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Population Genetic Structure And Parasite Communities In A Nomadic Songbird, The Red Crossbill (Loxia Curvirostra)

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Abstract

Although much speciation occurs in allopatry, populations with overlapping geographic ranges may also experience reduced gene flow due to ecological differences. Parasites are an important feature of the biotic environment, and place important selective pressures on their hosts, potentially reducing gene flow among geographically separated host populations. However, virtually nothing is known about host-parasite interactions in systems where hosts have nomadic distributions, and where ecologically distinct populations exist in sympathy. I examined population genetic structuring and characterized bloodborne parasite communities across four ecologically distinct, but partially sympatric, “vocal types” of nomadic red crossbills (*Loxia curvirostra*) sampled at multiple sites. I found evidence for isolation by distance in one vocal type, but vocal types were not genetically differentiated from one another, nor were they characterized by significantly different parasite communities. Despite differences in foraging ecology, crossbill vocal types do not appear to be incipient species or subject to different parasite communities.

Keywords

Parasite-host interactions, avian haematozoa, bloodborne parasites, red crossbill, nomadic, population genetic structure, ecological speciation
Co-Authorship Statement

My supervisor, Beth MacDougall-Shackleton, and my collaborators, Dr. Jamie Cornelius (Eastern Michigan University) and Dr. Tom Hahn (University of California, Davis) will be co-authors on any publications resulting from this thesis. Dr. MacDougall-Shackleton contributed to the development of ideas and data analysis in this thesis, and funded and oversaw my genetic analyses. Dr. Cornelius and Dr. Hahn conducted the fieldwork to collect all the blood samples used in this study; Dr. Cornelius conducted the blood-smear microscopic analysis of infection status, under the supervision of Dr. Hahn.
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List of Abbreviations

CC: Northern California Coast

CR: Columbia River

DNA: Deoxyribonucleic acid

LB: Lysogeny broth

EDTA: Ethylenediaminetetraacetic acid

OP: Olympic Peninsula

PCR: Polymerase Chain Reaction

SOC: Super optimal broth containing glucose

TE: Tris-HCL EDTA buffer

TM: Teton Mountain

WM: Warner Mountain
Chapter 1

1 General Introduction

1.1 Speciation and ecological speciation

Biological diversity is generated through speciation events, whereby one lineage diverges into two or more distinct lineages that are reproductively isolated (Mayr, 1947). Many speciation events result from a single population becoming geographically subdivided, due to distance or other physical barriers that prevent gene flow from occurring; this is referred to as allopatric speciation. However, even when populations have overlapping geographic ranges and thus are not physically prevented from gene flow, divergence may still occur; this is referred to as sympatric speciation. In particular, divergent ecological conditions can induce disruptive selection on various traits, generating reduced fitness of hybrid offspring. This may favour the evolution of prezygotic isolation mechanisms, and ultimately result in reproductive isolation (Schluter, 2001; Hatfield et al., 1999). The selective environment to which populations adapt includes both abiotic features (e.g., weather patterns, geographical features) and biotic features (e.g., interactions between predators and prey or hosts and parasites). Even when two populations are sympatric in distribution, differences in their ecological niches may lead to different selection pressures, which can drive the fixation of different advantageous alleles in each ecological niche and eventually lead to the formation of new species (Mayr, 1947).

Despite considerable interest in ecological speciation, there are relatively few established examples of ecological speciation in wild populations. One well-known example was documented in populations of Darwin’s finches (Geospiza spp.) that underwent a rapid period of divergence in response to fluctuations in climate and associated food availability (Grant & Grant, 1993). A strong El Niño event reduced the abundance of large-sized seeds and ultimately favoured the evolution of divergent beak sizes and morphologies. Hybridization events between populations of different beak morphologies were only recorded when strong selective pressures had relaxed, after food supplies had returned to pre-El Niño levels (Grant & Grant, 1993). Another classic example of ecological
speciation involves limnetic and benthic morphs of three-spined sticklebacks (Gasterosteus aculeatus). Although both forms of stickleback occupy the same freshwater lake, an individual’s location in the water column may predict its morphology and diet composition (Hatfield et al., 1999). Hybrid offspring are intermediate in phenotype and appear not to be well suited to either the benthic or the limnetic niche (Hatfield et al., 1999). Disruptive selection, i.e., selection against hybrids, in this system appears to be consistent over time, and perhaps as a result, there is evidence of strong premating isolation between different morphs (Nagel & Schluter, 1998). Larger-bodied benthic females preferred benthic males, and smaller-bodied limnetic females preferentially mated with limnetic males (Nagel & Schluter, 1998). Both of these examples emphasize the potential importance of ecological factors in generating reproductive isolation in wild populations.

