sparrows were taken between the spring of 2009 and the fall of 2012.

I captured adult song sparrows of both sexes with seed-baited potter traps that I checked at least every hour, and also lured birds (mainly males) into mist-nets using conspecific song playback. All birds were handled for less than 3 minutes, during which time I collected approximately 20 µl of blood via brachial venipuncture. A portion of the blood was blotted onto high wet strength filter paper, and later treated with a drop of 0.5 M EDTA (pH 8) and allowed to air dry awaiting genetic analyses. With the remaining blood sample I prepared a thin-film blood smear for microscopic analysis of parasite load. Birds were then banded and their sex assessed by the presence/absence of a cloacal protuberance. Most birds were then released at the site of capture, with the exception of 35 individuals retained for a captive study (see Chapter 5).

Table 3.2. Sites at which song sparrows were captured between the spring of 2009 and the fall of 2012. Sites 6 and 7 were pooled together due to their geographical proximity, and the low number of individuals captured at each site.

Site #	Site name	Coordinates	Years sampled	# Birds sampled	# Males	# Females	# Sex unknown
1	Bracken	44.633°N, 76.330°W	2009, 2010, 2012	157	112	44	1
2	Station	44.567°N, 76.324°W	2009, 2010	34	29	5	0
3	HP property	44.475°N, 76.430°W	2010	21	21	0	0
4	TresGrids	44.521°N, 76.385°W	2009	7	6	1	0
5	Murphys Point	44.781°N, 76.236°W	2010	20	19	1	0
6	Silver Lake	44.830°N, 76.579°W	2010	11	10	1	0
7	Sharbot Lake	44.783°N, 76.715°W	2010	4	4	0	0
8	Frontenac	44.508°N, 76.543°W	2010	21	20	1	0
9	Charleston Lake	44.501°N, 76.035°W	2010	19	17	2	0
10	Little Cataraqui	44.289°N, 76.511°W	2009	11	6	5	0
11	Lemoine Point	44.226°N, 76.612°W	2009	20	17	3	0
12	London	43.008°N, 81.291°W	2011, 2012	19	15	4	0
Total				344	276	64	1

3.2.2 Microscopic analysis of parasitism

In the field, after sampling blood from each bird I placed a single drop of whole blood onto a glass microscope slide, then used a second clean slide to gently pull the blood over the surface of the first slide. The resultant thin film blood smear was allowed to air dry and then fixed in 100% methanol. Following the field season, slides were stained using Wright-Giemsa stain following the protocol from HARLECO® Hemacolor® Stain Set. I examined each song sparrow's blood smear under a light microscope using a 100X objective, and counted the number of haematozoan parasites found per 10,000 erythrocytes. I recorded whether the parasites belonged to the genera *Plasmodium* or *Haemoproteus* (for the purposes of my project undistinguishable from each other under the microscope, thus these two genera were pooled for purposes of microscopy), or the genus *Leucocytozoon*, with reference to Valkiunas (2005).

3.2.3 Genetic analysis

DNA was extracted from blood samples with an ammonium acetate-based protocol (Bruford *et al*. 1998). This procedure allowed me to extract DNA from both the bird and any bloodborne parasites that the bird may have (Bensch *et al*. 2000). I used polymerase chain reaction (PCR) to genotype the birds at 17 microsatellite loci: Mme 1, Mme 2, Mme 7 (sex-linked) and Mme 12 (Jeffrey *et al*. 2001); Escμ1 (Hanotte *et al*. 1994); Pdoμ5 (Griffith *et al*. 1999); SOSP 1, SOSP 2, SOSP 4, SOSP 5, SOSP 7, SOSP 13, SOSP 14 (Sardell *et al*. 2010); SOSP 3, SOSP 9 (Dr. Lukas Keller, pers. comm. to Dr. Beth MacDougall-Shackleton); and Zole B03, and Zole C02 (Poesel *et al*. 2008). One

primer at each locus was dye-labelled (Integrated DNA Technologies or Applied Biosystems).

Each PCR reaction was conducted in a total volume of 10 µL and included 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100, 0.2 mg/mL BSA, 2.5 mM MgCl2, 0.2 mM of each dNTP, 0.1-0.4 mM of each primer, 0.5U *Taq* polymerase (Fisher Scientific) and approximately 25 ng of genomic DNA. Cycling conditions included an initial step of either 180 s at 94 °C (SOSP 1, 2, 3, 4, 5, 7, 9, 13, 14, Zole B03, C02, Mme 1, 12) or 270 s at 94 °C (Mme 2, 7, Escµ1, Pdoµ5), followed by 28 cycles of either 30 s at 94 °C, 90 s at the annealing temperature, and 60 s at 72 °C (SOSP 1, 2, 3, 4, 5, 7, 9, 13, 14, Zole B03, C02, Mme 1, 12) or of 30 s at 94 °C, 40 s at the annealing temperature, and 40 s at 72 °C (Mme 2, 7, Escµ1, Pdoµ5). All reactions had a final step of 270 s at 72 °C. Annealing temperatures were 57 °C for SOSP 2, 3, 4, 9, 13, 14, Mme 1, 12, Zole B03, C02, 55 °C for SOSP 1, 5, 7 and dropped from 52 °C to 48 °C using a touchdown reaction for Mme 2, 7, Escµ1 and Pdoµ5. PCR products were then subject to capillary electrophoresis on an Applied Biosystems 3130 (year 2009) or 3730 (years 2010-2012) Genetic Analyzer. I scored allele sizes manually using Gene Mapper software with reference to LIZ size standard.

The same extracted DNA was used to amplify a 480 bp portion (excluding primers) of an intron on the cytochrome b from genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*. I used a two-stage, nested PCR protocol following Hellgren *et al*. (2004) and Waldenström *et al*. (2004). The first-stage primers used were HaemNFI and HaemNR3 (Hellgren *et al*. 2004). The reaction had a total volume of 25 µl and included approximately 50 ng of genomic DNA, 1.25 mM of each nucleotide, 1.5 mM $MgCl_2$, 10x

PCR buffer (10 mM Tris-HCL pH 9.0; 50 mM KCL; 0.1% Triton X100; 0.2 mg/ml BSA), 0.6 mM of each primer and 0.5 U Taq DNA polymerase (Fisher Scientific). First-stage PCR conditions included an initial denaturing step of 94 ºC for 3 min; 20 cycles of 30 s at 94 °C, 30 s at 50 °C, and 45 s at 72 °C; then a final step of 10 min at 72 °C.

The second-stage PCR reactions used 2µl of first-stage PCR product, together with one of two internally nested primer pairs. Primers HaemF and HaemR2 (Bensch *et al*. 2000) amplify sequence from *Haemoproteus* and *Plasmodium*, while primers HaemFL and HaemR2L (Hellgren *et al*. 2004) amplify sequence from *Leucocytozoon*. Thus, three separate PCR reactions were conducted for each individual: first-stage (amplifies sequence from all three genera), second-stage *Haemoproteus*/*Plasmodium*, and second-stage *Leucocytozoon*. The total volume, recipe and PCR conditions for second-stage PCR reactions were otherwise the same as for the first-stage PCR reaction. Final product sizes were 480 bp long (excluding primers) for both *Haemoproteus*/*Plasmodium* and *Leucocytozoon* second-stage reactions.

I ran these final PCR products on two separate gels, one for the *Leucocytozoon* amplification, and one for *Haemoproteus* and *Plasmodium*. Each gel was made with 2% agarose and ran for 90 minutes at 100 volts. On each gel I included a ladder (Kplus DNA ladder, GeneDirex) as a size standard, as well as a negative control (using water in place of template in the PCR reaction) to check for contamination in the reagents. I visualized bands under UV light, excised bands of the correct product size, and purified them with a DNA purification kit (Gel/PCR DNA Extraction Kit, FroggaBio). Samples were then sent for sequencing on an Applied Biosystems 3730 DNA Analyzer at the London Regional Genomics Centre. Products were sequenced with primer HaemF (*Haemoproteus* and

Plasmodium) or HaemFL (*Leucocytozoon*) yielding 476 base pairs of readable sequence for the three parasite genera.

I edited and aligned parasite cytochrome b sequences with BioEdit version 7.1.3.0. (Hall 1999), and determined the number of different haplotypes found. To identify which Haemosporidian species my haplotypes belonged to, I used a Basic Local Alignment Search Tool (BLAST) search implemented in GenBank (http://www.ncbi.nlm.nih.gov) to compare my sequences with other sequences from GenBank that had been positively identified to morphospecies. For each of my haplotypes I noted which GenBank lineage that had been identified to morphospecies had the highest sequence similarity to my haplotype. When two published haplotype (morphospecies) were equally similar in sequence to my haplotype (n=2 cases), I used morphology and previous information on prevalence following Valkiunas (2005) to decide which morphospecies my haplotype most likely belonged to.

3.2.4 Data analyses

3.2.4.1 Parasite prevalence, diversity and the relationship between lineages

As noted above, I could confidently assign haemosporidia to genus *Leucocytozoon* based on microscopy, but could not confidently distinguish between *Plasmodium* vs *Haemoproteus* under the microscope. Thus, to estimate the prevalence of *Leucocytozoon* parasites in song sparrows I combined both the microscopy data (*i.e.*, individuals with one or more *Leucocytozoon* per 10,000 erythrocytes were considered “infected”) and the molecular data (*i.e.*, individuals with a band of the expected product size for second-stage

Leucocytozoon PCR were considered "infected") for an overall estimation of prevalence. For *Plasmodium* and *Haemoproteus* prevalence, I used only molecular data to determine prevalence. I then calculated the prevalence of each parasite genus for each sampled location.

To examine the relationship between lineages and to identify groupings of lineages that correspond to species, I constructed a phylogeny using the software MEGA5 (Tamura *et al*. 2011). I tested several algorithms, and the UPGMA (Sneath & Sokal 1973) was the one that gave me a phylogeny that grouped each genus together, which is consistent with previous knowledge of these organisms (Valkiunas 2005). Bifurcating phylogenies may be misleading when trying to represent relationships of extant haplotypes, since one haplotype may be the direct ancestor of another (Posada & Crandall 2001). Thus, as a complementary analysis to further visualize relationships among parasite lineages, I constructed an unrooted haplotype network for *Plasmodium* haplotypes and one for *Leucocytozoon* haplotypes using the software TCS version 1.21 (Clement *et al*. 2000).

3.2.4.2 Population genetic structure of host and parasites

In order to compare patterns of population genetic structuring in song sparrows and in parasites, I used the software Arlequin (ver 3.5.1.2; Excoffier & Lischer 2010) to calculate genetic distance between all pairwise combinations of sample sites. I calculated one pairwise matrix of F_{ST} values for song sparrows (based on microsatellite allele frequencies) captured at different sites; one matrix of Φ_{ST} values for all *Plasmodium*

haplotypes (based on cytochrome b sequence) detected at the different sites; and one matrix of Φ_{ST} values for all *Leucocytozoon* haplotypes (based on cytochrome b sequence) detected at the different sites. *Haemoproteus* was detected at only two locations, so I did not calculate pairwise Φ_{ST} for this genus. I then conducted an AMOVA (Distance method: Kimura 2P; significance test: 1000 permutations) in Arlequin to determine whether these distances were significantly different than what I would expect in the absence of population (host) or community (parasite) genetic structuring. As a complementary analysis, I also calculated pairwise Φ_{ST} values as above for a subset of *Plasmodium* haplotypes that formed a well-supported clade likely to correspond to a single morphospecies, and for a subset of *Leucocytozoon* haplotypes that likewise formed a well-supported clade likely to correspond to a single morphospecies. I used AMOVA as described above to examine "species" level population genetic structuring in each of these parasite morphospecies.

I tested for isolation by distance using the subprogram ISOLDE implemented in GENEPOP ON THE WEB (http://genepop.curtin.edu.au/; Rousset 2008). This analysis compared all pairwise values of (F_{ST}/1 - F_{ST}) to geographic distance between capture sites (Rousset 1997), using 10,000 permutations. Because multiple years of data were available at one of the sample sites (Bracken; Table 3.2), I also examined temporal variation in parasite haplotype frequencies at this site. Specifically, I compared haplotype frequencies at this site in 2009 and 2010 using an AMOVA implemented in Arlequin (Distance method: Kimura 2P; significance test: 1000 permutations).

3.2.4.3 Comparing host and parasites

To test whether pairwise genetic distance between host subpopulations (capture sites) was correlated with pairwise genetic distance among parasite subpopulations, I used a Mantel test (1000 permutations; Genalex ver 6.5: Peakall & Smouse 2006) to quantify the association between pairwise matrices of host vs parasite genetic distance. I performed four separate Mantel tests, comparing pairwise genetic distances between song sparrow sampling sites to the corresponding pairwise genetic distances between (a) the entire *Plasmodium* community; (b) the entire *Leucocytozoon* community; (c) the subset of *Plasmodium* lineages corresponding to a single morphospecies; (d) the subset of Leucocytozoon lineages corresponding to a single morphospecies.

To evaluate concordance between host and parasite phylogenies at each site sampled, I created phylogenies using the UPGMA algorithm in the software Phylip (ver 3.6: Felsenstein 2005), with pairwise F_{ST} values from song sparrows, and pairwise Φ_{ST} values from *Plasmodium* and *Leucocytozoon*. *Haemoproteus* was not included in these analyses due to its low prevalence overall.

Finally, to compare the degree of population genetic structuring in song sparrow hosts and in *Plasmodium* and *Leucocytozoon* morphospecies, I did a paired t-test with SPSS version 21 (SPSS, Inc.) comparing each pairwise F_{ST} value of song sparrows to the Φ_{ST} values of the respective parasite morphospecies.

3.3 Results

3.3.1 Parasite prevalence, diversity and the relationship between lineages

I found a general prevalence of parasitism of 35% (based on microscopy and molecular analyses combined), with 121 of 344 birds infected by one or more bloodborne parasites (Table 3.3). Infection prevalence also varied between sites, ranging from 0% (out of 20 birds) in Lemoine Point to as high as 52% in Charleston Lake (10 of 19 birds; Table 3.3). When molecular methods only were used to detect infections, observed prevalence dropped to 26.4% (91 of 344), whereas the use of microscopy only resulted in observed prevalence of 16.6% (57 of 344; Table 3.3). Thus, molecular detection of infection appears to be more sensitive than microscopy in this system, but both methods apparently fail to detect some infections and a combined approach is thus most effective in detecting parasitism.

The most prevalent parasite found to be infecting song sparrows was *Leucocytozoon*, followed by *Plasmodium*, while *Haemoproteus* was present only in two locations (Table 3.4). However, as noted above, prevalence of *Leucocytozoon* parasites was calculated combining information from microscopy as well as PCR, thus increasing my likelihood of detecting *Leucocytozoon* infections. I detected 16 cases of co-infections with *Plasmodium* and *Leucocytozoon* (4.6% of birds), and one co-infection with *Haemoproteus* and *Leucocytozoon* (0.3% of birds). Six birds (1.7%) showed double peaks on *Leucocytozoon*-specific cytochrome B sequences, indicating that these individuals were infected with two or more different *Leucocytozoon* lineages. These cases were scored positive for *Leucocytozoon* infection but specific haplotypes could not be

identified. Microscopic examination also detected one instance of infection with nematode microfilariae. This parasite was, however, omitted from subsequent analyses due to low prevalence.

Among the 91 individuals found to be infected with haemosporidia based on PCR analysis, I observed 11 different lineages of *Plasmodium*, 7 different lineages of *Leucocytozoon*, and 1 lineage of *Haemoproteus*. Figure 3.2 shows the UPGMA phylogeny of the lineages detected. This algorithm identified *Plasmodium* and *Leucocytozoon* as monophyletic groupings, and *Haemoproteus* as a sister taxon to *Plasmodium*, consistent with previous information from morphology and genetic analysis (Valkiunas 2005).

Table 3.3. Haemosporidian prevalence in song sparrows (number of individuals infected by one or more Haemosporidia of any genus in a scan of 10,000 erythrocytes) among the different capture sites, as determined by microscopy of thin-film blood smears, PCR amplification, and both methods combined.

Site #	Site name	Number of birds screened	Prevalence determined by blood smears	Prevalence determined by PCR amplification	Prevalence determined by both methods
1	Bracken	157	28 (17.8%)	47 (29.9%)	62 (39.5%)
2	Station	34	2 (2.9%)	4 (14.7%)	6 (17.6%)
3	HP property	21	5 (23.8%)	9 (42.8%)	10 (47.6%)
4	TresGrids	7	1 (14.3%)	2 (28.6%)	2 (28.6%)
5	Murphys Point	20	2 (10%)	3 (15%)	4 (20%)
6 & 7	Silver-Sharbot Lake	15	2 (13.3%)	5 (33.3%)	6 (40%)
8	Frontenac	21	6 (28.6%)	6 (28.6%)	9 (42.8%)
9	Charleston Lake	19	5 (26.3%)	8 (42.1%)	10 (52.6%)
10	Little Cataraqui	11	3 (27.3%)	0	3 (27.3%)
11	Lemoine Point	20	0	0	0
12	London	19	3 (15.8%)	6 (31.6%)	9 (47.4%)
Total		344	57 (16.6%)	91 (26.4%)	121 (35.2%)

Table 3.4. Prevalence (% of birds infected) of *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* parasites in song sparrows captured at sites throughout southern Ontario. For *Plasmodium* and *Haemoproteus*, values reported represent only infections detected by PCR amplification. For *Leucocytozoon*, values reported include infections detected by microscopy or PCR amplification, or both. Mixed infections comprised of two parasite genera were scored as positive for each parasite genus detected.