1.2 An overview of parasite-host interactions

For many species, parasites represent an important part of their biotic environment. Parasites comprise approximately 40% of all known species (Dobson et al., 2008) and can have important negative effects on host fitness. These effects include reduced expression of secondary sexual traits (e.g., in blackbirds Turdus merula (Biard et al., 2010)), reduced reproductive success (e.g., in male aquatic warblers Acrocephalus paludicola (Dyracz et al., 2005)), and reduced survivorship (e.g., in Pacific treefrogs Hyla regilla (Johnson et al., 1999)). Beyond these individual-level effects, parasites can also affect the evolutionary trajectories of host populations. For example, the persistence of sex has been attributed to host-parasite interactions, in that asexual host lineages are selected against when exposed to coevolving parasites (Hamilton, 1980; Lively, 1987). The strength of sexual selection can also be influenced by parasite-host dynamics, ultimately affecting the types of sexual ornaments favoured (Hamilton & Zuk, 1982). Moreover, parasite-mediated effects on host evolution have been proposed as an important causal mechanism behind latitudinal patterns of host biodiversity (Fincher & Thornhill, 2008). In general, parasite abundance and diversity increases when approaching the tropics. Fincher and Thornhill (2008) propose that distant outbreeding is maladaptive in areas where parasite diversity is high, in that it may lead to the loss of locally adaptive immune responses and
to a higher risk of encountering novel parasites. They suggest genetic diversity between nearby populations might be greater in areas with high parasite levels, and ultimately drive host diversification.

Just as parasites may affect host evolution, hosts may also affect the evolutionary trajectories of their parasites. In particular, selection on parasites favours the spread of adaptations allowing them to successfully infect and proliferate within hosts (Krüger & Kolss, 2013). The resulting co-evolutionary arms race between hosts and parasites can influence rates of parasite speciation and extinction (Krüger et al., 2009). For example, speciation by hosts may result in a corresponding pattern of speciation by parasites (co-speciation), such that phylogenies of parasites correspond to those of their respective hosts (e.g., simian foamy virus and Old World primates; Switzer et al., 2005). However, the evolutionary result of host-parasite interactions depends on the ecologies of hosts, parasites, and in the case of vector-borne parasites, that of the vector. Factors including relative migration rates of hosts and their parasites (Gandon & Michalakis, 2002), parasite virulence, and the degree to which parasites are host-specific (Gandon, 2002) may all influence host-parasite interactions.

Most studies exploring host-parasite dynamics have done so in the context of host species with predictable geographic ranges (Cornuault et al., 2013), and in host species that either do not migrate or species in which migratory patterns are predictable and well-characterized. Host migratory behaviour can influence evolutionary relationships between hosts and parasites; resident species show stronger evidence of coevolution with their parasites, whereas migratory species harbour a greater diversity of parasites and generally do not show co-evolutionary patterns with parasites (e.g., in 97 parasite lineages and 53 host bird species; Jenkins et al., 2011). Parasites vary geographically, and migratory hosts are exposed to a greater range of parasites along their migratory routes. The diversity of parasites migratory hosts encounter may allow for more host-switching events to occur, and may ultimately discourage strong patterns of coevolution (Thompson, 2005). However, little is known about host-parasite dynamics in host species with unpredictable migratory patterns, making it important to characterize these relationships in avian hosts with such unusual migratory behaviour.
1.3 Nomadic migrants

Migration has evolved independently in many taxonomic groups (e.g., in the monarch butterfly *Danaus plexippus* (Urquhart & Urquhart, 1977); loggerhead turtle *Caretta caretta* (Bowen *et al*., 1995); and caribou *Rangifer tarandus* (Ferguson & Elkie, 2004)). Many animals migrate seasonally to track changing ecological conditions needed for survival and reproduction, such as in temperature and food abundance (Alerstam *et al*., 2003), and in most cases these movements are highly predictable. However, in some cases individuals migrate unpredictably, and may vary the geographic location of reproductive events among-years; such species are known as nomadic migrants (Jonzén *et al*., 2011). Nomadic migration is thought to have evolved in response to unpredictable resources that vary in space and time. To exploit these resources, some species move long distances in search of resource-dense ‘patches’ (Cornelius *et al*., 2013). Nomadic migrants remain in an area until the food source has been exploited, and then travel to another area to locate an alternate food source.

Nomadism is taxonomically widespread, and has been documented in invertebrates, such as army ants (*Neivamyrmex nigrescens*; Mirenda & Topoff, 1980), and vertebrates, such as grey-headed flying-foxes (*Pteropus poliocephalus*; Eby *et al*., 1999). Nomadic species (e.g., Eurasian siskins (*Carduelis spinus*)) may maintain high mobility even in winter, and within a single breeding season (Senar *et al*., 1992). These species may also time their breeding with the availability of unpredictable resource abundances, such that timing of breeding may be less constrained by season and more responsive to environmental changes (Hahn, 1998). These are known as opportunistic breeding strategies (Anderson, 1980), and they provide flexibility in response to local resources.

Nomadic migrants present an interesting system in which to study host-parasite interactions because the hosts themselves may be unpredictably distributed though space and time (Valkiūnas, 2005). The irruptive movements of the nomadic hosts may reduce the opportunity for co-evolutionary arms races to occur between parasites and their host (Jenkins *et al*., 2011).
1.4 Red crossbills

Red crossbills (*Loxia curvirostra*) are nomadic songbirds distributed across the Holarctic. In North America their range extends from southern Yukon to southern Mexico, and spans from the coast of British Columbia east to Newfoundland (Groth, 1988). Red crossbills are irruptive migrants; it is difficult to predict the timing of their movements, although they are thought to track the fluctuating abundance of conifer cones, their primary food source. Crossbills travel in large flocks in search of nutrient-rich conifer patches, and can move upwards of 3000 km per year to do so (Newton, 2006). Although several subspecies of red crossbill are currently recognized (Adkisson, 1996), unlike most subspecies of bird, the subspecies do not correspond to strict and non-overlapping geographic ranges.

Ten different crossbill vocal types have been described in North America (Groth, 1993), each primarily associated with a different conifer species. This dietary exclusivity has facilitated a predator-prey arms race between crossbills and their conifer food source (Benkman, 2003). The close association crossbills have with their food source is reflected in their beak morphology; distinctive crossed mandibles are thought to be an adaptive tool used to extract conifer seeds (Summers & Broome, 2012). Indeed, beak morphology can be used to predict the predominant food source that crossbill vocal types exploit (Benkman, 2003).

Body size, wing size, and vocalizations also differ among crossbill vocal types, indicating that vocal types are ecologically and behaviourally isolated (Groth, 1993). For example, each vocal type is characterized by a distinctive flocking call, which is thought to unite flocks and signal to conspecifics the initiation of movement to new food patches. Moreover, female crossbills prefer males from their same vocal type (Snowberg & Benkman, 2007). Evidence of assortative mating within vocal types, along with ecological and behavioural differences between populations suggests that crossbill vocal types are genetically isolated. Indeed, recent genetic studies suggest that crossbill vocal types are genetically differentiated (Parchman et al., 2006), although it should be noted that these findings are based on low sample sizes (vocal type 2; n= 41, type 3; n=10, type 4; n=9 and type 5; n=13). Little is known about how other aspects of crossbill ecology, such as interactions with parasites, may differ among vocal types. Their distinctive
breeding phenology, nomadic migratory behavior, and inter-population variation in behaviour and ecology make red crossbills an exciting system in which to study host-parasite interactions.

1.5 Avian bloodborne parasites

Approximately 68% of bird species are infected by haemosporidian parasites (Atkinson & van Riper, 1991), which are unicellular Apicomplexa that are transmitted between avian host individuals by blood-sucking dipteran insect vectors. Evolutionary relationships within this group have been resolved based on their mitochondrial cytochrome b sequences (Hellgren et al., 2008), indicating that avian Haemosporidia include three genera: *Leucocytozoon*, and sister taxa *Plasmodium* and *Haemoproteus*.

Despite their well-established evolutionary relationships, the ecology of these parasites varies among and within genera. *Plasmodium* and *Haemoproteus* are generally thought to be less host-specific than *Leucocytozoon* (Bensch et al., 2000). However, new molecular techniques have revealed greater diversity and host specificity in these parasite lineages than previously thought (Bensch et al., 2004). Host specificity may vary within each genus; thus, caution should be used when making generalizations across genera.