Site #	Site	Number of birds screened	Number infected with *Plasmodium*	Number infected with H*aemoproteus*	Number infected with *Leucocytozoon*
1	Bracken	157	25 (15.9%)	5 (3.2%)	36 (22.9%)
2	Station	34	1 (5.9%)	0	3 (8.8%)
3	HP property	21	3 (14.3%)	0	8 (38.1%)
4	TresGrids	7	2 (28.6%)	0	0
5	Murphys Point	20	1 (5%)	0	3 (15%)
6 & 7	Silver-Sharbot Lake	15	4 (26.7%)	0	1 (6.7%)
8	Frontenac	21	4 (19%)	0	8 (38.1%)
9	Charleston Lake	19	4 (21%)	2 (10.5%)	5 (26.3%)
10	Little Cataraqui	11	0	0	3 (27.3%)
11	Lemoine Point	20	0	0	0
12	London	19	5 (26.3%)	0	2 (10.5%)
Total		344	49 (14.2%)	7 (2%)	69 (20.0%)

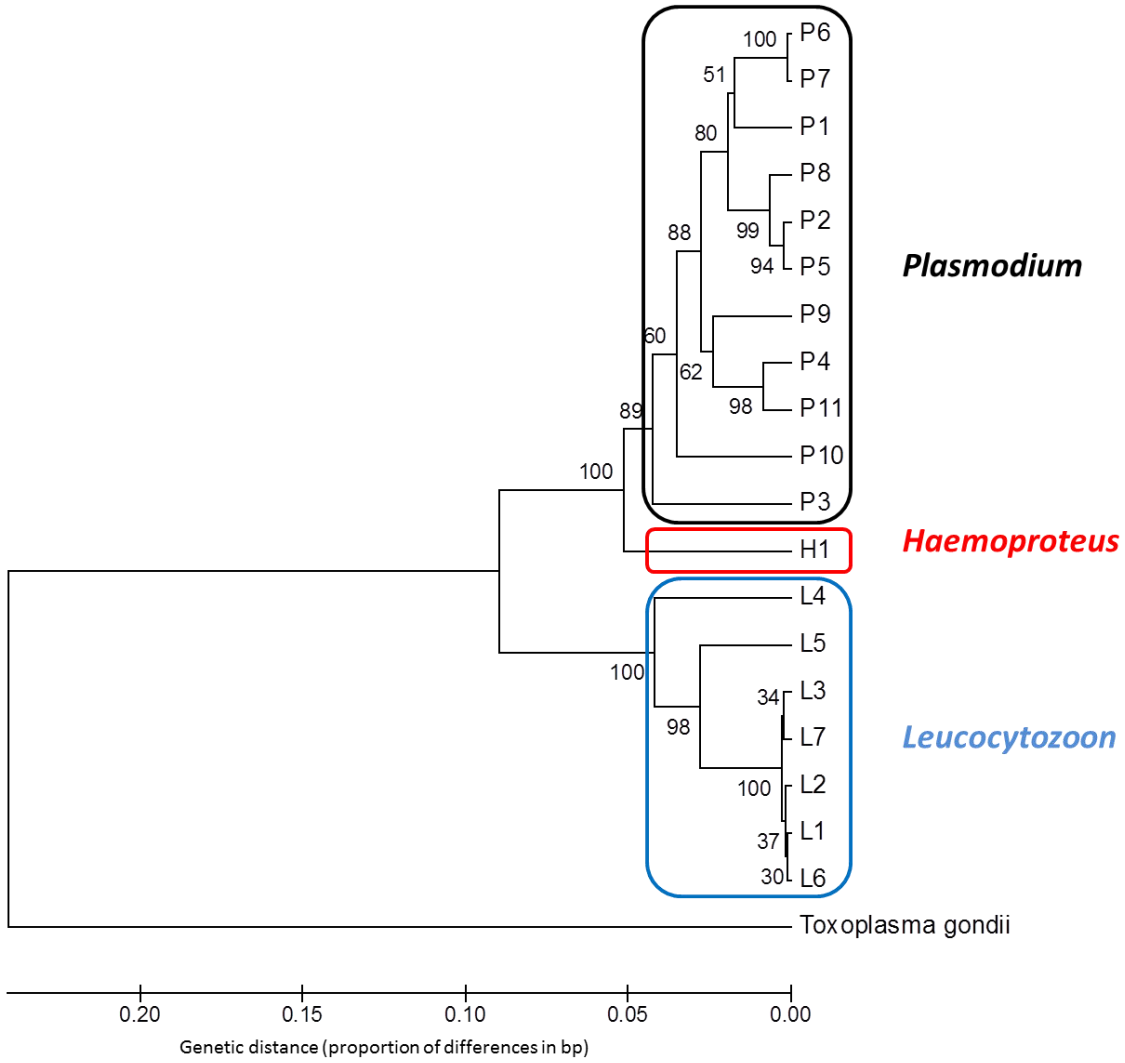

Figure 3.2. Phylogeny of three Haemosporidian genera: *Plasmodium*, *Haemoproteus* and *Leucocytozoon*, constructed from a 476 base pair sequence of cytochrome b obtained from song sparrows captured in southern Ontario, between 2009 and 2012. A UPGMA algorithm was used, with 1000 bootstraps replications and a Kimura 2-parameter substitution model. *Toxoplasma gondii* was used as the outgroup. Branch length indicates amount of genetic change, and numbers in the internodes represent bootstrap support.

3.3.1.1 *Plasmodium*

Most of the *Plasmodium* haplotypes identified were represented at the Bracken site (Fig. 3.3). However, sample size (number of birds screened) was not consistently associated with the observed diversity of *Plasmodium* haplotypes. For example, five distinct haplotypes were observed in London (19 birds screened) and only one haplotype was observed at the Station (34 birds screened; Table 3.4).

The 11 *Plasmodium* haplotypes detected correspond to at least seven described morphospecies (Fig. 3.4; Table 3.5). Most of these species are known to have a worldwide distribution, parasitizing many species (Table 3.5). Haplotypes P1, P2, P5, and P8 were most similar to *P. circumflexum* (96-99% sequence identity to GenBank accession number JN164734; Fig. 3.4) and were thus treated as a single species for population-level analyses and comparison of population genetic structure. Although UPGMA analysis grouped P1 with haplotypes P6 and P7 in 51% of simulations (Fig. 3.4), bootstrap support for this grouping was low and I grouped haplotype P1 along with P2, P5 and P8 based on sequence similarity (Fig. 3.5).

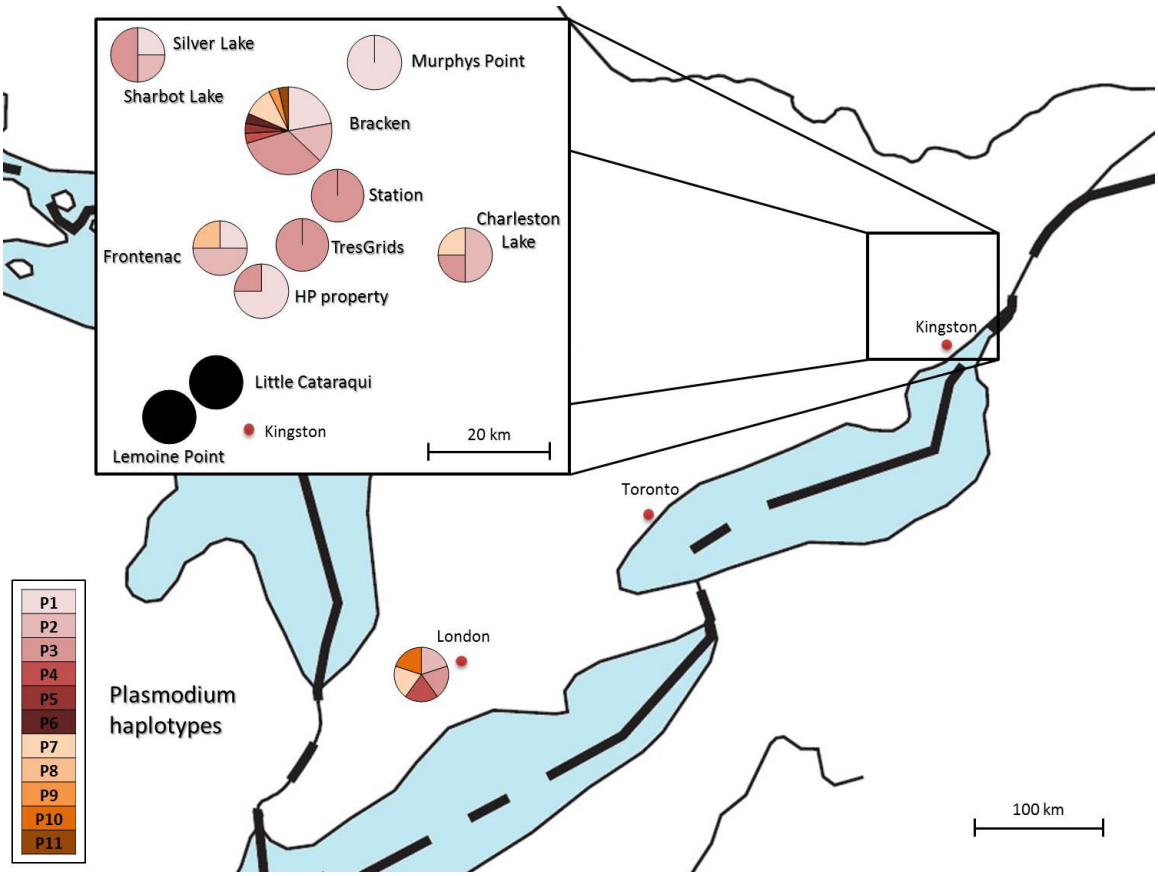

Figure 3.3. Capture sites at which different *Plasmodium* haplotypes were detected in song sparrow host individuals. Each circle indicates the relative abundance of the different haplotypes at a given sampling site. Different colours correspond to different *Plasmodium* haplotypes. The Bracken site is represented with a bigger circle because sample size was much bigger for that site. Black circles indicate sites that were sampled but no *Plasmodium* haplotypes detected.

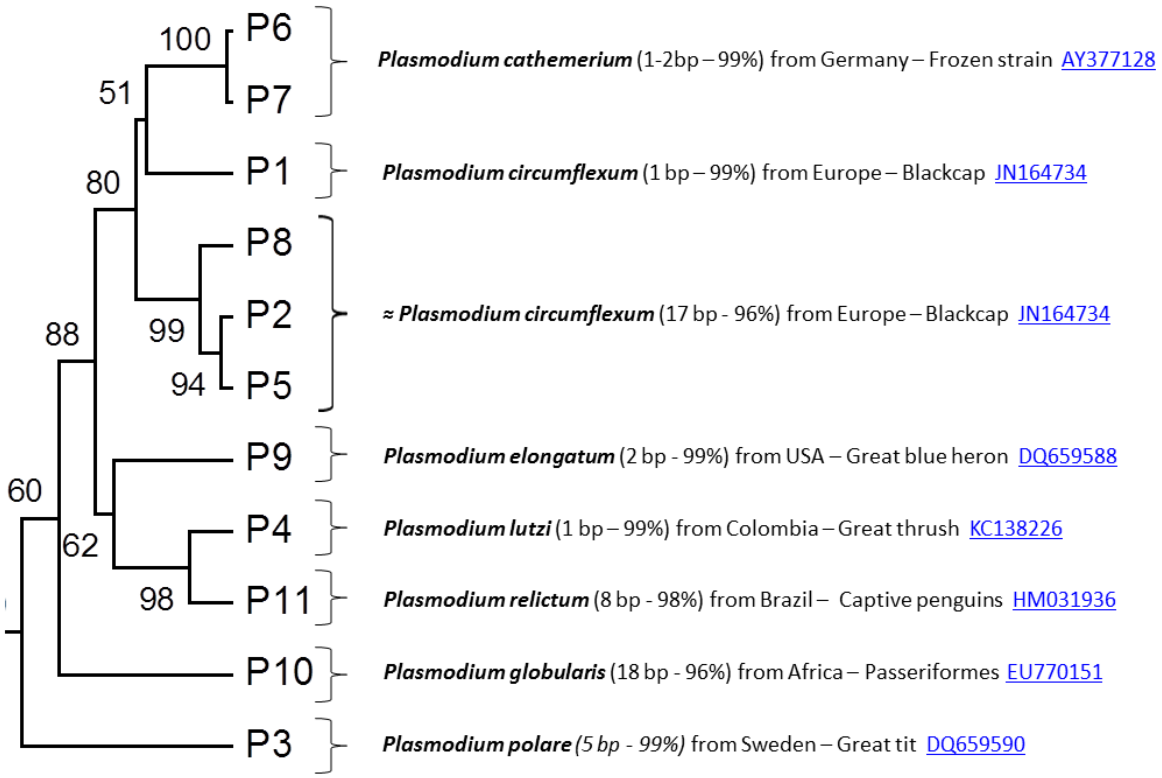

Figure 3.4. UPGMA phenogram of *Plasmodium* cytochrome b sequences (P1-P11) obtained from song sparrows across southern Ontario. Numbers in the internodes represent bootstrap support. For each observed sequence, I used GenBank to identify the most similar previously-published lineage that had been identified to morphospecies. Number of base pair differences between my observed sequence and the GenBank sequence, and % sequence identity, are reported in parentheses. Sampling location, host species and GenBank accession numbers are also reported for each GenBank sequence.

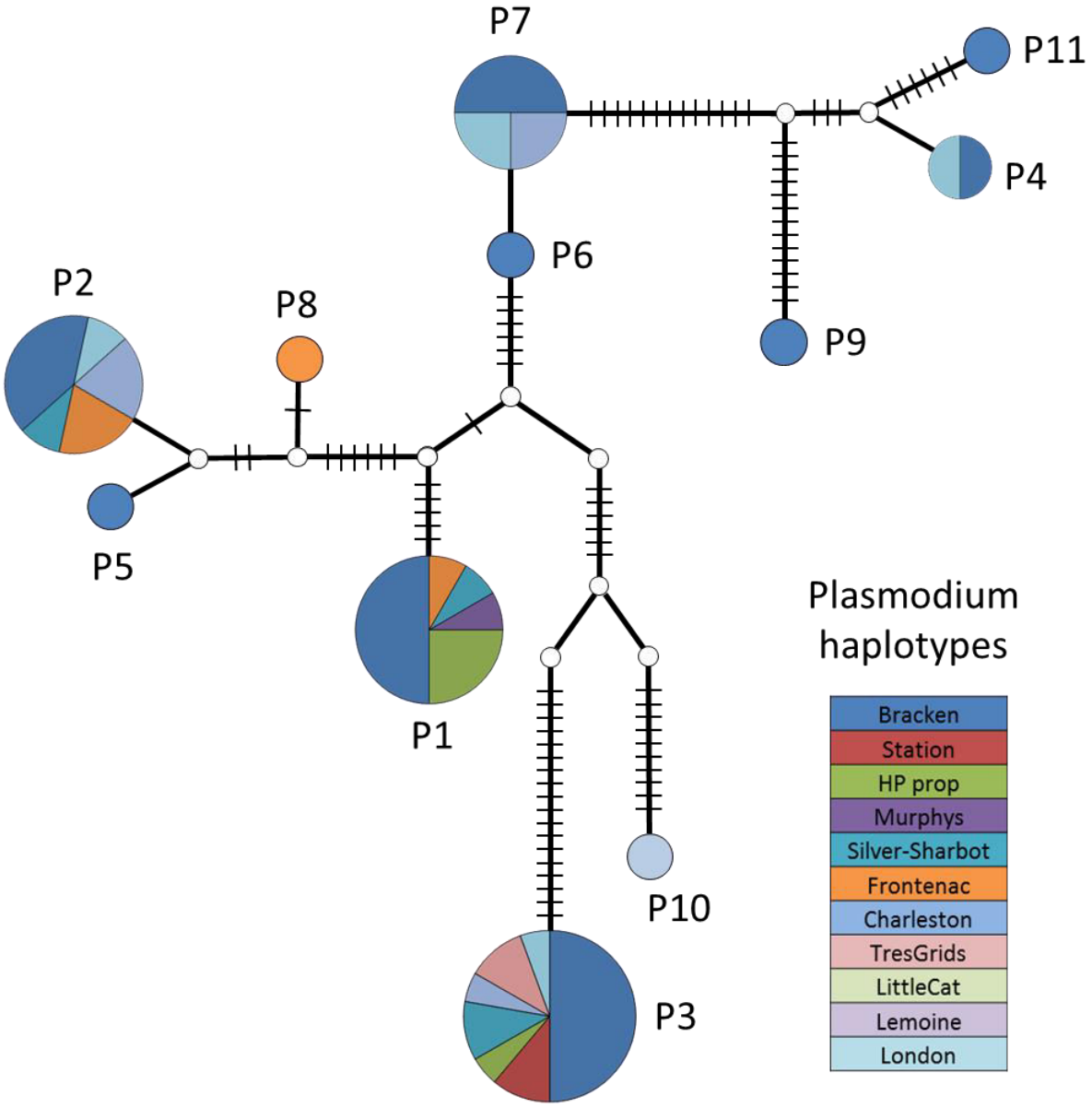

Figure 3.5. Unrooted haplotype network depicting the relationship among *Plasmodium* haplotypes found in song sparrows from Southern Ontario. Each circle (P1 – P11) represents a distinct haplotype and the colours indicate the location where they were found. The size of the circle is proportional to the overall frequency of the haplotype. Nodes are represented by white circles, and each dash denotes one base pair change.

Table 3.5. Recognized species from *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* genera that had the highest similarity to the lineages found in this study from song sparrows across southern Ontario. Information taken from Mantilla *et al*. 2013, Valkiunas 2005, and Valkiunas *et al*. 2008.

Species	Subgenus	Parasitizes Passeriformes	Parasitizes other orders	# of hosts	Distribution
P. cathemerium	Haemamoeba	Yes, mainly	Yes	50 species	All regions except Australia and Antarctica
P. circumflexum	Giovannolaia	Yes, mainly	Yes	100 species	All regions except Antarctica
p. elongatum	Huffia	Yes, mainly	Yes	60 species	All regions except Australia and Antarctica
P. lutzi	Haemamoeba	Yes	Gruiformes	2 species	Neotropic
P. relictum	Haemamoeba	Yes, mainly	Yes	310 species	All regions except Antarctica
P. globularis	Novyella	Yes	No	1 species	Africa
P. polare	Giovannolaia	Yes, mainly	Yes	23 species	All regions except Australia and Antarctica
H. tartakovskyi	Paraheamoproteus	Yes	No	3 species	Palearctic
L. fringillinarum	Leucocytozoon	Yes	No	200 species	All regions except Antarctica
L. majoris	Leucocytozoon	Yes	No	80 species	All regions except Neotropics and Antarctica

3.3.1.2 *Leucocytozoon*

Despite a relatively high prevalence of *Leucocytozoon* infections, most sites showed little haplotype diversity (Fig. 3.6). The seven *Leucocytozoon* lineages found in this study were more similar genetically to morphospecies *L. fringillinarum* (95-97.5% sequence similarity; haplotypes L4, L5) or *L. majoris* (98% sequence similarity; haplotypes L1, L2, L3, L6, L7) than to other described morphospecies (Fig. 3.7, Table 3.5). Based on UPGMA analysis (Fig. 3.7) and sequence similarity (Fig. 3.8) I treated this latter group of haplotypes (*i.e.*, L1, L2, L3, L6, and L7) as a single species for population-level analyses and comparison of population genetic structure.

3.3.1.3 *Haemoproteus*

I identified only a single lineage of *Haemoproteus* in this study, with 98% sequence similarity to morphospecies *H. tartakovskyi* (Genbank accession number GU289673). Due to the low prevalence and diversity of *Haemoproteus* infections, further population-level analyses were not conducted for this genus.

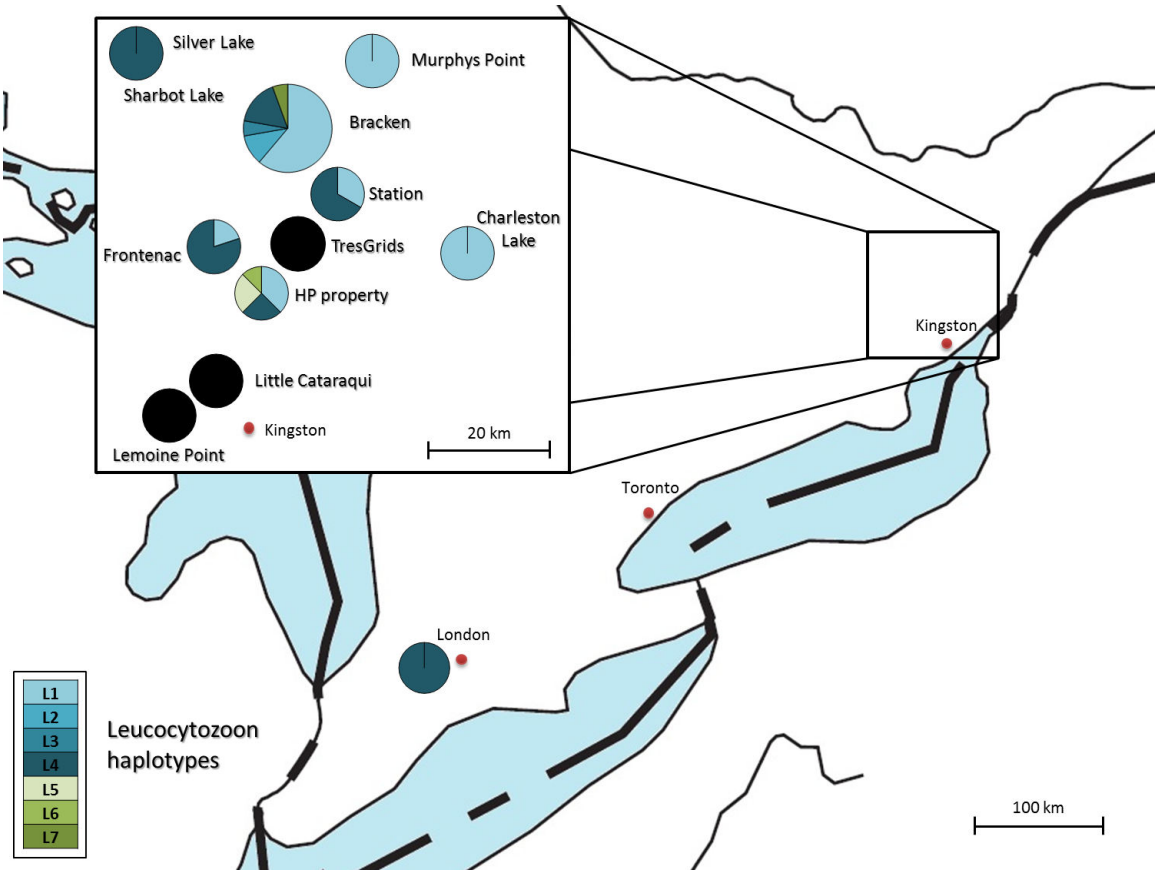

Figure 3.6. Capture sites at which different *Leucocytozoon* haplotypes were detected. Each circle indicates the relative abundance of the different haplotypes at a given sampling site. Different colours correspond to different *Leucocytozoon* haplotypes. The Bracken site is represented with a bigger circle because sample size is much bigger for that site. Black circles indicate sites that were sampled but no *Leucocytozoon* haplotypes detected.

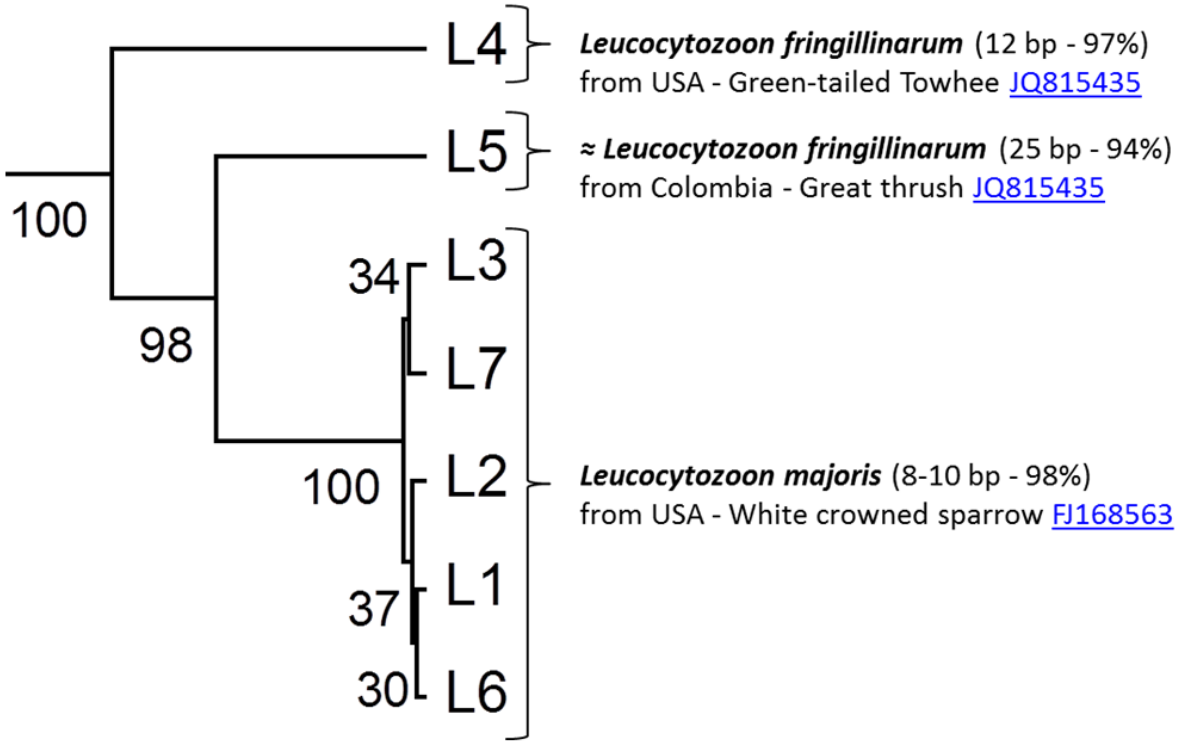

Figure 3.7. UPGMA phenogram of *Leucocytozoon* cytochrome b sequences (L1-L7) obtained from song sparrows across southern Ontario. Numbers in the internodes represent bootstrap support. For each observed sequence, I used GenBank to identify the most similar previously-published lineage that had been identified to morphospecies. Number of base pair differences between my observed sequence and the GenBank sequence, and % sequence identity, are reported in parentheses. Sampling location, host species and GenBank accession numbers are also reported for each GenBank sequence.

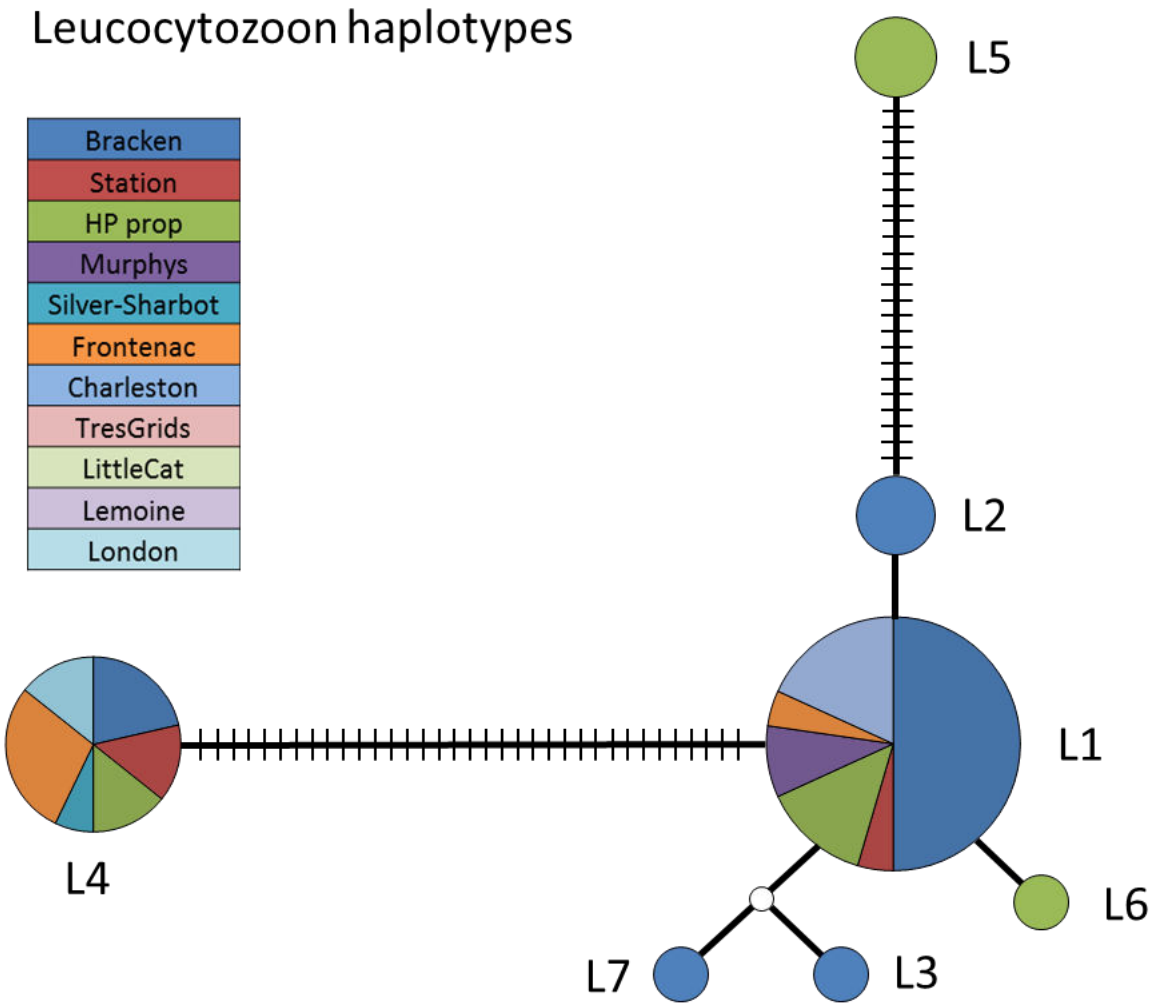

Figure 3.8. Unrooted haplotype network showing the relationship among *Leucocytozoon* haplotypes sampled in song sparrows from southern Ontario. Each circle (L1 – L7) represents a distinct haplotype and the colours indicate the location where they were sampled. The size of the circle is proportional to the overall frequency of the haplotype. Nodes are represented by white circles, and each dash denotes one base pair change.

3.3.2 Genetic structure of host and parasites

3.3.2.1 *Plasmodium*

Pairwise genetic distances (Φ_{ST}) for *Plasmodium* haplotypes observed at all possible pairs of sites are summarized in Table 3.6. An AMOVA conducted on *Plasmodium* haplotype frequencies at this geographic scale showed no significant genetic structuring at this community level (Φ_{ST}=0.057, p= 0.166). Specifically, only 5.7% of variation in haplotype frequencies was between sites, with the remaining 94.3% of variation within sites. At the species level, an AMOVA conducted on the grouped *Plasmodium* lineages P1, P2, P5 and P8 also showed no significant population genetic structuring at this geographic scale (Φ_{ST}=0.108, p= 0.259), with 11% of variation in haplotype frequencies occurring between sites.

I found no evidence for isolation by distance at this geographic scale in *Plasmodium*, either at the community level (all *Plasmodium* lineages; one-tailed p= 0.981; Table 3.6) or at the species level (lineages P1, P2, P5, P8; one tailed p= 0.474). I did, however, detect a significant difference in *Plasmodium* haplotype frequencies between 2009 and 2010 at the Bracken site, with 47.5% of variation in haplotype frequencies occurring between years (AMOVA, Φ_{ST} = 0.4748, p= 0.017). Notably, in 2009 only *Plasmodium* haplotype P3 was found at Bracken, whereas in 2010 seven Plasmodium haplotypes were present (P1 – P7).

Table 3.6. Pairwise matrix showing geographic distances (km; above diagonal), and genetic distances (Φ_{ST}; below diagonal) for *Plasmodium* lineages detected in song sparrows captured at sites in southern Ontario. Sites at which no *Plasmodium* were detected were excluded from the matrix. Statistically significant Φ_{ST} values are denoted with an asterisk.

	Bracken	Station	HP property	TresGrids	Murphys Point	Silver-Sharbot Lake	Frontenac	Charleston Lake	London
Bracken	0	8	20	14	18	31	22	28	436
Station	0.2506	0	13	7	25	37	19	24	435
HP property	-0.0475	0.5294	0	6	38	41	10	31	423
TresGrids	0.2506	0.0000	0.5294	0	31	38	13	27	428
Murphys Point	-0.2969	1.0000	-1.0000	1.0000	0	33	40	35	451
Silver-Sharbot Lake	-0.1090	0.0390	-0.0935	0.0390	-0.2856	0	34	59	422
Frontenac	0.1118	0.8511	0.2543	0.8511*	0.1863	0.2457	0	41	415
Charleston Lake	-0.0974	0.4114	0.0062	0.4114	-0.3132	-0.1450	-0.0375	0	453
London	-0.0560	0.3145	-0.0017	0.3145	-0.3912	-0.0735	0.0964	-0.1639	0

3.3.2.2 Leucocytozoon

Pairwise genetic distances (Φ_{ST}) for *Leucocytozoon* haplotypes observed at all possible pairs of sites are summarized in Table 3.7. An AMOVA conducted on *Leucocytozoon* haplotype frequencies showed significant genetic structuring at this community level (Φ_{ST} = 0.284, p<0.001). A total of 28.4% of variation in *Leucocytozoon* haplotype frequencies occurred between sites. At the species level, however, an AMOVA conducted on the grouped lineages L1, L2, L3, L6 and L7 showed no significant population genetic structuring (Φ_{ST} = 0.190, p= 0.114), with 19% of variation in haplotype frequencies occurring between sites.

I found some marginal evidence for isolation by distance at the community level (all *Leucocytozoon* lineages; one tailed p= 0.057; Table 3.7), but not at the species level (lineages L1, L2, L3, L6 and L7; one tailed p= 0.474). Haplotype frequencies of *Leucocytozoon* (all lineages) did not differ significantly between 2009 and 2010 at the Bracken site (Φ_{ST} = -0.030, p= 0.414).

Table 3.7. Pairwise matrix showing geographic distances (km; above diagonal), and genetic distances (Φ_{ST}; below diagonal) for *Leucocytozoon* lineages detected in song sparrows captured at sites in southern Ontario. Sites at which no *Leucocytozoon* were detected were excluded from the matrix. Statistically significant Φ_{ST} values are denoted with an asterisk.

	Bracken	Station	HP property	Murphys Point	Silver-Sharbot Lake	Frontenac	Charleston Lake	London
Bracken	0	8	20	18	31	22	28	436
Station	0.2446	0	13	25	37	19	24	435
HP property	0.0596	0.1985	0	38	41	10	31	423
Murphys Point	-0.1570	0.3684	-0.0800	0	33	40	35	451
Silver-Sharbot Lake	0.5318	-1.0000	0.4087	1.0000	0	34	59	422
Frontenac	0.4399*	-0.2992	0.3860*	0.6226	-1.0000	0	41	415
Charleston Lake	0.0011	0.5790	0.0751	0.0000	1.0000	0.7203*	0	453
London	0.5937*	-0.2000	0.5088	1.0000	0.0000	-0.2903	1.0000*	0

3.3.2.3 Song sparrows

Pairwise F_{ST} values for song sparrows captured at different sites are summarized in Table 3.8. Although only 1.49% of the variation in microsatellite allele frequencies occurred between sites, AMOVA indicated that among-site differentiation was marginally significant (F_{ST}= 0.015, p= 0.049). I also found some evidence for isolation by distance in song sparrows: pairs of sites separated by greater geographic distances tended to be more genetically different as well (one tailed p= 0.048; Table 3.8).

Table 3.8. Pairwise matrix showing geographic distances (km; above diagonal) and genetic distances (F_{ST}; below diagonal) for song sparrows captured at 11 sites throughout southern Ontario. Statistically significant F_{ST} values are denoted with an asterisk.

	Bracken	Station	HP property	TresGrids	Murphys Point	Silver-Sharbot	Frontenac	Charleston	Little Cataraqui	Lemoine	London
Bracken	0	8	20	14	18	31	22	28	41	51	436
Station	-0.0029	0	13	7	25	37	19	24	35	44	435
HP property	0.0369*	0.0628*	0	6	38	41	10	31	22	31	423
TresGrids	-0.0268	-0.0100	-0.0395	0	31	38	13	27	28	11	400
Murphys Point	0.0040	0.0087	0.0169	-0.0313	0	33	40	35	59	69	451
Silver-Sharbot Lake	0.0435*	0.0119	0.1593	0.0541	0.0500	0	34	59	59	65	422
Frontenac	-0.0057	0.0049	0.0035	-0.0468	0.0044	0.0760	0	41	25	31	415
Charleston Lake	-0.0069	-0.0010	0.0026	-0.0460	-0.0062	0.0741	-0.0264	0	45	55	453
Little Cataraqui	-0.0072	0.0088	-0.0121	-0.0663	0.0005	0.0746	-0.0284	-0.0251	0	11	410
Lemoine Point	-0.0116	-0.0107	0.0282	-0.0402	-0.0033	0.0328	-0.0163	-0.0150	-0.0220	0	400
London	0.0644*	0.0848*	0.0031	0.0073	0.0497*	0.2060*	0.0206	0.0170	0.0286	0.0604	0

3.3.3 Comparing host and parasites

Mantel testing revealed no significant association between pairwise genetic distances of song sparrows (F_{ST} values) and of the *Plasmodium* (Φ_{ST} values) community (all *Plasmodium* lineages; Mantel r = −0.301, p= 0.130), nor between genetic structuring of song sparrows (F_{ST}) and of the *Leucocytozoon* (Φ_{ST}) community (all *Leucocytozoon* lineages; Mantel r =0.054, p= 0.410). At the species level, Mantel testing did not show an association between pairwise genetic distances of song sparrows and of *Plasmodium* lineages P1, P2, P5 and P8 (Mantel r = − 0.399, p= 0.070), nor between song sparrows and *Leucocytozoon* lineages L1, L2, L3, L6 and L7 (Mantel r = 0.367, p= 0.210).

UPGMA phenograms for song sparrows, *Plasmodium*, and *Leucocytozoon* sampled at different capture sites are shown in Figure 3.9. Topologies and branch lengths were generally non-congruent between song sparrows and the *Plasmodium* community, and between song sparrows and the *Leucocytozoon* community (Fig. 3.9). Similarly, topology and branch lengths for song sparrows were not congruent to those of *Plasmodium* lineages P1, P2, P5 and P8, or of *Leucocytozoon* lineages L1, L2, L3, L6 and L7 (data not shown).

Genetic distances between sites were not significantly different between song sparrows (pairwise F_{ST}) and the *Plasmodium* (pairwise Φ_{ST}) lineages P1, P2, P5, P8 (paired t-test, t_{73} = -0.667, p= 0.507; Fig. 3.10), or between song sparrows and the *Leucocytozoon* lineages L1, L2, L3, L6, L7 (t_{68} = 1.220, p= 0.227; Fig. 3.10).

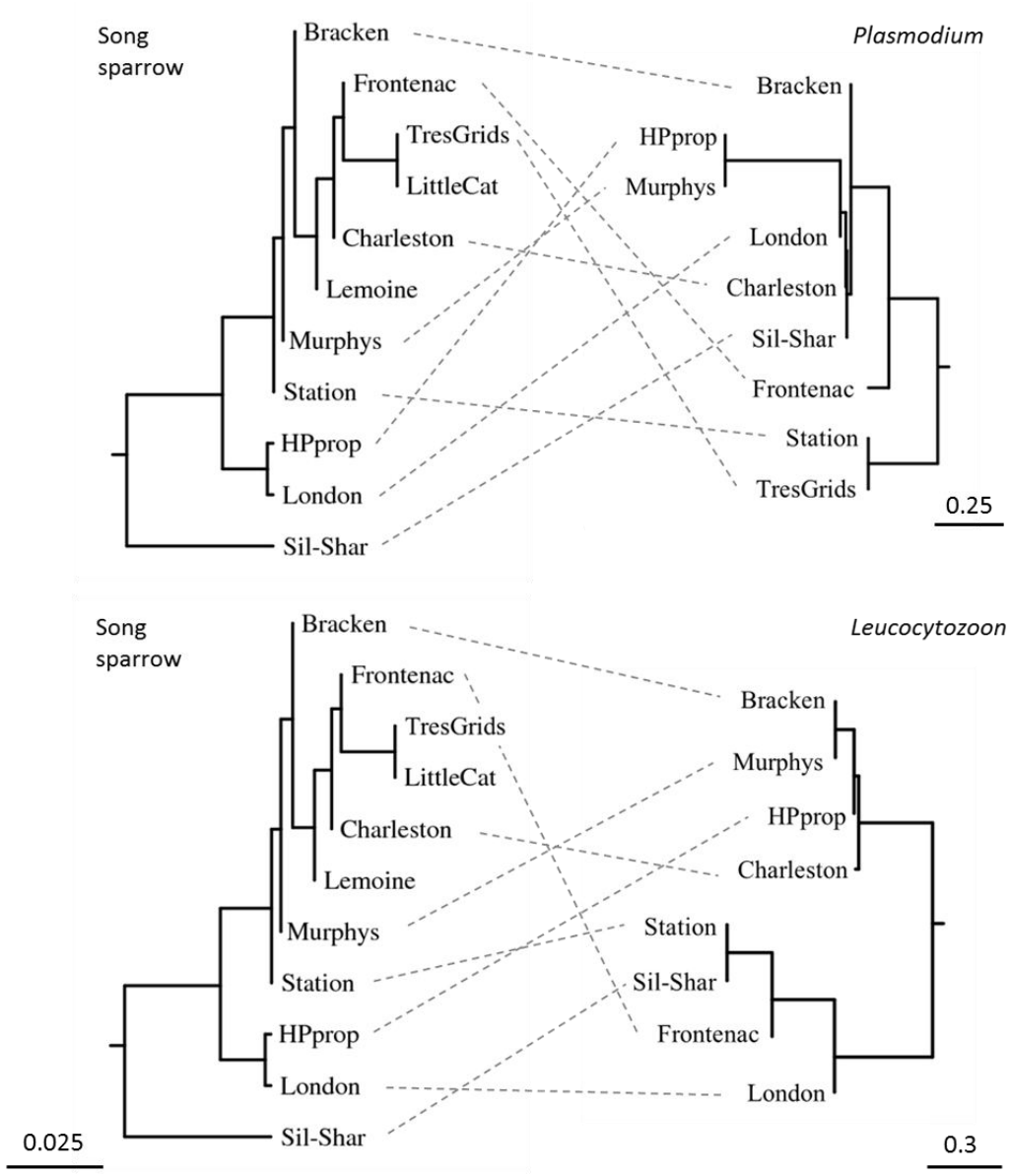

Figure 3.9. UPGMA phenograms of song sparrows (left) and parasites (right), constructed based on pairwise F_{ST} values between capture sites. Each terminal branch represents a site at which song sparrows were captured. Scale bar represents F_{ST} for each phenogram. Parasite phenograms have fewer terminal branches because parasites were not detected at all sites. The upper two phenograms compare song sparrows with *Plasmodium* and the lower two phenograms compares song sparrows with *Leucocytozoon*. Phenograms of song sparrows were generally not congruent to those of their parasites.