Moreover, the life cycles of Haemosporidians are complex and involve different hosts, different forms of reproduction, and multiple morphological stages. As obligate parasites they require two groups of hosts to complete their life cycles: vertebrates (birds), and invertebrates (dipteran vectors).

Within the three Haemosporidian genera, the life cycles of *Plasmodium* spp. have been better characterized due to parallels with *Plasmodium* species that infect humans, and successful inoculation of vectors in laboratory settings (Atkinson & van Riper, 1991). In brief, transmission occurs when a dipteran vector consumes a blood meal from an infected avian host. The parasite reproduces in the midgut of the dipteran before travelling to the salivary gland. The avian source of the next blood meal becomes infected, where the parasites undergoes intracellular growth in tissues and gametocytes mature in the red blood cells until being consumed by another blood-feeding dipteran vector (Atkinson & van Riper, 1991).
Haemosporidian parasites differ in the invertebrate vectors by which they are transmitted. *Haemoproteus* species are transmitted by louse flies (family Hippoboscidae; Kettle, 1982) and biting midges (family Ceratopogonidae; Wirth *et al*., 1974), and *Plasmodium* by mosquitoes (family Culicidae; Atkinson & van Riper, 1991). *Leucocytozoon* species are transmitted by blackflies (family Simuliidae; Rickels *et al*., 2004). The ecologies of vectors are important to consider when determining the host-parasite interactions between birds and their bloodborne parasites.

Louse flies are globally distributed, and are associated with bird populations across their entire range. In North America, adult louse flies are most abundant in July and August, and overall peak prevalence occurs in August and September (Corbet, 1956). Variation in louse fly peak prevalence can be determined, in part, by variation in average minimum temperatures and precipitation. There is little information on factors that influence abundance or avian host-selection by biting midges in the wild. Recent evidence from pied flycatchers (*Ficedula hypoleuca*) suggests that increased nest temperature and humidity are associated with increased biting midge abundance (Martínez-de la Puente *et al*., 2010).

The majority of mosquito species implicated as avian *Plasmodium* vectors belong to the genera *Culex*, *Aedes*, and *Culiesta* (Atkinson & van Riper, 1991). Mosquitoes search for suitable hosts and oviposition sites in similar ways. Both behaviours rely on the integration of many chemical and physical cues at long and short ranges (Bentley & Day, 1989). Host and oviposition site specificity vary across mosquito species; some species are selective (*e.g*., black-tailed mosquito (*Culiseta melanura*) prefer avian hosts), while others are opportunistic (*e.g*., *Culex nigripalpus*). Habitat preference of host-seeking female mosquitoes is influenced by small-scale (< 200 m) differences in vegetation, and may differ among mosquito species (O’Brien & Reiskind, 2013). Some species prefer open grassland (*e.g*., *Culex quinquefasciatus*), or dense woodlands (*e.g*., *Aedes albopictus*, *Culex erraticus*), whereas some species show even distributions across open and woodland vegetation types (*e.g*., *Aedes vexans*).
Habitat selection by blackflies is largely determined by water flow (current). Blackfly larvae attach to substrate submerged near the upper surface of the stream, and are influenced by changes in water flow (Mellor et al., 2000). This is, in part, due to the feeding strategy and diet of blackfly larvae. A streamline flow maintained at a strong current is needed for filter-feeding diatoms, desmids, and plant material (Mellor et al., 2000). Host selection in blackflies may be influenced by host condition. Nestling weight predicted the presence/absence of blackflies in pied flycatcher nests (Martínez-de la Puente et al., 2010); blackflies were associated with nests that housed heavier nestlings. Timing of breeding may also influence host selection. In blue tits (Cyanistes caeruleus), blackfly abundance increased in nests with later hatching dates (Tomás et al., 2008). Although it is difficult to determine the extent to which vectors influence host-parasite interactions in avian haematozoa, the ecologies of the vectors, parasites, and hosts all influence the outcome of these dynamics.

1.6 Research rationale

Despite the potential importance of ecological factors, such as selection imposed by different parasite communities, in shaping evolutionary trajectories of wild populations (Mayr, 1942; 1947; Schluter, 2001), relatively little is known about how these processes play out in nomadic and irruptive migrants. Even less is known about the evolutionary interactions these species may have with their parasites. Ranges of vectors and parasites are projected to shift and expand as a result of changing climate, making it increasingly important to understand host-parasite interactions. In this thesis I characterize the population genetic structure of a nomadic migrant, the red crossbill (Loxia curvirostra), and that of their bloodborne parasites.