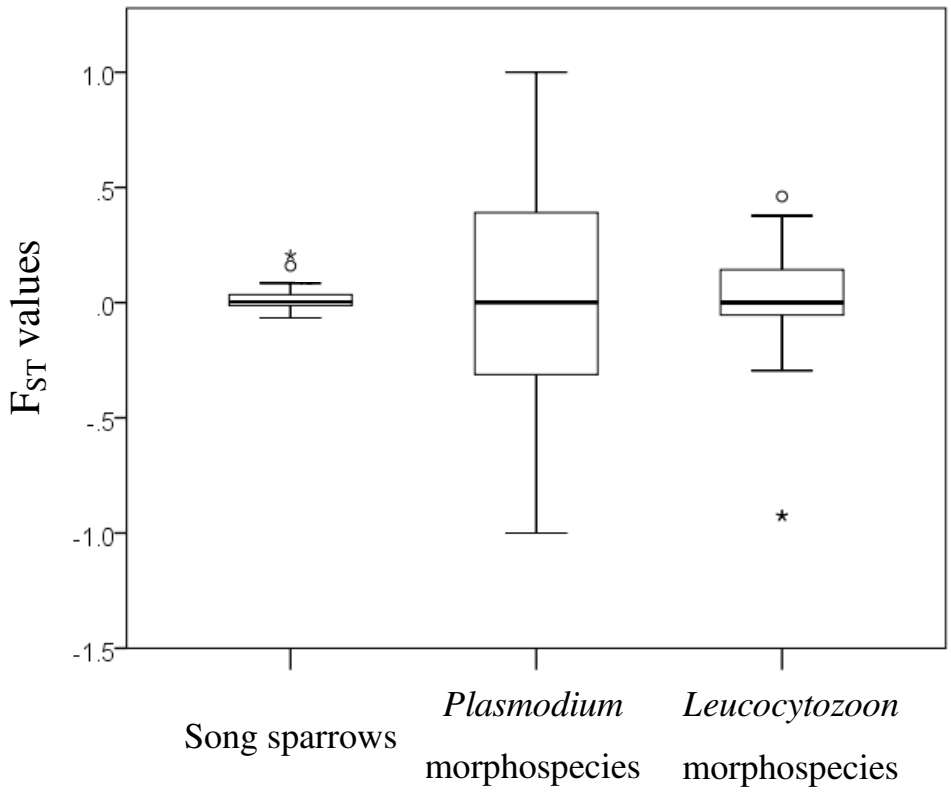

Figure 3.10. Range of F_{ST} values between sites for population analysis of song sparrows, *Plasmodium* morphospecies (comprising lineages P1, P2, P5, P8), and *Leucocytozoon* morphospecies (comprising lineages L1, L2, L3, L6, L7). The box represents the data between the first and third quartile and the median is shown by the black line. Whiskers show 95% confidence interval. Data points outside whiskers are outliers.

3.4 Discussion

The main objective of this study was to compare patterns of genetic structure in song sparrows to those of the birds' major bloodborne parasites. Song sparrows showed significant population genetic structuring over the geographic scale investigated, and evidence for isolation by distance. However, this population genetic structure did not coincide with patterns of genetic variation in the local parasite communities. This suggests that haematozoan parasites of song sparrows are relatively host-generalist and may not have coevolved appreciably with this host species. The two major genera of parasites (*Plasmodium* and *Leucocytozoon*) infecting song sparrows also differed from one another in patterns of genetic structuring. Overall, *Plasmodium* had little community-level genetic differentiation over the landscape whereas *Leucocytozoon* had significant community-level genetic differentiation. This suggests that *Plasmodium* communities were similar across the landscape, having high gene flow in this area, while *Leucocytozoon* communities varied in species composition, suggesting restricted gene flow. Moreover, haplotype frequencies in the *Plasmodium* community varied between years whereas *Leucocytozoon* haplotype frequencies were relatively stable in time, suggesting that there might be other factors influencing *Plasmodium* community besides gene flow, while the *Leucocytozoon* community seems more stable in its genetic structuring.

3.4.1 Genetic structure of host and parasites

I found significant population genetic structuring among song sparrows throughout southern Ontario, and evidence for isolation by distance. These patterns are consistent

with previous work on this and other bird species. Indeed, some studies have shown genetic differentiation at much finer geographic scales than that investigated here. For example, in great tits (*Parus major*) genetic similarity is greater among individuals living within 700 m than among individuals living 1500 m away from one another (Garroway *et al*. 2013). In song sparrows, Wilson *et al*. (2011) detected significant genetic structuring among west-coast populations (subspecies *M. melodia morphna*) separated by as little as 10 km, suggesting that landscape barriers such as small bodies of water can restrict gene flow in this species (Wilson *et al*. 2011). Similarly, MacDougall-Shackleton *et al*. (2011) reported population genetic structuring and isolation by distance among west-coast song sparrow populations separated by 4 km or less. Unlike the western *morphna* subspecies, song sparrows in Ontario (subspecies *M. melodia melodia*) are seasonally migratory, a phenomenon often associated with increased natal dispersal distance and reduced population genetic structure. Still, my findings provide support for geographic variation in allele frequencies among Ontario song sparrow populations.

In contrast to my findings for song sparrows, frequencies of *Plasmodium* lineages did not vary geographically, nor did the *Plasmodium* community show isolation by distance at the geographic scale examined. Similarly, finer-resolution examination of *Plasmodium* lineages that presumably comprise the same morphospecies (*i.e.*, lineages P1, P2, P5 and P8) revealed no evidence for geographic variation in lineage frequencies or isolation by distance. Collectively, these findings suggest that *Plasmodium* spp. are highly mobile, with a dispersal capacity that may exceed that of their avian hosts. This might be achieved if *Plasmodium* infections are transmitted in both breeding and wintering grounds. However, transmission at both ends of a migratory route is

uncommon (Hellgren *et al*. 2007, Waldenström *et al*. 2002). The absence of geographic variation in *Plasmodium* lineage frequencies, and of isolation by distance within this parasite genus, contrasts with findings of studies in the Southern Melanesia Islands. In those studies, *Plasmodium* lineages in multiple bird species were found to be geographically structured and positive relationships were seen between geographic distance and *Plasmodium* community dissimilarity (Ishtiaq *et al*. 2010) and lineage divergence (Olsson-Pons *et al*. 2015). Similarly, in the lesser Antilles, avian haemosporidia such as *Plasmodium* are geographically structured in their community composition (Fallon *et al*. 2003, Fallon *et al*. 2005). Thus, dispersal capacity of *Plasmodium* may vary between different parts of the world, perhaps due to variation in host migratory behaviour or the ecology and abundance of insect vectors.

Frequencies of *Leucocytozoon* lineages, on the other hand, showed significant geographic variation such that site pairs separated by longer geographic distances also tended to have more dissimilar *Leucocytozoon* communities. However, finer-resolution examination of *Leucocytozoon* lineages that presumably comprise the same morphospecies (*i.e.*, lineages L1, L2, L3, L6 and L7) did not detect geographic variation in lineage frequencies or isolation by distance. Geographic variation in *Leucocytozoon* lineage frequency may thus involve mainly variation in abundance between, rather than within, *Leucocytozoon* species. In contrast to the community-level geographic structuring of *Leucocytozoon* found here, European lineages of *Leucocytozoon* infecting blue tits (*Cyanistes caeruleus*) and great tits (*Parus major*) are not phylogeographically structured across Europe (Jenkins & Owens 2011). As with *Plasmodium*, the dispersal capacity of *Leucocytozoon* may differ regionally.

Isolation by distance within parasite-host systems, such that parasite communities are more similar among geographically close host populations than among geographically distant host populations, is generally expected to be common (Krasnov *et al*. 2005, Poulin 2003, Poulin & Morand 1999, Poulin *et al*. 2011). However, empirical evidence for this pattern is mixed and may vary geographically as well as among host species. In the Lesser Antilles, geographic distance predicts *Plasmodium* and *Haemoproteus* lineage composition in lesser Antillean bullfinches *Loxigilla noctis*, but not in bananaquits (*Coereba flaveola*) or black-faced grassquits (*Tiaris bicolor*; Svensson-Coelho & Ricklefs 2011). In Asia, haemosporidian (*Plasmodium* and *Haemoproteus*) community similarity is not associated with geographic distance between populations of Hume's warbler (*Phylloscopus humei*) or of greenish warblers (*P. trochiloides*; Scordato & Kardish 2014). My findings add to the diversity of patterns observed, and suggest that the relationship between geographic distance and parasite community similarity may vary between parasite genera, in that geographic structuring was observed for *Leucocytozoon* but not *Plasmodium* communities.

Plasmodium lineage frequencies varied significantly between years at the same capture location, with lineage diversity relatively high in one year whereas the following year only one lineage was observed. Such temporal variation might result from competitive exclusion of one lineage over others (Fallon *et al*. 2005, Santiago-Alarcon *et al*. 2011) and/or from host-parasite interactions including acquired immunity by host individuals (Fallon *et al*. 2004). In contrast, lineage composition of *Leucocytozoon* appears to be more stable in time, showing no significant difference in lineage frequencies between years at the same location. Temporal fluctuation in the prevalence

and lineage composition of haemosporidian parasites is not uncommon. For example, prevalence and lineage composition of haematozoa infecting 42 bird species in southern Missouri varied significantly over a four year period (Ricklefs *et al*. 2005). Similarly, on the islands of Puerto Rico and St. Lucia, sampling of *Haemoproteus* and *Plasmodium* spp. over a ten year period revealed multiple losses and gains of parasite lineages (Fallon *et al*. 2004). Haemosporidian lineages infecting great reed warblers (*Acrocephalus arundinaceus*) show 3-4 year cycles of high and low prevalence for the most abundant parasite lineages (Bensch *et al*. 2007). Other studies, however, have noted no temporal variation in parasite prevalence and lineage composition (Ishak *et al*. 2008). Collectively, these studies suggest that host-parasite systems vary in the degree to which parasite lineage composition is stable over time.

3.4.2 Comparing host and parasites

Mantel tests indicated that pairwise genetic distances between song sparrow populations (capture sites) were generally not correlated with dissimilarity of the parasite communities or morphospecies, for either *Plasmodium* or *Leucocytozoon*. Overall, I found little evidence for cospeciation between song sparrows and their haemosporidian parasites. Furthermore, phenograms created with genetic distances among sites from host (F_{ST}) and parasites (Φ_{ST}) were not concordant. My findings suggest that haemosporidian parasites infecting song sparrows are likely to be host-generalists.

Host-generalist parasites typically display no genetic structure within any single host species, because parasite gene flow can occur between co-occurring host individuals

of different species (Archie & Ezenwa 2011). This pattern is consistent with my findings for *Plasmodium*, *i.e.*, lack of population genetic structuring combined with lack of concordance to host genetic structuring, although not with my findings for *Leucocytozoon* in which significant population genetic structure was detected. However, under certain ecological conditions, it is still possible to find genetic structure in a generalist parasite, as in the seabird tick (*Ixodes uriae*; McCoy *et al*. 2001). Host-generalist species are also characterized by frequent host-switching, and indeed, host-switching events have frequently been observed (Beadell *et al*. 2006) or inferred (Bensch *et al.* 2000, Medeiros *et al.* 2013, Ricklefs & Fallon 2002, Ricklefs *et al*. 2004, Ricklefs *et al*. 2005, Svensson *et al*. 2007, Waldenström *et al*. 2002) in Haemosporidia. This pattern is important in the light of emerging infectious diseases, since Haemosporidians seem to readily colonize novel host species. Conversely, however, some Haemosporidian lineages are highly host-specific, restricted to a single host or to a group of closely related host species (Bensch *et al*. 2000, Merino *et al*. 2008, Perez-Tris *et al*. 2007, Ricklefs *et al*. 2005). Thus, some lineages may be involved in relatively close host-parasite associations, potentially enhancing divergence of host populations that are exposed to different parasite communities (Ricklefs 2010, Thornhill & Fincher 2013).

An additional objective of this study was to compare the magnitude of population genetic structuring in song sparrows to that of their haemosporidian parasites. This can be useful in predicting whether host or parasite is likely to show local adaptation to the other in evolutionary arms races. On average, F_{ST} and Φ_{ST} values were not significantly different between song sparrows and either *Plasmodium* or *Leucocytozoon*, respectively.

This suggests no clear advantage to either host or parasite in becoming locally adapted to the other.

In conclusion, this study established that song sparrows breeding throughout southern Ontario are infected with a diverse community of Haemosporidian parasites. None of the 19 lineages detected in this study had been described previously, although most lineages observed were reasonably genetically similar to described morphospecies. Population genetic structure of song sparrows was not correlated to community genetic structure of their haemosporidian parasites. These findings suggest that these Haemosporidian lineages may be relatively host-generalist with a history of host-switching, which is consistent with most findings on these parasites (Beadell *et al*. 2006, Bensch *et al*. 2000, Fallon *et al*. 2005, Medeiros *et al*. 2013, Ricklefs & Fallon 2002, Ricklefs *et al*. 2004, Ricklefs *et al*. 2005, Svensson *et al*. 2007, Waldenström *et al*. 2002). If so, evolutionary arms races with song sparrows (or any other individual host species) may represent a relatively weak selection force on haemosporidian parasites. Finally, the magnitude of genetic structuring was not significantly different between hosts and parasites in this system, potentially suggesting similar adaptive potential, *i.e.*, that local adaptation of hosts to parasites or of parasites to hosts may be equally likely (Greischar & Koskella 2007).

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Chapter 4

4 Effects of Haemosporidians on Territorial Defense by Male Song Sparrows: An Experimental Approach

4.1 Introduction

Parasites are thought to have important effects on the evolutionary trajectories of their hosts, with effects potentially including increasing the intensity of sexual selection (Hamilton & Zuk 1982), favouring sexual over asexual reproduction (Hamilton 1980, Lively 2010) and maintaining high levels of genetic variation in host populations (Haldane 1949). These models rely on the assumption that parasites impose substantial fitness costs on host individuals. Surprisingly, however, empirical evidence that parasites reduce host survivorship and/or reproductive success remains mixed.

Haematozoan parasites (genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*, Phylum Apicomplexa) and their interactions with avian hosts have been well studied (Valkiunas 2005). These unicellular parasites use vertebrates as their intermediate hosts, where their asexual life stage occurs, and are transmitted between vertebrate host individuals by bloodsucking insects (Valkiunas 2005). Haematozoa can be very harmful to their hosts. When insects release sporozoite-stage parasites into the host's bloodstream, the sporozoites invade organs such as the liver, kidney, lungs, heart, and brain, potentially causing haemorrhages due to capillary blocking, enlargement and ultimately malfunction of these organs (Valkiunas 2005). Moreover, merozoite-stage parasites reduce oxygen transport through the body because they invade and destroy red blood cells (Valkiunas 2005).

Despite substantial mechanistic knowledge of how avian Haemosporidians affect their hosts, many observational studies have failed to document negative effects of these parasites on wild birds. For example, brown-headed cowbirds (*Molothrus ater*) parasitized with *Plasmodium* or *Leucocytozoon* did not differ from unparasitized birds in body condition (mass corrected for wing length) or in survival between years (Weatherhead & Bennett 1992). Similarly, in the great reed warbler (*Acrocephalus arundinaceus*), infection with *Haemoproteus* and *Plasmodium* parasites was not associated with survival from one year to the next, or with number of offspring produced that returned the following year to breed at the site (Bensch *et al.* 2007). Several studies have even found positive associations between parasitism and fitness measures, potentially suggesting positive effects of being parasitized. For example, breeding pairs of Hawaiian amakahi (*Hemignathus virens*) in which at least one individual was infected with *P. relictum* produced a greater number of offspring that were re-sighted eight months later, relative to uninfected breeding pairs (Kilpatrick *et al*. 2006). In the great tit (*Parus major*), breeding males infected by *Haemoproteus* spp. had higher fledging success than did uninfected males, and females infected with *Plasmodium* spp. laid heavier eggs than did uninfected females (Norte *et al*. 2009). Likewise, blue tit (*Parus caeruleus*) females that laid larger clutches were more likely to be parasitized with at least one haemosporidian parasite (Fargallo & Merino 2004). In mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*), females infected with *Haemoproteus* spp. had higher overwinter return rates, and fledged twice as many nestlings as uninfected females (Zylberberg *et al*. 2015).

In contrast to the findings reviewed above, some observational studies have indeed found the expected negative relationship between infection by Haemosporidian parasites and fitness measures. In Tengmalm's owls (*Aegolius funereus*), for example, females infected with *L. ziemanni* laid smaller clutches than did uninfected females (Korpimaki *et al*. 1993). In great reed warblers, individuals parasitized with *H. payevskyi* produced fewer fledglings than did unparasitized birds, and parasitized females also arrived later to the breeding sites than did unparasitized females (Asghar *et al*. 2011). Also in great reed warblers, chronic *Haemoproteus* and *Plasmodium* spp. infections were associated with reduced life span (possibly resulting from telomere degradation) and as a result, a smaller number of offspring fledged during their lifetime (Asghar *et al*. 2015).

An important limitation of observational studies as reviewed above is that such studies fail to sample individuals that died as a result of parasitic infection. Thus, birds that are found to be harbouring infections in the wild are individuals that have already experienced parasite-mediated selection (Goater & Holmes 1997) and may in fact be of superior quality. These individuals may have already survived the acute phase of the infection (where parasite intensities are the highest, and where most host deaths occur) and are now in the chronic stage, where infection intensities are low. Therefore, an experimental approach is needed to quantify the effect of haematozoan parasites on host fitness components. This may involve either experimentally infecting birds and comparing survival or reproductive success to that of uninfected controls (Knutie *et al*. 2012), or alternatively, treating individuals with anti-parasitic drugs and comparing survival or reproductive success to that of untreated controls (Merino *et al*. 2000, Marzal *et al*. 2005, Knowles *et al*. 2010, Martinez-de la Puente *et al*. 2010).

The effects of parasites on host fitness may be more intense in one sex than another (Korpimaki *et al*. 1993, Fargallo & Merino 2004, Norte *et al*. 2009, Asghar *et al*. 2011) or more intense in one context (e.g. reproductive success: Merino *et al*. 2000, Marzal *et al*. 2005, Knowles *et al*. 2010) than another (e.g. survivorship: Martinez-De la Puente *et al*. 2010, Asghar *et al*. 2015). In most socially monogamous songbirds, a major determinant of male reproductive success is the ability to defend a breeding territory. Unlike nest success, which is heavily influenced by condition of both partners, territory defence can be attributed largely to male condition and should be much less confounded by female condition.

4.1.1 Objectives

To investigate effects of malarial parasites on the ability of their hosts to defend a territory, I experimentally treated breeding male song sparrows (*Melospiza melodia*) with the anti-malarial drug Primaquine. This drug has been used previously in other species (Merino *et al*. 2000, Marzal *et al*. 2005, Martinez-de la Puente *et al*. 2010) to reduce loads of *Haemoproteus* and *Leucocytozoon* spp., which infect approximately 2% and 18% (respectively) of song sparrows in my study population. I then compared behavioral response of treated versus untreated males to a simulated territorial intrusion. If parasites do in fact affect this aspect of host fitness, I predict that birds in the experimental group (where parasites have been removed) should be able to mount a stronger behavioural response to simulated intrusions relative to untreated males, who may still be harbouring parasites.

4.2 Methods

Study subjects were 45 male song sparrows defending breeding territories at a long-term study site near Newboro, Ontario, Canada (44.633°N, 76.330°W). Between May 4th and May 29th, 2011, I captured males in seed-baited Potter traps that I checked every hour, or using mist-nets with conspecific song playback. Song sparrows are multi-brooded (Arcese *et al*. 2002), and this period of time corresponds roughly to the laying, incubation and early post-hatch periods of the first brood in the study population

From each male I collected a small (approx. 35 μL) blood sample via brachial venipuncture and used this to prepare a thin film blood smear. I placed a drop of whole blood onto a clean glass microscope slide and used another clean slide to gently pull the blood over the surface of the first slide (Bennett 1970). Smears were allowed to air dry, fixed in 100% methanol within 24 h of collection, and then stored at room temperature awaiting staining and microscopic examination of parasite loads. Birds were colour banded with individually unique combinations of coloured plastic leg bands to allow individual identification during simulated territorial intrusions.

Upon capture, I assigned each male into one of two groups: Drug and Control. Group assignment alternated with each male captured to ensure similar reproductive timing in each group. For each male in the Drug group (n=23), I injected 0.1 mg of Primaquine (Fisher Scientific) dissolved in 0.1 ml of phosphate-buffered saline (PBS) subcutaneously into the wing web, following Martinez-de la Puente *et al*. (2010). This drug has been shown to reduce the intensity and lower the prevalence of *Haemoproteus*

and *Leucocytozoon* spp. in blue tits and house martins (Merino *et al*. 2000, Marzal *et al*. 2005, Martinez-de la Puente *et al*. 2010). Males in the control group (n=22) received 0.1 ml of PBS, again through subcutaneous injection into the wing web. Following injections, I released birds at the site of capture.

4.2.1 Simulated territorial intrusion

One week after each individual was injected (*i.e.*, between May 11th and June 4$^{th)}$, a simulated territorial intrusion was performed. This timeframe coincides generally with the incubation and nestling periods of reproduction. The mean date of simulated intrusion for Drug males was May 26 (SE=1.76 days), and that for Control males was May 27 (SE=1.77 days), so presumably they were at similar reproductive stages. Likewise, the time of day of the simulated intrusion was similar between Drug (Mean=7:28 am; SE=10 min) and Control (Mean=8 am; SE=11 min).

In preparation for each simulated intrusion ("trial"), a taxidermied song sparrow was placed in the center of the focal bird's territory (inferred by observations during the catching and injection of the bird, and the week that followed) between ground level and 1 m height, with a speaker beside it. Distances of 1 m, 2 m, and 5 m from the mount were marked with one piece of flagging tape so that the focal bird's distance from the mount could be estimated. Each trial consisted of 5 minutes of conspecific song playback, broadcast from the speaker at a rate of seven songs per minute for 5 minutes. Speaker volume was held constant across trials. In most cases the focal male appeared and responded immediately after the start of the playback. However, seven males (2

experimental, 5 control) did not appear in response to playback. In these cases I waited two days then repeated the trial.

During the 5 minute trial I recorded the number of flights in which the bird did not cross the speaker, number of flights crossing over the speaker, number of songs produced, number of attacks to the mount, and the time spent within 1 m, 1-2 m, 2-5 m, and greater than 5 m from the mount. I verbally recorded all behavioural observations onto a solid-state recorder, and later transcribed recordings. These methods and measures of territory defence are consistent with standard practice on song sparrows and other bird species (e.g. Amy *et al.* 2010, Gilman *et al*. 2007, Hollander *et al*. 2008, Jacobs *et al*. 2014). At the conclusion of each trial I opened up a mist net, recaptured the study subject, and took a second small blood sample for microscopic analysis of parasite load, in order to confirm that the drug treatment was effective in reducing parasite load. Birds were then released at the site of capture.

Of the 45 males initially captured and dosed with primaquine or saline, two disappeared from the study site before simulated territorial intrusions could be performed; these individuals were excluded from further analysis. Of the 43 remaining males on which simulated territorial intrusions were conducted, I was able to recapture 22. These 22 males (n=11 Drug, 11 Control) were used to confirm the effectiveness of the drug in reducing parasite load. The remaining 21 males that were not able to be recaptured (n=12 Drug, 9 Control) each responded successfully to the simulated intrusion trials and their identities were confirmed by visual inspection of colour bands with the aid of binoculars. Thus, all 43 males on which simulated territorial intrusions were performed were included in the behavioural analyses.

4.2.2 Microscopy

Following the field season, I treated all blood smears using Wright-Giemsa stain, following instructions in HARLECO® Hemacolor® Stain Set (Fisher Scientific). I then examined every smear under a light microscope using a 100X objective until 10,000 red blood cells (RBC) had been screened. I counted the number of Haemosporidian parasites present in these 10,000 RBC and classified them as either *Leucocytozoon* spp. or *Haemoproteus*/*Plasmodium* spp. following Valkiunas 2005. Because *Haemoproteus* and *Plasmodium* spp. are morphologically similar at some life cycle stages (Valkiunas 2005) I could not confidently distinguish between these two genera. In any case, because I was interested in behavioural effects of haematozoa in general (rather than comparing effects between genera), for each male I determined the total parasite load per 10,000 RBC. Both first and second blood smears were examined and scored in the same way, and all smears were examined blind to treatment and behavioural trial result.

4.2.3 Data analyses

To confirm whether males treated with Primaquine had lower prevalence of bloodborne parasites, I categorized each of the 22 recaptured males (*i.e.*, each male with known post-treatment parasitism status and load) as either unparasitized (no haemosporidia detected in a screen of 10,000 RBC post-treatment) or parasitized (one or more haemosporidia detected in the post-treatment screen). I used Fisher's exact test (due to low sample sizes) to compare post-treatment parasite prevalence of drug-treated versus control males. To determine whether males treated with Primaquine had lower loads of bloodborne

parasites, I compared post-treatment haemosporidian parasite loads (number of parasites combined across all genera, per 10,000 RBC) of drug-treated versus control males. Males with no parasites observed post-treatment were included in this analysis with a parasite load of zero.

All 43 males for which simulated territorial intrusions were performed were included in the analysis evaluating the effects of Primaquine treatment on territorial defence. Preliminary analyses revealed no relationship between trial date and any of the behavioural measures recorded (Pearson's correlation: flights crossing over the mount, $r_{1,41} = -0.005$, $p = 0.724$; flights not crossing over the mount, $r_{1,41} = -0.179$, $p = 0.250$; number of songs produced, $r_{1,41} = -0.183$, $p = 0.240$; number of attacks to the mount, $r_{1,41} = -0.225$, $p = 0.146$; time spent more than 5 m from mount, $r_{1,41} = 0.219$, $p = 0.159$; time between 2-5 m of mount, $r_{1,41} = -0.223$, $p = 0.151$; time between 1-2 m of mount, $r_{1,41} = -0.235$, $p = 0.129$; time within 1 m of the mount, $r_{1,41} = -0.283$, $p = 0.066$). Thus, to avoid model overfitting I did not include date in subsequent analyses.

To reduce the dimensionality of measurements of territorial defence, I conducted principal components analysis (PCA) using a correlation matrix. All behavioural measures (flights crossing over the mount, flights not crossing over the mount, number of songs produced, number of attacks to the mount, time spent more than 5 m from mount, time between 2-5 m of mount, time between 1-2 m of mount, time within 1 m of the mount) were entered into the PCA. Principal components with eigenvalues greater than 1 were retained for subsequent analysis. The results below are derived from analyses of unrotated principal components, but repeating the analyses with Varimax rotation of

principal components yielded qualitatively identical results (data not shown). I used an independent-samples t-test to compare the intensity of territory defence (*i.e.*, principal component scores) in Primaquine-treated versus control males. All analyses were performed in SPSS version 21 (2012) and all tests were two-tailed.

4.3 Results

4.3.1 Effects of Primaquine on parasite prevalence and load

Males treated with Primaquine did not appear to have lower prevalence (likelihood of being infected) or load of bloodborne parasites, relative to controls. Figure 4.1 shows pre- and post-treatment prevalence and intensity of haemosporidian infections in the recaptured birds (*i.e.*, those for which both pre- and post-treatment blood smears were available). The proportion of birds categorized as infected in the post-treatment screen did not differ between Primaquine-treated and control groups (Figure 4.1a; $p = 0.361$, $N=22$, Fisher's exact test). Post-treatment parasite loads (including all birds regardless of infection status) also did not differ between Primaquine-treated and control groups (Figure 4.1b; $t=0.613$, $N=22$, $p= 0.547$). The initial prevalence of haemosporidian parasites was 18% (4/22 birds parasitized pre-treatment; 2 in each of the Primaquine-treated and control groups). All four initial infections comprised *Leucocytozoon* spp. only. Final prevalence of haemosporidia in the recaptured birds was 32% (7/22). Of the four birds that were initially infected, three remained so and one lost its infection (Primaquine-treated), and four more (1 Primaquine-treated, 3 control) became infected. Of the seven birds found to be parasitized post-treatment, six were infected with

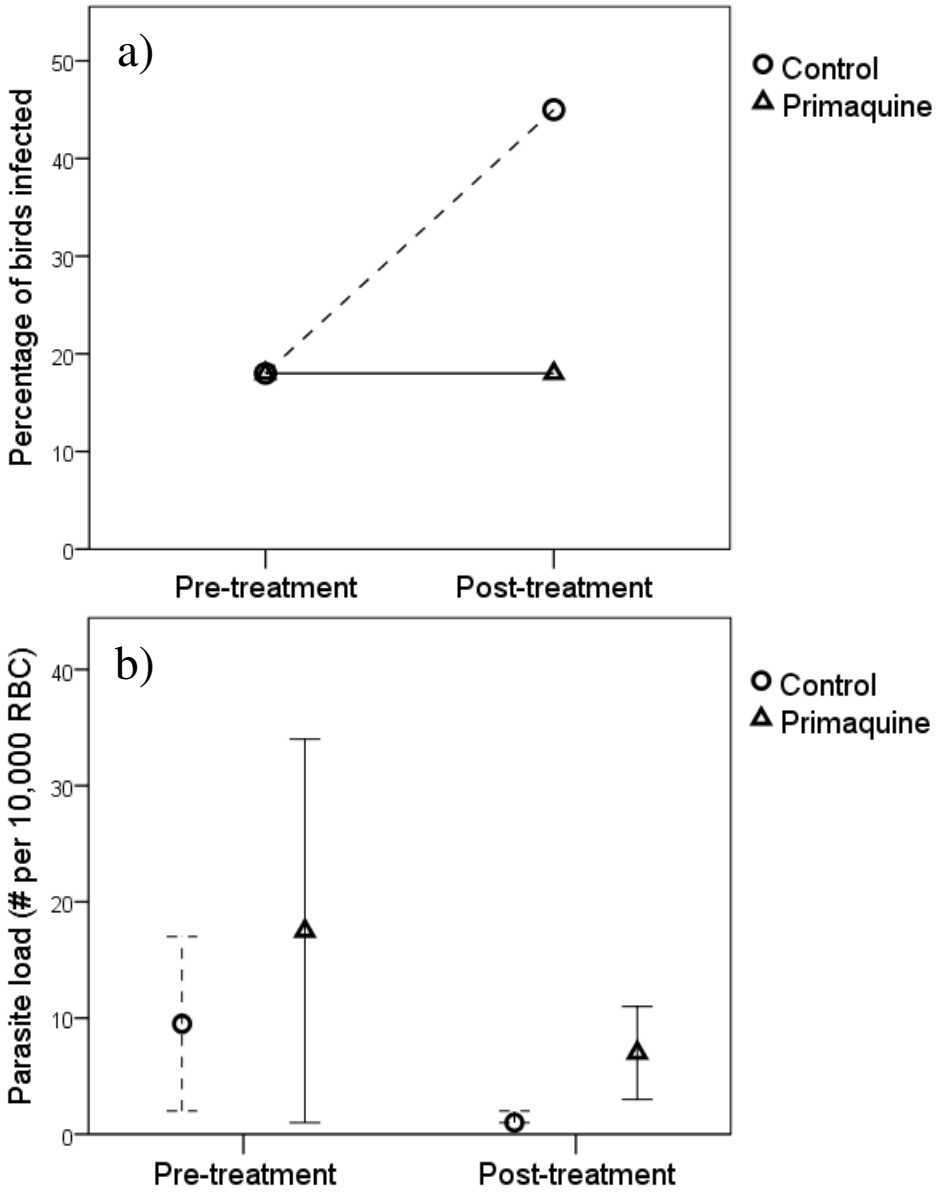

Figure 4.1. Pre and post-treatment prevalence (a) and intensity (b) of haemosporidian infections in male song sparrows treated with an antiparasitic drug (Primaquine) or saline solution (Control). In panel b) symbols represent median values, and error bars indicate 95% confidence intervals; birds with parasite loads of zero (*i.e.*, unparasitized) are excluded from this panel. Overall I detected no significant effect of Primaquine in reducing parasitism.

Leucocytozoon and one with *Haemoproteus* or *Plasmodium*; no mixed infections were detected.

4.3.2 Effects of Primaquine on response to simulated territorial intrusion

PCA of the eight behavioural measures collected during simulated territorial intrusions identified three principal components with eigenvalues greater than 1 (Table 4.1), collectively explaining 66.7% of the variation observed, which were retained for further analysis. PC1 had high positive loadings for flights not crossing the mount and time spent within close proximity to the mount; PC2 had high positive loadings for songs and attacks to the mount; and PC3 had high positive loadings for flights crossing over the mount and time spent more than 5 m from the mount. However, primaquine treatment had no significant effect on any of these principal components (PC1, t_{41}=-0.085, p= 0.933, Figure 4.2a; PC2, t_{41}=1.009, p= 0.319, Figure 4.2b; PC3, t_{41}=-1.938, p= 0.059, Figure 4.2c).

Table 4.1. Factor loadings of behavioural measures of territorial defence, resulting from simulated territorial intrusions onto the territories of 43 male song sparrows. Principal component analysis (PCA) extracted eight principal components, three of which had eigenvalues greater than one. These three principal components (PC1, PC2, PC3) were retained for further analyses.

	Component							
	1	2	3	4	5	6	7	8
Flights crossing over the mount	0.328	-0.522	0.693	-0.205	0.026	-0.220	0.107	-0.192
Flights not crossing over the mount	0.645	0.045	0.325	0.586	0.179	0.195	-0.249	-0.029
Number of songs	-0.340	0.622	0.277	0.362	-0.251	-0.469	0.060	0.051
Number of attacks	0.113	0.815	0.265	-0.192	-0.067	0.403	0.149	-0.160
Time more than 5 m from mount	-0.739	-0.214	0.407	0.154	0.234	0.246	0.182	0.265
Time between 2-5 m from mount	0.737	-0.134	0.155	-0.123	-0.562	0.125	0.002	0.260
Time between 1-2 m from mount	0.790	-0.069	-0.313	0.330	0.142	-0.049	0.377	-0.004
Time within 1 m of mount	0.593	0.434	0.103	-0.375	0.462	-0.218	-0.061	0.211
Cumulative % variation explained	33.971	53.769	66.653	77.172	85.982	93.422	96.896	100.000
Eigenvalue	2.718	1.584	1.031	0.842	0.705	0.595	0.278	0.248

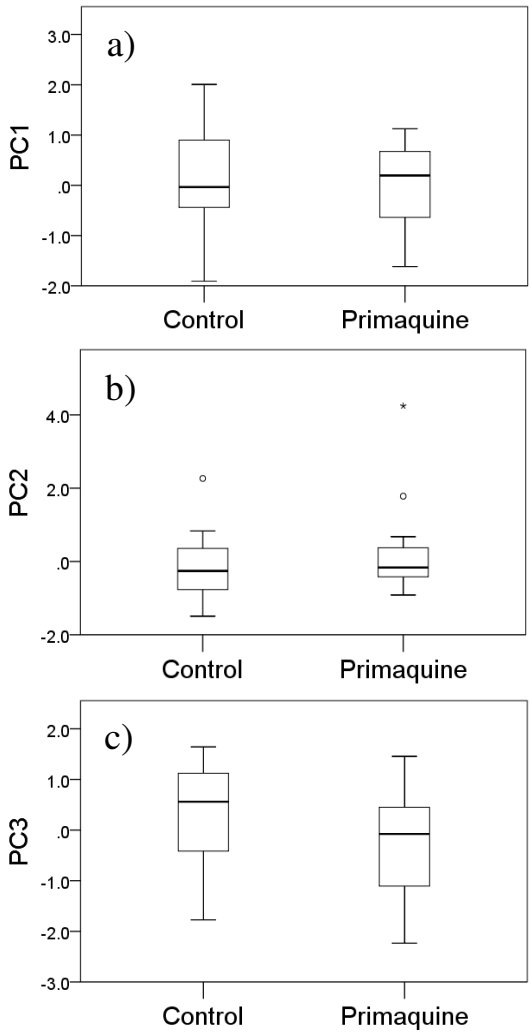

Figure 4.2. Behavioural responses of 43 male song sparrows treated with saline solution (Control; n=20) or an antiparasitic drug (Primaquine; n=23) and exposed to simulated territorial intrusions. Principal components analysis identified three components with eigenvalues greater than one. Control and Primaquine-treated males did not differ significantly in (a) PC1, which had positive loadings for flights not crossing the mount and time spent within close proximity to the mount; (b) PC2, which had positive loadings for songs and attacks; or (c) PC3, which had positive loadings for flights crossing over the mount and time spent more than 5 m away from the mount. The top of the box represents the 75th percentile, the inner line is the median, and the bottom of the box indicates the 25th percentile. Bars show 95% confidence limit, and points beyond this limit are outliers.

4.4 Discussion

The objective of this study was to use antiparasitic manipulation of haemosporidian parasite load to determine whether haemosporidian parasites affect territorial defence behaviour of male song sparrows. I found no significant differences in response to simulated territorial intrusions between males that were treated with the antiparasitic drug Primaquine and control males. However, I also observed no difference between these groups in the prevalence or intensity of haemosporidian parasites. Thus, this study cannot determine whether or not haemosporidian parasites influence this component of male fitness.

Unexpectedly, and in contrast to previous studies on other songbird species (Martinez-de la Puente *et al*. 2010, Marzal *et al*. 2005, Merino *et al*. 2000), Primaquine treatment did not reduce load or prevalence of haemosporidian infection. Several potential reasons may help to explain this lack of effect. First, the initial level of parasitism in the study birds was relatively low (4/22, or 18%) and most infections observed were not very intense (1 to 34 parasites). Low initial prevalence and intensity of infection may have contributed to a "floor effect" such that even if the drug had the intended effect, I would have little power to detect this effect. Moreover, Primaquine may be more effective in controlling infection by *Haemoproteus* than other haemosporidian genera. For example, in blue tits, Primaquine treatment reduced levels of infection by *Haemoproteus* but not *Leucocytozoon* (Martinez-de la Puente *et al*. 2010, Merino *et al*. 2000). In the present study, all of the pre-treatment infections detected and

most of post-treatment infections consisted of *Leucocytozoon* spp., which likely explains the failure to reduce parasite load.