1.7 Hypotheses and predictions

In chapter 2 of this thesis I examine the population genetic structure of four red crossbill vocal types (Types 2,3,4 and 5; Groth, 1993), caught throughout the western United States (Cornelius & Hahn, 2012). Red crossbill vocal types are ecologically and behaviourally isolated, and therefore I predicted that different vocal types should be genetically distinct, even where they occur in sympatry. As a secondary objective, I
investigated population genetic structure of red crossbills from vocal type 3, specifically the degree to which isolation by distance occurs within this vocal type. Red crossbills are nomadic, and because crossbills of the same vocal type are presumably similar in behaviour and ecology, I predicted that red crossbills of vocal type 3 would not show genetic isolation by distance.

In chapter 3, I examine the bloodborne parasite communities of those same four red crossbill vocal types using genetic techniques. My primary objective was to determine whether different vocal types are subject to different parasite communities. Red crossbill vocal types are ecologically distinct (Benkman, 2003), and therefore I hypothesized that different vocal types would be subject to different haematozoan parasite communities and that geographic distance and ecological differences are important in predicting parasite community similarity among red crossbill vocal types. I predicted that red crossbills of the same vocal type would have similar parasite communities despite the geographic distances (up to 1200 km) between trapping locations. Finally, in chapter 4 of this thesis I synthesize the findings from chapters 2 and 3 in a general discussion.
1.8 References


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2 Population genetic structure of red crossbills (*Loxia curvirostra*)

2.1 Introduction

Ecological factors can produce divergent selection on populations, with the result that different populations will have different adaptive traits in part because they exploit different resources (Mayr, 1947). If divergent selection persists through time, it can diversify populations and lead to the formation of new species, in some cases even when populations have overlapping (sympatric) geographic ranges (Schluter, 2001). Ecological isolation has been hypothesized to contribute to reproductive isolation and may thus be associated with population genetic structure (Knox, 1992). However, it remains unclear if ecologically-isolated populations are always genetically-isolated as well (Hairston *et al*., 2005).

Red crossbills (*Loxia curvirostra*) are polytypic songbirds found throughout most of the Holarctic. In North America their range extends from southern Yukon to southern Mexico, and from coastal British Columbia east to Newfoundland (Groth, 1988). Unlike most migratory birds, which make predictable seasonal migrations and return to particular areas, crossbills make irruptive nomadic movements driven by the unpredictable nature of conifer cone production (Adkisson, 1996). Seeds derived from conifer cones are their primary food source, and different populations of crossbills are predictably associated with different conifer species. This dietary exclusivity has set the stage for a predator-prey arms race between crossbills and local conifer species, such that divergent selection has given rise to ecologically-isolated crossbill vocal types (Benkman, 2003; Edelaar *et al*., 2012; Groth, 1988).

Crossbill vocal types can be differentiated by beak size and curvature, and in some cases by body size (Groth, 1993). Vocal types differ in their learned flight calls, with as many as ten vocal types described across North America (Groth, 1993). Vocal types remain stable after fledging and throughout the birds’ lifetime, suggesting that these vocalizations may be a cultural isolating mechanism (Sewall, 2010). Indeed, although different vocal types can occur in sympatry, females prefer to mate with males of the same vocal type.
Previous work on crossbill vocal types has revealed some evidence for genetic
differentiation among eight vocal types (types 1, 2, 3, 4, 5, 6, 7, and 9; Parchman et al.,
2006), suggesting incipient ecological speciation. However, that study included relatively
small sample sizes (9–41 individuals of each vocal type). Studies with a greater
sampling effort are needed to resolve the genetic structure of red crossbill vocal types.

Ecological differences among crossbill vocal types can be predicted by the primary
conifer species they are associated with. Type 2 crossbills feed exclusively on ponderosa
pine (Pinus ponderosa), which is associated with a mountainous environment. Type 3
crossbills feed on hemlock (Tsuga spp.), which is adapted to moist temperate areas where
water stress is minimal (Taylor, 1993). Type 4 crossbills are associated with Douglas fir
(Pseudotsuga menziesii), which can be morphologically and ecologically diverse, and
tolerant of dry habitats (Rehfeldt, 1989). Type 5 crossbills feed exclusively on lodgepole
pine (Pinus contorta), which is limited to upper montane to subalpine regions. Ecological
and behavioural differences between these vocal types, together with their nomadic
distribution, make red crossbills an exciting and novel system in which to explore
population genetic structuring and the potential for incipient ecological speciation.