Because the drug treatment did not reduce parasite load, it is impossible to determine whether or not haemosporidian parasites reduce the ability of song sparrows to defend breeding territories. Among studies that have successfully used medication to experimentally reduce parasite loads, several (e.g. Knowles *et al*. 2010, Martinez-de la Puente *et al*. 2010, Marzal *et al*. 2005, Merino *et al*. 2000) have shown that experimentally reducing blood-borne parasites enhances various fitness components. However, other studies that experimentally reduced parasite load have failed to detect improvements to fitness components. For example, female blue tits treated with Primaquine did not show increased fledging success or body condition of nestlings produced, despite the drug successfully decreasing *Haemoproteus* parasite load (Tomas *et al*. 2007). In rock pigeons (*Columba livia*), nestlings experimentally infected with *Haemoproteus columbae* showed no difference in body mass and fledging success compared to uninfected nestlings (Knutie *et al*. 2012).

Compared to studies examining fitness correlates of naturally-occurring variation in parasitism, experimentally manipulating parasite load represents a valuable method of disentangling effects of inherent individual quality from effects of parasites on fitness. However, as this study illustrates, a risk of this approach is that the drug may not necessarily have the intended effect on parasite loads, thus recapturing subjects to confirm drug efficacy is essential. In sum, even though this study did not successfully manipulate parasite load and thus cannot assess effects of parasites on territorial defense,

manipulative experiments in general should allow us to quantify fitness costs of parasites on individual fitness and the impact that they could ultimately have on wild populations.

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Chapter 5

5 Song Sparrows *Melospiza melodia* have a home-field advantage in defending against sympatric malarial parasites

5.1 Introduction

Parasites and their hosts are key features of one another's environments, interacting on both evolutionary timescales (through local arms races) and ecological timescales (through parasite effects on host fitness and the development of resistance within the lifetime of host individuals). The outcome of these interactions, *i.e*, whether parasites are better able to infect hosts from the same area (sympatric) than hosts from a different area (allopatric) or whether hosts are more resistant to infection by sympatric than allopatric parasites, can have important consequences for host diversification (Fincher & Thornhill 2008). For example, if parasites are better able to infect sympatric than allopatric hosts, selection on hosts may promote natal dispersal as a means of escaping sympatric parasites and contribute to the success of invasive species (Keane & Crawley 2002). Conversely, if hosts have a "home-field advantage" in that they are more resistant to sympatric than allopatric parasites, the risk of encountering allopatric parasites may represent a cost of dispersal (Moller *et al.* 2004) and selection on hosts may thus promote philopatry.

The reciprocal selection forces that hosts and parasites impose on one another can generate local cycles of antagonistic coevolution (Gandon & Nuismer 2009). Selection on parasites favours the ability to exploit locally common host genotypes, potentially

resulting in parasite local adaptation, whereby parasites are better able to infect sympatric than allopatric hosts (Lively & Dybdahl 2000). At the same time, selection on hosts favours resistance to locally common parasite strains, potentially resulting in host local adaptation, *i.e.* greater resistance to sympatric than allopatric parasites (Oppliger *et al*. 1999). In general, larger population sizes and shorter generation times in parasites than hosts are thought to provide parasites with an evolutionary advantage (Gandon 2002). However, host-parasite coevolution can also be influenced by factors such as relative rates of gene flow, virulence, and host-specificity of the parasite (Greischar & Koskella 2007). Thus, although parasite local adaptation appears to be common, in some systems hosts show local adaptation to parasites, and others show a mosaic pattern such that the outcome of host-parasite interactions varies across populations (Schulte *et al.* 2011).

In addition to evolutionary interactions between hosts and parasites, the exposure history of individual hosts can also influence their resistance to particular parasites. In jawed vertebrates, the acquired immune response facilitates the development of immunological memory. Following exposure to antigens, particularly during early life, individual hosts develop antigen-specific lymphocyte lines and antibodies (Janeway *et al.* 2001), essentially vaccinating them against repeated encounters with the same antigen (Moller & Szép 2011). Such immunological memory permits a rapid and efficient secondary response to pathogens that an individual host has encountered previously. In humans, for example, exposure to malaria parasites (*Plasmodium falciparum*) during early life confers protection against subsequent infection by similar strains (Doolan *et al*. 2009). Thus, vertebrate hosts may have a home-field advantage over sympatric parasites due to population-level evolutionary processes (*i.e.*, host local adaptation) and/or

individual-level ecological processes (*i.e.*, immunological memory). Changing ranges of parasites and their vectors, associated with a changing climate (Harvell *et al*. 2002), emphasize the importance of predicting the outcome of evolutionary and ecological interactions between hosts and parasites.

Interactions between birds and their parasites are of keen interest from a diversity of perspectives including conservation (McCallum & Dobson 2002), sexual selection (Hamilton & Zuk 1982), speciation (Ricklefs 2010), and zoonotic disease (Prugnolle *et al*. 2011). Correlative evidence from natural populations suggests that at least in some cases, birds may be more resistant to sympatric than to allopatric parasites. Male white-crowned sparrows (*Zonotrichia leucophrys*) that sing local songs, and thus presumed to be of local origin, have lower haematozoan parasite loads than males singing non-local songs and thus presumed to be immigrants (MacDougall-Shackleton *et al*. 2002). Similar patterns have been reported for song sparrows (*Melospiza melodia*) (Stewart & MacDougall-Shackleton 2008). Moreover, female barn swallows (*Hirundo rustica*) that remain to breed in their natal colony have lower ectoparasite (louse fly) infestations than do females that disperse to breed outside their natal colony (Saino *et al*. 2014). Lower parasite loads in philopatric than in dispersing individuals suggest that philopatry may confer home-field advantage in dealing with the local parasite fauna. However, other potential explanations for this pattern cannot be excluded. Philopatric and dispersing individuals may differ in morphology, behaviour or other traits that may affect susceptibility to parasites (Debeffe *et al*. 2014, Saino *et al*. 2014). Thus, conclusively determining whether low parasite loads in philopatric birds reflect home-field advantage

or simply differences in quality between philopatric and dispersing hosts requires an experimental approach.

Here I report findings of a reciprocal cross-infection experiment testing whether migratory song sparrows have an advantage in defending against their local malarial parasites (*Plasmodium* spp.), or conversely whether parasites in this system have an advantage in infecting their local hosts. I captured sparrows from two different breeding sites, identified locally-confined *Plasmodium* lineages in each, and assessed resistance to sympatric versus allopatric lineages by measuring infection status and infection severity (*i.e.*, parasite load, body mass, fat). If parasites have a home-field advantage in infecting their local hosts, *Plasmodium* spp. cultured from one site should be better able to infect and/or proliferate in its sympatric than allopatric hosts. Conversely, if hosts have a home-field advantage in resisting their local parasites, birds should be more resistant to sympatric than to allopatric *Plasmodium* lineages.

5.2 Methods

5.2.1 Study system: hosts and parasites

I captured song sparrows from two breeding locations separated by 437 km: an eastern site at Newboro, Ontario, Canada and a western site at London, Ontario, Canada (Table 5.1). Song sparrows in these areas are seasonally migratory, and show moderate natal philopatry combined with high adult philopatry. At the eastern site, where song sparrow breeding biology has been studied since 2002, approximately 10% of breeding adults each year were first banded as nestlings on the site; and nearly 50% of breeding adults

(presumably all those surviving the winter) return to the site the following year (Potvin *et al*. 2015).

Some of the most commonly observed bloodborne parasites in these birds are *Plasmodium* spp. (Apicomplexa). These intracellular parasites are normally transmitted between avian hosts by mosquitoes (Valkiunas 2005). However, because asexual reproduction (schizogony) occurs in circulating erythrocytes as well as in fixed tissues of the vertebrate host, *Plasmodium* spp. are highly amenable to infectivity experiments, because infections can be transmitted to new hosts through inoculation with infected blood (Atkinson & van Riper 1991; Dimitrov *et al*. 2015).

Using mist nets and seed-baited Potter traps, I captured 16 adult song sparrows (8 males, 8 females) from the eastern site, and 18 adult song sparrows (14 males, 4 females) from the western site. Six birds (4 males, 2 females) were captured in October 2011, and 28 (16 males, 12 females) between July-September 2012. I transported birds in individual cages to the Advanced Facility for Avian Research (AFAR) at the University of Western Ontario. Once at AFAR, birds were maintained in individual cages, in rooms free of insect vectors and kept at 20-22 ^{o}C under natural photoperiod with *ad libitum* access to food and water.

Table 5.1. Sites at which song sparrows were captured and screened for infection with *Plasmodium* spp. Numbers include birds used in the current study. GenBank accession numbers for each *Plasmodium* lineage are reported in Fig. 1. Asterisks denote lineages used for experimental infection (P-SOSP9, P-SOSP10).

Site	Coordinates (°N, °W)	Distance from Newboro (km)	Birds sampled	Birds infected with *Plasmodium* spp.	*Plasmodium* lineages detected (# birds with each lineage)
			Eastern sites		
Newboro	44.633, 76.330	--	160	27	P-SOSP1 (6) P-SOSP2 (4) P-SOSP3 (9) P-SOSP4 (1) P-SOSP5 (1) P-SOSP6 (1) P-SOSP7 (3) P-SOSP9* (1) P-SOSP11 (1)
Biology Station	44.567, 76.324	7	38	2	P-SOSP3 (2)
Swallow Grids	44.521, 76.385	13	6	2	P-SOSP3 (2)
Murphy's Point	44.781, 76.236	18	20	1	P-SOSP1 (1)
Elbow Lake	44.475, 76.430	19	21	4	P-SOSP1 (3) P-SOSP3 (1)
Frontenac Park	44.508, 76.543	22	21	4	P-SOSP1 (1) P-SOSP2 (2) P-SOSP8 (1)
Charleston Lake	44.501, 76.035	28	19	4	P-SOSP2 (2) P-SOSP3 (1) P-SOSP7 (1)
Silver Lake	44.830, 76.579	29	11	3	P-SOSP1 (1) P-SOSP2 (1) P-SOSP3 (1)
Sharbot Lake	44.783, 76.715	35	4	1	P-SOSP3 (1)
Little Cataraqui	44.289, 76.511	41	11	0	--
Lemoine Point	44.226, 76.612	51	21	0	--
			Western site		
London	43.008, 81.291	437	19	5	P-SOSP2 (1) P-SOSP3 (1) P-SOSP4 (1) P-SOSP7 (1) P-SOSP10* (1)

5.2.2 Characterizing naturally-occurring infections

To identify birds that were already naturally infected with *Plasmodium* spp., I collected a small (25 μL) blood sample via brachial venipuncture of each individual. A drop of this sample was used to prepare a thin-film blood smear. Smears were air-dried, fixed in 100% methanol, treated with Wright-Giemsa stain, and examined under a light microscope with a 100x objective. For each bird, I scanned 10,000 erythrocytes and noted the number of cells containing one or more haematozoan parasites.

From the remainder of the blood sample, I extracted DNA using an ammonium-acetate based protocol then used a two-stage, nested PCR approach to amplify parasite cytochrome *b* (Hellgren *et al*. 2004). The first stage used primers HAEMNFI and HAEMNR3, which amplify cytochrome *b* of genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* (Hellgren *et al*. 2004), to amplify an initial 571 bp fragment (excluding primers). The second stage used 1 μL of product from the initial PCR as template, along with the internally nested primers HAEMF and HAEMR2 (Bensch *et al*. 2000) to amplify 480 bp (excluding primers) of cytochrome *b* of *Haemoproteus* and *Plasmodium*. PCR was conducted in 25 μL volumes following Hellgren *et al*. (2004). Second-round PCR products were run at 100 V for 90 min on 2% agarose gels, stained with ethidium bromide, and visualized under UV light. Bands of the expected product size were excised and purified using a Gel/PCR DNA Extraction Kit (FroggaBio, North York) then sequenced from the 5' end with primer HAEMF on an ABI 3730 Genetic Analyzer (Applied Biosystems) at the London Regional Genomics Centre. Sequences were aligned using ClustalW, trimmed to 476 bp, and identified to genus (*i.e.*, *Plasmodium* or *Haemoproteus*) using the Basic Local Alignment Search Tool (BLAST) implemented in

GenBank. I observed only one instance of double peaks on electropherograms, suggesting that co-infections were rare (although see Valkiunas *et al*. 2006).

5.2.3 Identifying locally-confined lineages

Additional surveys of song sparrows and their *Plasmodium* parasites were not possible throughout western Ontario. Thus, I cannot exclude the possibility that parasite lineages detected only at the eastern site might also occur (at least at low frequency) at the western site. However, I conducted wider sampling of song sparrows throughout eastern Ontario to identify which, if any, lineages detected at the western site were absent from the eastern site. Between 2009 and 2012, I collected blood samples as described above from an additional 316 song sparrows captured at Newboro (the eastern site) and other sites within 50 km (Table 5.1). Cytochrome b was amplified and sequenced as described above.

Of the 350 song sparrows screened (*i.e.*, 34 used in the present study plus 316 in the expanded survey), 53 were infected with *Plasmodium* spp. In all, I identified eleven unique *Plasmodium* lineages (P-SOSP1 through P-SOSP11), defined as sequences that differed by at least 1 bp (Fig. 5.1). These lineages showed 96-99% sequence identity to other published *Plasmodium* lineages and have been deposited in GenBank (accession numbers KT193627-KT193637; Fig. 5.1).

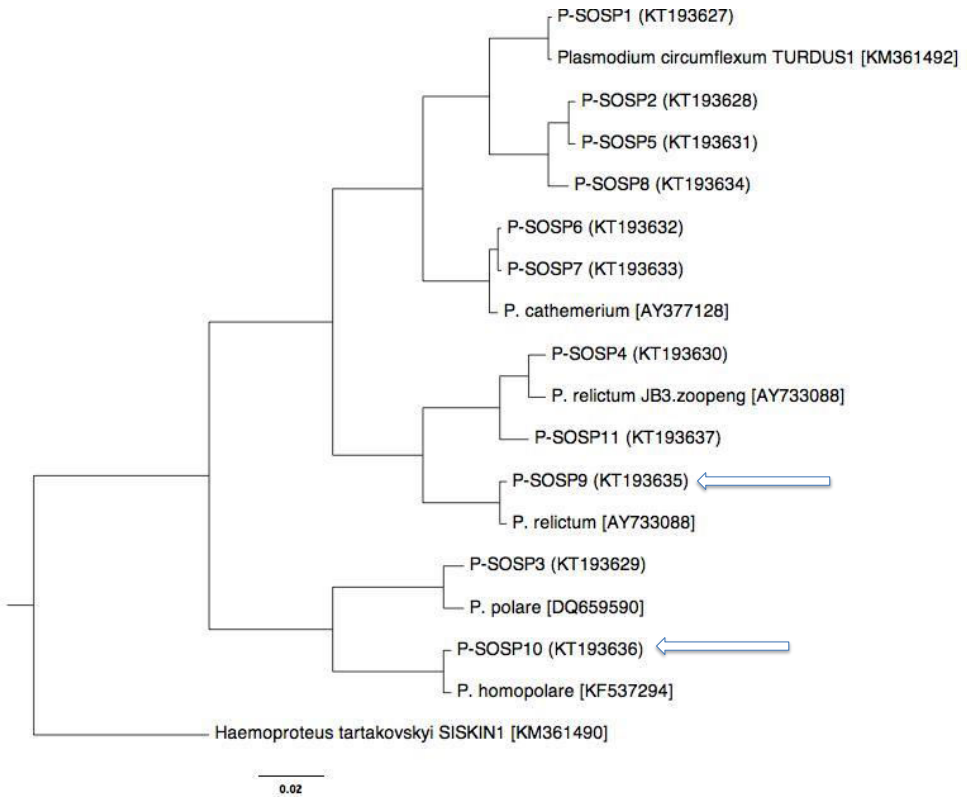

Figure 5.1. UPGMA phylogeny of 11 cytochrome b lineages of *Plasmodium*, detected in song sparrows in southeastern and southwestern Ontario (GenBank accession numbers reported in parentheses); plus 6 similar sequences previously identified to morphospecies (GenBank accession numbers reported in square brackets). *Haemoproteus tartakovskyi* was used as an outgroup. Arrows denote lineages used for experimental infection (P-SOSP9, P-SOSP10).

Several lineages were observed at both the eastern and the western site, but lineage P-SOSP10 (99% sequence identity to morphospecies *P. homopolare*; Fig. 5.1) was detected only at the western site and not at the eastern site or surrounding locations (Table 5.1). Thus, I am confident that this lineage is absent or at least very rare at and around the eastern site, and used it as the "western" lineage in the cross-infection experiment described below. Conversely, lineages P-SOSP9 and P-SOSP11 were detected only at eastern site and not at the western site. I arbitrarily selected P-SOSP9 (99% nucleotide sequence identity to morphospecies *P. relictum*; Fig. 5.1) as the "eastern" lineage, although as noted above, I cannot exclude the possibility that this strain may also occur in western Ontario. Nucleotide sequence divergence between P-SOSP9 and P-SOSP10 was 8%.

5.2.4 Cross-infection experiment

In October 2012, I collected 200 μL of blood by brachial venipuncture from each of two naturally-infected "parasite donors", *i.e.*, an eastern bird infected with P-SOSP9 and a western bird infected with P-SOSP10. Donors showed no evidence of co-infections with other lineages. I used donor blood to inoculate a total of four, previously uninfected, "amplifier" birds, each captured from the same site as its respective donor. Thus, blood from the eastern donor harbouring P-SOSP9 was injected into two eastern amplifiers, and blood from the western donor harbouring P-SOSP10 was injected into two western amplifiers.

I used a sterile, single-use syringe and 26 gauge needle to inject 200 μL of a mixture containing 50 μL freshly-collected (within 5 min) blood, 3.7% sodium citrate, and 0.9% saline, into the pectoralis muscle of each amplifier over 5-10 s. I monitored infection status of the four amplifiers by collecting 20 μL blood samples every three days between 08:00-10:00, and prepared thin-film blood smears as described above. By 18 days after inoculation, all amplifiers showed 1-3 parasites per 10,000 erythrocytes (average for eastern amplifiers = average for western amplifiers = 2.0 parasites per 10,000 erythrocytes). Asexual (infectious) stages of *Plasmodium* were present in similar concentrations (1-2 meronts per 10,000 erythrocytes) in all amplifiers. Amplifiers were euthanized by overdose of isofluorane vapours and 600 μL of blood immediately collected into a syringe through cardiac puncture. I combined blood from the two eastern amplifiers, and from the two western amplifiers, and mixed each with saline/sodium citrate buffer as described above. Each infected blood mixture was inoculated into "experimental" birds from both the eastern and western site, with 200 μL of infected blood/buffer mixture injected into the pectoralis muscle. "Experimental" birds comprised six eastern birds and six western birds inoculated with the eastern parasite P-SOSP9; and six eastern birds and six western birds inoculated with the western parasite P-SOSP10. As controls, one eastern and one western bird were inoculated with blood from an uninfected eastern bird; and another western bird received no inoculation.

Ten of the experimental birds (5 eastern, 5 western) and one (western) control bird were determined to be naturally infected with *Plasmodium* spp. prior to the start of the experiment. Although none of these naturally-occurring infections consisted of P-SOSP9, P-SOSP10 or closely related lineages (5.2% minimum sequence divergence

between lineages observed in naturally-infected experimental or control birds and lineages P-SOSP9 and P-SOSP10), I included prior infection status as a factor in subsequent analyses.

Beginning six days after experimental and control birds were inoculated, I monitored their bloodborne parasite loads every three days until 30 days post-inoculation. I collected approximately 20 μL of blood from each bird via brachial venipuncture, between the hours of 08:00 and 10:00. I prepared and screened thin-film blood smears as described above, and scored them blind as regards experimental treatment. Immediately after blood sampling, I also measured each bird's mass to the nearest 0.001 g using a digital scale, and scored subcutaneous furcular fat on a scale of 0 (no visible fat) through 5 (bulging deposits of fat). After blood sampling on post-inoculation day 30, I euthanized experimental and control birds by inhaled overdose of isofluorane followed by decapitation. I removed each bird's liver and spleen, and weighed these organs to the nearest 0.001 g using a digital scale.

5.2.5 Data analysis

In the control bird that tested positive for naturally-occurring *Plasmodium* infection, no parasites were detected through microscopy throughout the duration of the experiment. Parasite loads among naturally-infected experimental birds prior to the start of the experiment ranged from 0-0.02% (mean ± SE = 0.40 ± 0.21 parasites per 10,000 erythrocytes). Based on these values, which presumably reflect chronic rather than acute infections, I established an arbitrary threshold for infection success of twice the

maximum observed chronic-stage parasitaemia, *i.e.*, 0.04%. Thus, experimentally inoculated birds with at least one observation of at least four infected erythrocytes per 10,000 examined were considered successfully infected. I note that even a single observed gametocyte of the inoculated strain is evidence of successful infection, but because some subjects had previous natural infections, I used a higher threshold to be conservative and to reduce reliance on identifying parasite species through microscopy.

5.2.5.1 Infection risk

To compare birds' risk of becoming infected with sympatric versus allopatric parasites, I constructed generalized linear model regressions with binomial error distributions in JMP 10. The dependent variable was infection success, *i.e.*, whether or not an individual became successfully infected as defined above. I first constructed a fully-loaded initial model with predictor variables including bird origin (eastern or western); parasite origin (eastern or western); sex; prior infection status (*i.e.*, whether or not the individual was naturally-infected with *Plasmodium* spp. before the start of the experiment); and the interaction between bird origin × parasite origin. After running the initial model, to improve model fit and maintain parsimony (West *et al*. 2006) I calculated the minimal adequate model by sequentially removing non-significant predictors ($p \geq 0.05$), beginning with the least significant.

5.2.5.2 Infection severity

For the 15 birds that became infected, I constructed linear mixed models to compare measures of infection severity (*i.e.*, parasite load, and body condition as assessed by mass and fat) associated with sympatric versus allopatric *Plasmodium* infections. Mass and fat were examined because birds infected with *Plasmodium* may show anorexia and weight loss (Williams 2005). I constructed initial models with parasite load, body mass and fat as dependent variables; and bird origin, parasite origin, sex, prior infection status, experimental date, and the interaction between bird origin × parasite origin entered as fixed effects. Because each bird was measured multiple times, models included bird ID as a random effect. After running the initial model, I calculated the minimal adequate model as described above. I report results from minimal adequate models using restricted maximum likelihood estimation.

Plasmodium infections in birds may be associated with enlarged liver and spleen (Williams 2005), so I compared the mass of these organs among treatment groups at the end of the experiment (30 days after inoculation). To correct for body size, I divided liver and spleen mass by body mass at experimental day 0. Size-corrected liver mass was normally distributed, and I log-transformed size-corrected spleen mass to achieve a normal distribution. I used factorial ANOVAs to evaluate effects of bird origin, parasite origin, and their interaction on size-corrected liver and spleen mass.

To assess the effects of parasitic infection on body composition, I compared body mass, fat score, and size-corrected mass of liver and spleen of birds that became successfully infected (n = 15) versus those that did not (n = 12, including the three

controls). I constructed linear mixed models with bird ID as a random effect and infection success as a fixed effect, in analyses of body mass and fat score. I used t-tests to compare size-corrected liver and spleen mass of infected versus uninfected birds. All analyses were conducted in JMP 10, and all tests were two-tailed.

5.3 Results

5.3.1 Infection risk

Of 24 birds experimentally inoculated with *Plasmodium* spp., 15 (63%) became successfully infected. Bird origin and parasite origin interacted to influence infection risk (Table 5.2), such that birds were more likely to be infected by allopatric than by sympatric parasites (Fig. 5.2). Post-hoc testing revealed a significantly lower risk of infection by allopatric than sympatric parasites among eastern birds ($\chi^2_{1,10} = 5.82$, $p = 0.016$) but not western birds ($\chi^2_{1,10} = 0.45$, $p = 0.50$).

Table 5.2. Predictors of infection risk (defined as one or more observations of at least 0.04% parasitemia) for 24 song sparrows inoculated with sympatric or allopatric *Plasmodium* spp. Results reported are from a minimal adequate model, retaining only terms that significantly predicted infection status and main effects involved in a significant interaction.

Predictor	χ^2	*df*	*P*
Bird origin	1.23	1, 20	0.27
Parasite origin	1.23	1, 20	0.27
Bird origin × parasite origin	4.53	1, 20	0.033
Whole model	7.90	3, 20	0.048

Eliminated variables: sex, prior infection status.

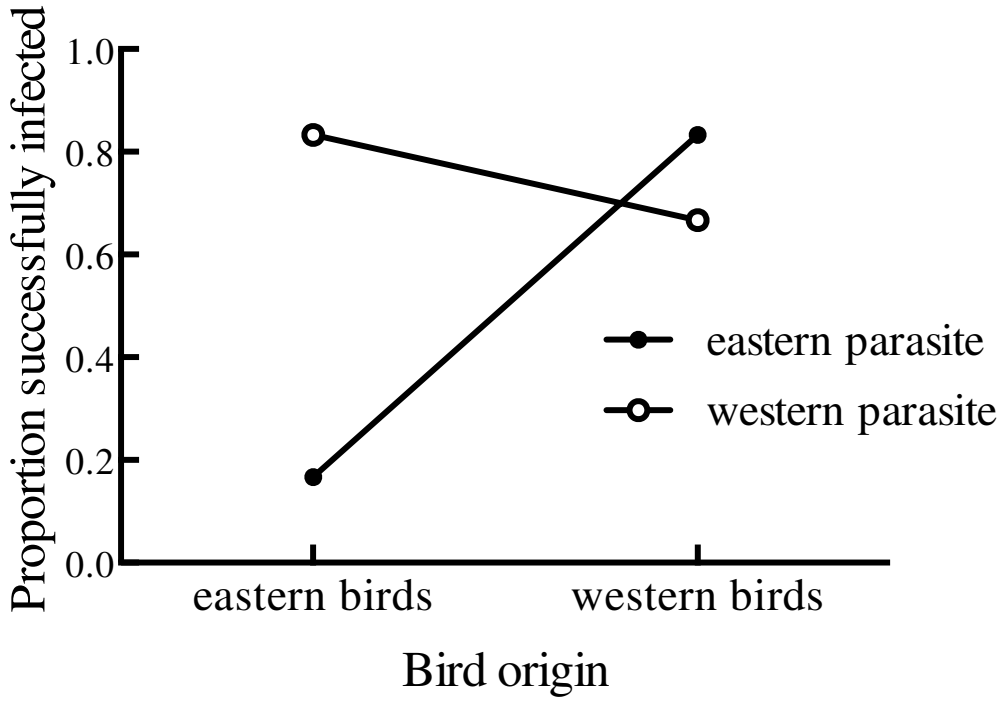

Figure 5.2. Proportion of song sparrows that became successfully infected (defined as one or more observations of at least 0.04% parasitemia) after inoculation with the eastern (P-SOSP9) or western (P-SOSP10) *Plasmodium* lineages. *N* = 24 (6 per group).

5.3.2 Infection severity

5.3.2.1 Parasite load

Figure 5.3 shows the time course of experimental infections. Maximum parasite load for infected birds was 31.1 ± 6.4 (average ± SE) infected erythrocytes per 10,000 examined. Average parasite load for these birds throughout the experiment was 7.9 ± 1.5 infected erythrocytes per 10,000 examined. By contrast, the maximum parasite load found in control birds was 2 infected erythrocytes per 10,000 examined (for two individuals determined through PCR screening to have been previously infected at the time of capture). For a third control bird that was uninfected at the time of capture, no infected erythrocytes were found at any time. All birds survived to the 30-day post-inoculation endpoint, by which time all parasite loads had returned to fewer than 0.04%.

Within the subset of 15 birds that became successfully infected, the only significant predictor of parasite load was experimental date ($F_{8,112} = 7.12$, $P < 0.0001$, Fig. 5.3A). I observed no main effect of bird origin, parasite origin, bird origin × parasite origin interaction, or sex on parasite load (all $P > 0.05$). Prior infection status did not significantly predict parasite load, although birds previously infected with *Plasmodium* spp. appeared to have somewhat higher peak loads than did previously-uninfected birds (Fig. 5.3B).

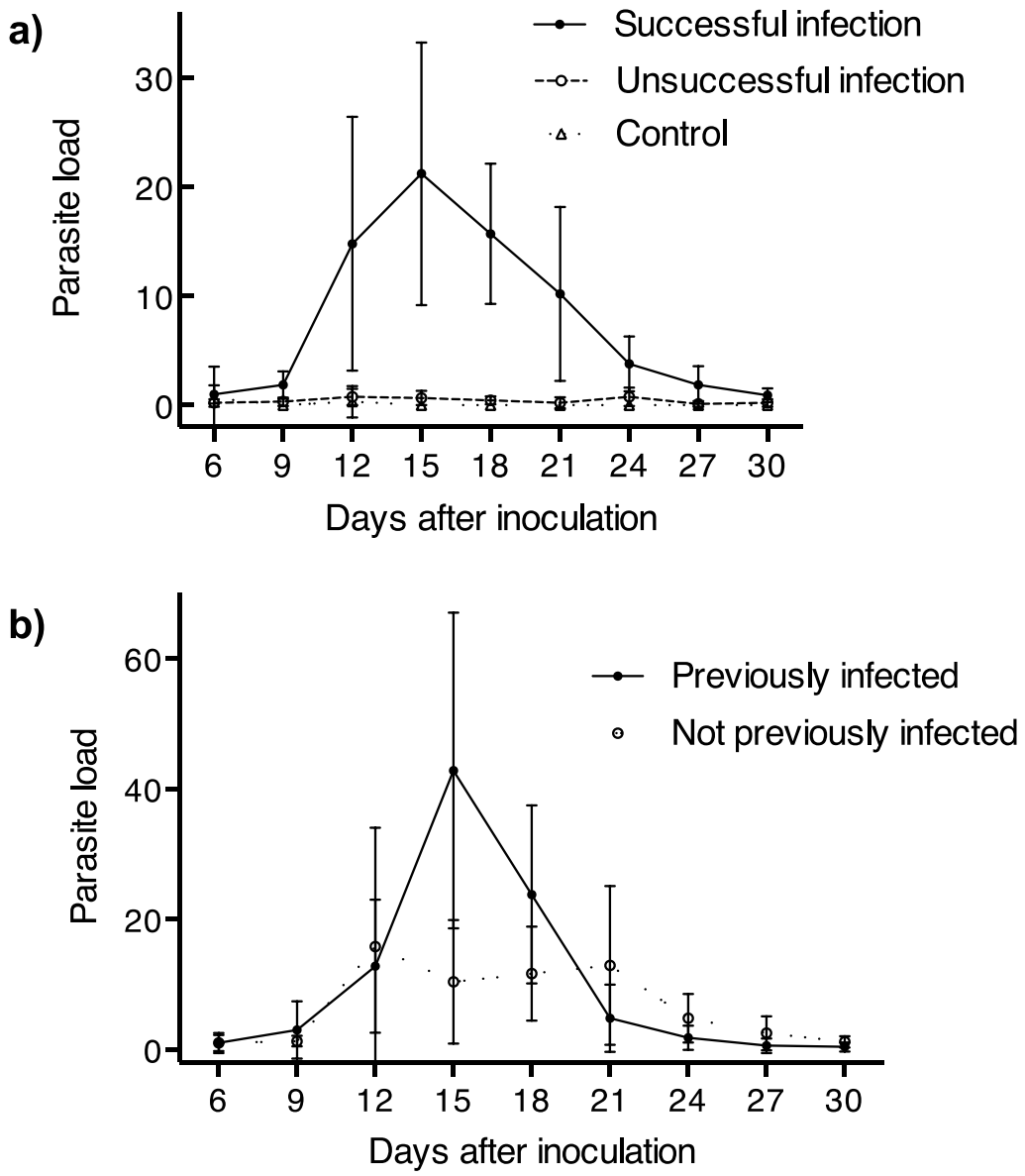

Figure 5.3. Parasite loads in song sparrows after experimental inoculation with *Plasmodium* spp. Values reported are mean (± 95% CI) # infected cells per 10,000 erythrocytes. (A) Parasite loads in birds categorized as successfully infected (one or more observations of at least 0.04% parasitemia after inoculation), unsuccessfully infected (inoculated but no observations of at least 0.04% parasitemia), or control (inoculated with uninfected blood or receiving no inoculation). (B) Parasite loads in the subset of birds categorized as successfully infected, separated by prior infection status with *Plasmodium*.

5.3.2.2 Body mass

Within the subset of birds that became successfully infected, the only significant predictor of body mass was experimental date ($F_{9,126} = 10.88$, $P < 0.0001$). Bird origin, parasite origin, bird origin × parasite origin interaction, sex and prior infection status were not significantly predictive of body mass (all $P > 0.05$) and were eliminated from the minimal adequate model.

5.3.2.3 Fat score

Within the subset of birds that became successfully infected, none of the predictors entered into the initial model (bird origin, parasite origin, bird origin × parasite origin interaction, sex, prior infection status, experimental date) significantly predicted fat score (all $P > 0.05$), thus none were retained in the minimal adequate model.

5.3.2.4 Comparison of infected vs. uninfected birds

Infected and uninfected birds (including the three controls) did not differ in mass ($F_{1,24.77} = 0.14$, p = 0.71), fat score ($F_{1,24.92} = 2.49$, p = 0.13), or size-corrected liver mass ($t_{25} = 1.07$, $P = 0.30$). However, size-corrected spleen mass was greater in infected than uninfected birds ($t_{25} = 2.07$, p = 0.049).

5.4 Discussion

I conducted reciprocal infection experiments to expose song sparrows to sympatric or allopatric lineages of *Plasmodium*. Host and parasite origin interacted to predict infection success, a pattern that is generally consistent with birds having a home-field advantage in defending against their sympatric parasites. However, the magnitude of this advantage appears to vary geographically: eastern, but not western, song sparrows were less susceptible to infection by sympatric than allopatric parasites. Evolutionary and ecological interactions with sympatric haematozoa may in some cases provide birds with a parasite-mediated home-field advantage, but the outcome of host-parasite interactions may not be constant over the landscape.

The vast majority of experimental infection studies have used plant or invertebrate hosts (Greischar & Koskella 2007). The minority of studies involving vertebrate hosts have yielded diverse findings. In some systems, parasites perform better on sympatric than allopatric hosts, for example in interactions between European minnows (*Phoxinus phoxinus*) and trematodes (Ballabeni & Ward 1993); Black-legged Kittiwakes (*Rissa tridactyla*) and ticks (McCoy *et al*. 2002); and clawed frogs (*Xenopus laevis*) and polystomatid parasites (Jackson & Tinsley 2005). In other systems, hosts are more resistant to infection by sympatric than allopatric parasites, as in Canarian lizards (*Gallotia galloti*) and their haemogregarine parasites (Oppliger *et al*. 1999), with similar findings in three-spined sticklebacks (*Gasterosteus aculeatus*) infected with trematodes (Kalbe & Kurtz 2006). Meanwhile, in interactions between great tits (*Parus major*) and parasitic fleas, neither hosts nor parasites appear to show local adaptation (Dufva 1996). One potential reason for the complex patterns observed across vertebrate hosts is that in

addition to evolutionary processes (*i.e.*, local arms races with sympatric parasite strains), ecological interactions (*i.e.*, acquired immune memory following exposure to sympatric parasite strains) likely also influence the outcome of host-parasite interactions.

Meta-analyses and theoretical simulations of evolutionary interactions between hosts and parasites identify relative dispersal ability as the key factor that determines whether parasites adapt to their local hosts or hosts to their local parasites (Gandon 2002; Greischar & Koskella 2007). Thus, if the typical dispersal distance for song sparrows exceeds that of *Plasmodium* spp., song sparrow populations may become locally adapted to sympatric *Plasmodium* strains. High adult philopatry in these birds (Potvin *et al*. 2015) suggests that most gene flow results from natal rather than adult dispersal. Natal dispersal distance varies among individuals and populations, but has been estimated at approximately 6 km in this species (Zink & Dittmann 1993). Dispersal of *Plasmodium* spp. occurs passively, through the movements of their invertebrate and vertebrate hosts. Mosquitoes are generally poor fliers (Verdonschot & Besse-Lototskaya 2014) and presumably contribute little to *Plasmodium* dispersal. By contrast, long-distance seasonal migrations of song sparrows and other birds may enhance dispersal distances of haematozoan parasites well beyond those of their hosts (Jourdain *et al*. 2007). In eastern song sparrows, for example, migratory distances of over 1000 km have been recorded (Brewer *et al*. 2006). However, if infected hosts are unable to migrate successfully (migratory culling: Bradley & Altizer 2005) then seasonal migration may contribute little to haematozoan dispersal, meaning that the dispersal capacity (and thus adaptive potential) of song sparrow populations could still exceed that of *Plasmodium*. If so, song sparrows may become locally adapted to sympatric *Plasmodium* strains, potentially

contributing to the observed home-field advantage of eastern birds in resisting infection by eastern parasites.

Cross-infection experiments represent a significant advance over comparing naturally-occurring parasite loads of dispersing versus philopatric individuals, because such experiments are not confounded by variation in individual quality. However, in hosts with the capacity for acquired immune training, reduced susceptibility to sympatric parasites might reflect either host populations being locally adapted to these parasites, and/or host individuals having previously encountered them. This is particularly true when using wild-caught subjects, because previous exposure history is not known. Thus, conclusively disentangling the relative contributions of evolutionary and ecological processes to home-field advantage is beyond the scope of this study. None of the experimental birds in my study were found to be naturally-infected with the experimental lineages or with other lineages within 5% sequence similarity, and previous infection by *P. relictum* does not appear to protect against infection by other *Plasmodium* morphospecies (Draper 1953). Similarly, in my study previous infection by *Plasmodium* spp. did not significantly reduce infection risk (Table 5.2). Nor were infections less intense in birds previously infected with *Plasmodium*; indeed, previously-infected birds tended to have higher peak parasite loads than did previously-uninfected birds (Fig. 5.3B). These observations undermine the role of prior immune experience in this study, and suggest that the home-field advantage I observed may reflect mainly adaptation to local parasites. However, in light of low sample sizes, as well as the use of only two parasite lineages, I do not rule out prior immune experience as a mechanism for generating home-field advantage. Moreover, both microscopy and PCR-based approaches

occasionally fail to detect existing infections (Valkiunas *et al*. 2008), so I cannot dismiss the possibility that some experimental birds might have previously encountered the experimental lineages or similar variants that could influence their resistance to the strains used in this experiment. Infectivity experiments conducted on wild-caught hosts permit distinguishing home-field advantage from differences in quality between philopatric and dispersing host individuals, but determining whether such advantage results from local adaptation and/or prior exposure will ultimately require inoculating immunologically naïve (*i.e.*, hand-reared) hosts.

The capture sites in this study represent two geographically distinct breeding locations, separated by a distance much greater than the typical natal dispersal distance for song sparrows. However, song sparrows at both locations are seasonally migratory, and the extent of population mixing at the wintering grounds or on migration is uncertain. Haematozoan parasites observed at the breeding grounds may thus have been acquired on the wintering grounds (Ricklefs *et al*. 2005). Although I cannot rule out a wintering-ground origin for the *Plasmodium* lineages used in this study, I note that avian *Plasmodium* can be transmitted as far north as Alaska, at latitudes up to 64 °N (Loiseau *et al*. 2012): thus my breeding-ground capture sites (43-44 °N) are well within the latitude at which *Plasmodium* can be transmitted. Moreover, I captured birds (including parasite donors) between July and October, several months after arrival to the breeding grounds, providing ample opportunity for donors to acquire local parasite lineages.

I did not confirm through PCR that successful infections represented the strains of interest. Thus, I cannot conclusively exclude the possibility that some of the observed infections represented a relapse of a previous infection (e.g., due to the stress of repeated

handling and blood-sampling) rather than a new, experimentally induced, infection. However, a control individual determined through PCR to be naturally-infected at the start of the experiment and subject to the same handling and blood collection regime showed no such relapse; microscopy revealed no parasites in this individual throughout the 30 day duration of the experiment. Still, even if PCR confirmation of the strains infecting experimental birds had been performed, molecular techniques may fail to amplify certain lineages and can thus underestimate the diversity of haematozoan infections within an individual host (Valkiunas *et al*. 2006, Zehtindjiev *et al*. 2012). Accordingly, I cannot dismiss the possibility that experimental birds may have been co-inoculated with other *Plasmodium* lineages in addition to the intended lineages. However, co-infections are frequently associated with very high parasite loads and increased mortality (Dimitrov *et al*. 2015), whereas I observed relatively low parasitemia (consistently below 1%) and no mortality even among successfully-infected birds. Thus I think it likely that the experimentally-induced infections represent the intended lineages, but alternative interpretations are possible.

Interestingly, the reduced infection risk associated with sympatric parasites was more pronounced for birds of eastern than western origin. Sampling effort was much higher at and around the eastern site than at the western site, thus while I am confident that P-SOSP10 appears to be absent from the eastern site, I cannot dismiss the possibility that P-SOSP9 may also be present at the western site. If so, western song sparrows may have coevolved with and/or previously encountered P-SOSP9 or similar strains, which may explain why these birds had similar risk of infection by their putatively allopatric lineage (P-SOSP9) as by their sympatric lineage (P-SOSP10). Alternatively, because

host-parasite interactions vary over space and time, this pattern could reflect local variation in the timing and outcome of antagonistic coevolutionary cycles. Moreover, the two experimental lineages appear to correspond to different morphospecies (Fig. 5.1) and may thus differ in virulence, host-specificity, or other factors likely to influence evolutionary arms races. Ecological differences between sites, such as abundance and encounter rates between hosts and parasites, may also contribute to the observed asymmetry of home-field advantage and help to explain why home-field advantage was more pronounced at the eastern site.

Whereas song sparrows from the eastern site were less likely to become infected by sympatric than allopatric parasites, in the subset of birds that became infected I observed no difference in the severity of sympatric versus allopatric infections. In white-crowned sparrows, different factors predict the likelihood that an individual will be infected versus the severity of infection should it occur (MacDougall-Shackleton *et al*. 2005), suggesting that these aspects of disease susceptibility may be somewhat independent. As well, one limitation of comparing infection severity is that the subset of host individuals that become infected by parasites are not necessarily representative of the wider population in terms of their immune function or condition. Finally, laboratory studies may be poorly suited to examine infection severity because captive conditions (e.g., handling stress and unrestricted access to food) do not reflect those experienced by free-living animals. Even under these benign captive conditions, relative spleen mass was greater in infected birds than their uninfected counterparts, suggesting a cost to infection. Such costs, particularly if they are intensified under natural conditions, may place a

selection pressure on birds favouring immune adaptations that improve resistance to the local malarial parasites.

My findings are relevant to the adaptive significance of dispersal, a key life history trait with implications for population connectivity. Studies of free-living animals often find that immigrants are in poor condition or have low fitness relative to philopatric individuals (e.g., Forero *et al*. 2002, MacDougall-Shackleton *et al*. 2002), but have not generally been able to distinguish between condition-dependent dispersal and home-field advantage. If sympatric parasites pose less of an infection risk than allopatric parasites, regardless of whether this pattern results from local adaptation and/or prior immune experience with the local parasites, home-field advantage may represent an important fitness benefit of philopatry.

In conclusion, song sparrows from the eastern site were less susceptible to infection by sympatric than allopatric malarial parasites, a pattern consistent with home-field advantage to the local parasite strains. This home-field advantage was not observed in birds from the western site, however, consistent with mosaic models of local arms races (Schulte *et al*. 2011). Key next steps in disentangling the relative contributions of local adaptation and prior immune experience include cross-infection experiments on hosts known to be immunologically naïve, such as birds captured as eggs and hand-raised, together with surveys of geographic variation at candidate immune loci such as MHC to identify locally protective alleles. Regardless of the relative contributions of local adaptation and previous immune experience, my findings suggest that parasites may impose fitness costs to dispersal and advantages to philopatry, and that free-living birds may be particularly vulnerable to unfamiliar parasites.

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Chapter 6

6 General Discussion

The overall objective of my dissertation was to investigate ecological and evolutionary interactions between hosts (song sparrows) and their bloodborne parasites. Specifically, I investigated these interactions within the context of local coevolutionary arms races and local adaptation. I asked whether host individuals of local origin enjoy a parasite-mediated "home field advantage" over their less philopatric counterparts, as predicted by the parasite-driven wedge hypothesis (Thornhill & Fincher 2013). My research involved both observational (Chapters 2, 3) and experimental (Chapters 4, 5) approaches, on both free-living (Chapters 2, 3, 4) and captive song sparrows (Chapter 5), at a variety of geographic scales throughout southern Ontario.

To test my prediction that song sparrows would show evidence of local adaptation to their bloodborne parasites, I investigated several complementary lines of evidence. In Chapter 2, I used genetic assignment tests to quantify naturally-occurring variation in natal dispersal at multiple breeding sites. I asked whether individuals of local origin had better body condition, lower parasite loads, and higher overwinter survivorship relative to less philopatric individuals breeding at the same site. I also investigated whether the relationship between philopatry and condition was consistent or variable over the landscape. In Chapter 3, I compared population genetic structuring in song sparrows to that of the birds' haematozoan parasites sampled over the same geographic scale, to investigate whether hosts (song sparrows) in this system show greater gene flow and potentially greater adaptive potential than do parasites. In this Chapter I also used

phylogenetic analysis to assess concordance in genetic structuring between song sparrows and haematozoa. In Chapter 4, I attempted to experimentally manipulate bloodborne parasitism in free-living male song sparrows in order to assess the effects of parasites on territorial defense. Finally, in Chapter 5, I used an experimental infectivity approach to determine whether song sparrows are more resistant to infection by sympatric than allopatric parasites.

6.1 Inferred philopatry and its relationship to condition, parasitism, and survivorship

In Chapter 2, I examined whether song sparrows of relatively local origin (as inferred by genetic assignment tests) had a home-field advantage (*i.e.*, better body condition, lower prevalence and intensity of haematozoan parasites, and greater overwinter survivorship) relative to individuals inferred to have immigrated from farther away. I evaluated the relationship between inferred philopatry and condition at multiple sites throughout southern Ontario, to see if any such "home-field" advantage was consistent across the landscape or conversely varied between sites.

I found some evidence for home-field advantage, in that individuals of local origin tended to have lower overall parasite loads (Figure 2.3). This suggests that song sparrows of local origin might have a home-field advantage over immigrants mediated by an ability to control infections with bloodborne parasite. However, this relationship was not statistically significant, and moreover, inferred philopatry was not a significant predictor of infection status, body condition, or overwinter return rates at the main study site. These results suggest that song sparrows of local origin might have a home-field

advantage over immigrants mediated by an ability to control infections with bloodborne parasites, but that the magnitude of the advantage may be relatively weak and moreover this advantage does not extend to other fitness-related traits.

These results are consistent with previous findings on song sparrows at the focal population that showed that philopatric males (as determined by high genetic similarity to the population) were in better physiological condition, including having lower bloodborne parasite loads, than immigrant males (less genetically similar to the population; Stewart & MacDougall-Shackleton 2008). Both findings suggest an immunological advantage of philopatric song sparrows in dealing with bloodborne parasites. These findings are also in line with results from two previous studies on birds (white-crowned sparrows and barn swallows), where philopatric individuals had lower parasite loads than immigrant individuals (MacDougall-Shackleton *et al*. 2002, Saino *et al*. 2014). These previous studies, as well as this present study, are consistent with host local adaptation to their parasites.

Contrary to what I would expect if individuals that disperse do so because they cannot compete for territories near their natal site, I found no main effect of inferred philopatry on skeletal size (assumed to reflect competitive ability). However, I found a near-significant interaction between inferred philopatry and capture site in predicting skeletal size: at some capture sites, individuals of local origin were relatively large, whereas at other sites, individuals of local origin were relatively small. As discussed in Chapter 2, this landscape variation in the relationship between skeletal size (and presumably competitive ability) and dispersal could reflect among-site variation in quality. My study design is an advance over previous studies that focused on a single

population, given that it allows me to measure the relationship between philopatry and performance at multiple sites. Thus, my study explicitly considers the possibility that home-field advantage may vary from site to site due to local variation in coevolutionary arms races (Thompson 2005).

6.2 Comparative population genetics of song sparrows and their haemosporidian parasites

In Chapter 3, I characterized the population genetic structure of song sparrows and compared it with the population genetic structure of its major bloodborne parasites (*Plasmodium* and *Leucocytozoon*) across southeastern Ontario. As discussed in Chapter 3, the prevalence of *Haemoproteus* spp. was too low to examine population genetic structuring. I found significant population genetic structure in song sparrows at this geographic scale, consistent with isolation by distance. I found contrasting patterns of genetic structuring in the *Plasmodium* and the Leucocytozoon parasite communities. I found no detectable genetic structuring in *Plasmodium* at the community level. Moreover, the prevalence of *Plasmodium* lineages varied significantly between years. Conversely, the *Leucocytozoon* parasite community had significant genetic structure, and prevalence of different lineages did not vary significantly between years.

Contrary to my original prediction and the expectations of the parasite-driven wedge hypothesis (Thornhill & Fincher 2013), population genetic structure of song sparrows was not correlated with that of either the *Plasmodium* or *Leucocytozoon* communities. However, song sparrow population genetic structure was borderline-

significantly associated with that of a monophyletic clade of *Plasmodium* lineages (Chapter 3).

The fact that the population genetic structure of song sparrows was not correlated with that of *Plasmodium* or *Leucocytozoon* significantly undermines the hypothesis of coevolution between song sparrows and their bloodborne parasites, instead suggesting that these parasites are relatively host-generalist and do not appear to be restricted by this particular host's gene flow.

6.3 Experimental administration of antiparasitics to assess haematozoan effects on territorial defense

In Chapter 4, my objective was to assess whether haemosporidian parasites negatively affected song sparrows' territorial defense behaviour: the parasite-driven wedge hypothesis implicitly assumes that parasites have detectable negative effects on host fitness components. As outlined in Chapter 4, conclusively establishing the effects of parasites on host fitness is best accomplished through an experimental approach. I used an antiparasitic drug in an attempt to lower bloodborne parasite load in free-living song sparrows, and later compared drug-treated and control males in their territorial responses to simulated territorial intrusions. Unfortunately, the drug failed to reduce infections. I found no difference in territorial defense behaviour between drug-treated and control males, but was thus unable to determine whether this lack of behavioural difference reflects parasites having no effect on this fitness component. Thus I could not conclusively determine whether haemosporidian parasites affect this aspect of male

fitness in song sparrows. In other avian species, however, haemosporidian parasites reduce reproductive success (Merino *et al*. 2000, Marzal *et al*. 2005, Knowles *et al*. 2010) and survival (Martinez-de la Puente *et al*. 2010, Asghar *et al*. 2015), so it seems possible that haematozoan parasites may represent an appreciable selection pressure on their song sparrow hosts.

6.4 Host resistance to sympatric versus allopatric parasites

In Chapter 5, I used a cross-infection experimental design to test a key component of the parasite-driven wedge hypothesis: specifically, whether song sparrows were better able to defend against infection by sympatric than allopatric parasites. Enhanced resistance to sympatric relative to allopatric parasites could reflect either host adaptation to the local parasite strains, and/or host individuals developing resistance to the local parasites through previous immune exposure. Conversely, if parasites exhibit local adaptation to their hosts in this system, song sparrows should be more resistant to allopatric than to sympatric parasite lineages. I used data from Chapter 2 to identify two apparently locally-confined *Plasmodium* lineages, one from eastern Ontario and the other from western Ontario. I captured birds from eastern and western Ontario, and inoculated them with *Plasmodium* cultured either from their capture site (sympatric) or from the other site (allopatric).

In the subset of birds that became infected, I found no difference in the severity of allopatric versus sympatric infections. However, infection risk was lower for birds

exposed to sympatric than to allopatric *Plasmodium*, suggesting that song sparrows may have a home-field advantage in defending against local parasite strains. This pattern was observed at one capture site (eastern Ontario) but not at the other (western Ontario), consistent with mosaic models of host-parasite interactions.

Home-field advantage may arise from evolutionary processes, whereby host populations become adapted to their local parasites, and/or from ecological interactions, whereby host individuals develop resistance to the local parasites through previous immune exposure. Distinguishing between these possibilities could be accomplished by conducting similar infection experiments on hand-raised birds, ideally captured and transported to a vector-free environment prior to hatch to rule out previous immune experience with local parasite strains. Regardless of the relative importance of local adaptation and prior immune experience, though, my findings suggest that greater susceptibility to (allopatric) parasites may be a fitness consequence of natal dispersal. If so, natural populations may be particularly vulnerable to novel and invasive parasites.

6.5 Which Haemosporidian genera could song sparrows be adapted to?

Taken together, the findings of my dissertation suggest that bloodborne parasites may mediate at least some of the fitness consequences of dispersal by their song sparrow hosts, although the magnitude of these effects may vary over the landscape. However, the three Haemosporidian genera found to infect song sparrows (*Haemoproteus*,

Leucocytozoon and *Plasmodium*) differ in their life histories and ecologies, and may not all have consistent effects on the evolutionary trajectories of their hosts.

The proportion of song sparrows infected by *Haemoproteus* spp. was particularly low (2%, Chapter 3), suggesting that this parasite may not represent a strong selection pressure on song sparrows. Conversely, *Haemoproteus* parasites could be an important selection pressure on song sparrows despite low prevalence, if *Haemoproteus* infections are so virulent that song sparrows that become infected die before they are sampled. *Haemoproteus* is very prevalent (e.g. up to 100% prevalence in 331 individuals of wild dove *Zenaida auriculata* in Brazil; Adriano & Cordeiro 2001) in other avian species (Bensch & Akesson 2003, Bensch *et al*. 2000, Bensch *et al*. 2007, Fallon *et al*. 2003, Fallon *et al*. 2005, Olsson-Pons *et al*. 2015), so it is notable that prevalence and diversity of these parasites were so low in song sparrows. Other studies have found evidence of *Haemoproteus* lineages being restricted to taxonomically similar hosts, suggesting that this genus is highly affected by its host's genetics (Beadell *et al*. 2004, Ishtiaq *et al*. 2007, Martınez-de la Puente *et al*. 2011, Olsson-Pons *et al*. 2015, Waldenström *et al*. 2002). For example, *Haemoproteus* infections are very rare among chiffchaffs (*Phylloscopus collybita*) breeding in northern Sweden, even though the closely related and sympatric willow warbler (*P. trochilus*) is commonly infected by *Haemoproteus* parasites (Bensch & Akesson 2003).

Leucocytozoon was the most commonly observed haematozoan parasite infecting song sparrows (20%, Chapter 3), making this genus a good candidate for potentially influencing host (song sparrow) evolution and the fitness consequences of dispersal. Moreover, *Leucocytozoon* lineages had significant community genetic structuring, and

tended to be isolated by distance, a pattern similar to that seen in song sparrows (Chapter 3). *Leucocytozoon* lineage frequencies also remained reasonably stable over time (Chapter 3). However, population genetic structure of song sparrows did not correspond to that found in the *Leucocytozoon* community at the same geographic scale, undermining the idea that coevolution has occurred between song sparrows and *Leucocytozoon* parasites.

The haematozoan genus *Plasmodium* was reasonably prevalent in song sparrows (14.2%, Chapter 3), tends to be relatively harmful at least in other avian species (Dimitrov *et al*. 2015, Palinauskas *et al*. 2011, Zehtindjiev *et al*. 2008) and may thus represent an important selection pressure on song sparrows. Consistent with this idea and with the general expectations of the parasite-driven wedge hypothesis (Thornhill & Fincher 2013), the population genetic structure of a focal *Plasmodium* morphospecies tended to be concordant with that of song sparrows (Chapter 3). However, it should be noted that the community genetic structure of *Plasmodium* (*i.e.*, all lineages combined) was not concordant with song sparrow population genetic structuring. (Chapter 3) Moreover, the relative frequency of different *Plasmodium* lineages varied significantly from year to year, suggesting they might not represent a constant selection pressure on their avian hosts (Chapter 3). Still, I discovered that song sparrows were better able to resist sympatric than allopatric *Plasmodium* infections (Chapter 5), suggesting that this genus may influence the relationship between host dispersal and fitness.

6.6 Final remarks

Despite widespread and growing concern over migratory birds as reservoirs of infectious disease, my research represents one of the first attempts to address local adaptation in birds and their parasites. Most studies of local adaptation in parasite-host systems have been done using either plants or invertebrates as hosts (Greischar & Koskella 2007), with only a small minority of studies using vertebrate hosts. Studies like this are important from the perspective of conservation and infectious disease, since they may allow predicting whether naïve hosts are likely to be vulnerable to novel parasites. This is particularly true now that climate change and anthropogenic forces are expanding vector ranges (Garamszegi 2011, Rosenthal 2009) or introducing invasive species carrying unfamiliar parasites to novel areas (Mack *et al*. 2000, Taraschewski 2006). The host-switching nature of haematozoan parasites infecting song sparrows (Chapter 3) further emphasizes the importance of monitoring these parasites from the perspective of emerging infectious diseases.

Finally, another important contribution of my dissertation is that it is among the first descriptions of the distributions, prevalence and diversity of Haemosporidian parasites in Canada (Svensson *et al*. 2007). Interestingly, no *Leucocytozoon* lineages had previously been reported in song sparrows, or in Canada, before this study. There is an intrinsic value in studying parasites unique diversity as important components of natural ecosystems, as well as from the perspective of predicting threats to host populations.

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Appendices

Appendix A: Animal Use Protocol 2008

AUP Number: 2008-054
PI Name: MacDougall-Shackleton, Elizabeth
AUP Title: Mating Signals, Gene Flow and Disease Resistance in Songbirds

Dear Dr. **MacDougall-Shackleton:**
Your Animal Use Protocol form entitled "Mating Signals, Gene Flow and Disease Resistance in Songbirds" has had its yearly renewal approved by the Animal Use Subcommittee.
This approval is valid from **June 1, 2009 to May 31, 2010**

The protocol number for this project remains as **2008-054**

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received. If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar
with the contents of this document.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

c.c. Approved Renewal - E. MacDougall-Shackleton, W. Lagerwerf

Appendix B: Animal Use Protocol 2011

AUP Number: 2011-084
PI Name: MacDougall-Shackleton, Elizabeth
AUP Title: Experimental Inoculation Of Songbirds With Local Versus Nonlocal Bloodborne Parasites

Official Notice of Animal Use Subcommittee (AUS) Approval: Your new Animal Use Protocol (AUP) entitled "Experimental Inoculation Of Songbirds With Local Versus Nonlocal Bloodborne Parasites" has been APPROVED by the Animal Use Subcommittee of the University Council on Animal Care. This approval, although valid for four years, is subject to annual Protocol Renewal.2011-084::1

1. This AUP number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this AUP number.
3. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Copeman, Laura
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University Council on Animal Care

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Sarquis-Adamson, Y. & MacDougall-Shackleton, E.A. (Submitted) Song sparrows (*Melospiza melodia*) have a home-field advantage in defending against sympatric malarial parasites. Ibis.

Kelly, T. R., MacGillivray, H. L., Sarquis-Adamson, Y., MacDougall-Shackleton, S.A., MacDougall-Shackleton, E. A. (Submitted) Seasonal migration distance varies with natal dispersal and predicts parasitic infection in song sparrows (*Melospiza melodia*). American Naturalist.

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Kelly, T., MacGillivray, H.L., Watson, M., Sarquis-Adamson, Y., Hobson, K., MacDougall-Shackleton, S.A. & MacDougall-Shackleton, E.A. 2016. Seasonal migration distance varies with natal dispersal and predicts parasitic infection in song sparrows (*Melospiza melodia*). The Society for Integrative & Comparative Biology (SICB), Portland (OR), USA. (Oral presentation)

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Sarquis-Adamson, Y. & MacDougall-Shackleton, E.A. 2012. Are song sparrows affected by avian malaria? Field experimental injections with an anti-parasitic drug. Evolution Conference. Ottawa (ON), Canada. (Poster)

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