My primary objective was to determine whether a selection of red crossbill vocal types
from western North America are genetically distinct. I hypothesized that vocal types are
ecological and behaviourally isolated, and therefore predicted that different vocal types
should be genetically distinct (characterized by different microsatellite allele frequencies),
even where they occur in sympathy. As a secondary objective, I also investigated
population genetic structure of red crossbills from vocal type 3, specifically the degree to
which isolation by distance occurs within this vocal type. Little is known about vocal
type 3 crossbills (Groth 1993), except that they are among the smallest vocal type and are
adapted to feed on hemlock species. Due to the nomadism of crossbills in general, and
because crossbills of the same vocal type are presumably similar in behaviour and
ecology, I predicted that red crossbills of vocal type 3 would not show genetic isolation
by distance.
2.2 Methods

2.2.1 Study animals and sampling

I extracted DNA from blood samples previously collected from 640 red crossbills, captured at 21 locations separated by up to 1177 km throughout Washington, Oregon, California, and Wyoming. I collapsed this to five locations (Figure 2.1; Table 2.1) based on the following criteria: a) sites with fewer than five birds captured that were also b) within 20 km of another sampling locale. The midpoint between all sampling locales that were collapsed into one location was used as the coordinate for the ‘new’ location. Geographic distances among locations were calculated using great-circle distance (i.e., the shortest distance between two locations on the spherical surface of the Earth) formula developed by Veness (2015), available at: http://www.movabletype.co.uk/scripts/latlong.html#ellipsoid.

All blood samples were collected between 2003 and 2012 (Cornelius & Hahn, 2012). Samples were collected from crossbills of four vocal types (types 2, 3, 4 and 5; Groth, 1993), identified prior to capture through vocalizations made in the field. I excluded all crossbills with unknown vocal types from all genetic and statistical analyses (n= 203), for a total of 437 birds included. Upon capturing each crossbill, a small blood sample was collected from each bird through brachial venipuncture, and blotted a portion of this onto high wet-strength filter paper, saturated with 0.5 M Na-EDTA (pH 8.0) then allowed to air-dry, for subsequent genetic analysis.

2.2.2 Genetic analysis

I extracted DNA from dried blood following an ammonium-acetate based salting-out protocol modified from Laitinen et al., (1994). Using scissors sterilized in bleach solution, I cut each blot into small (~1 mm²) square sections in preparation for extraction. I added ammonium acetate (5 M (pH 8.0)) to precipitate proteins out of the solution, centrifuged, and recovered the supernatant that contained the dissolved DNA. Using ice-cold isopropanol I precipitated the DNA and centrifuged to generate a pellet of DNA at the bottom of the microcentrifuge tube. After I discarded the supernatant, I washed the DNA pellet with 70% ice-cold ethanol, poured off the ethanol, inverted the tube and allowed the
tube to dry to ensure any excess ethanol had evaporated. I suspended the DNA pellet in 50 µL 1X TE buffer (10 mM Tris (pH 8.0) and 1.0 EDTA mM) and quantified DNA concentration using a NanoDrop 2000 Spectrophotometer (Thermo Scientific), and diluted samples with 1xTE buffer to a final working concentration of 50 ng/µL.

I genotyped red crossbills at ten microsatellite loci, developed for use in closely related species (*Loxia scotica, Carduelis tristis*; Table 2.2), using conditions described by Piertney *et al.*, (1998) and Tarvin (2006). However, I excluded three loci (LOX4, LOX5, and LOX8) in the statistical analysis because these loci contained missing information on more than 20% of the birds included. The reverse primer in each primer pair was labeled with fluorescent dye (Life Technologies), and for each individual crossbill I multiplexed up to four loci in a single polymerase chain reaction (PCR). PCRs were conducted in a final volume of 10 µL, to include approximately 50 ng of template DNA, 0.05 mM of each nucleotide, 2.5 mM MgCl₂, 1x PCR buffer without MgCl₂ (Sigma-Aldrich: 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.001% gelatin), 5 µM of each primer and 0.5 U *Taq* DNA polymerase (Fisher Scientific). Thermocycling profiles for PCR included an initial denaturing step of 92 ºC for three minutes; followed by 30 cycles of 92 ºC for 30 s, annealing temperature (see below) for 30 s, and a final elongation step of 72 ºC for five minutes.

PCR products were separated by capillary electrophoresis on an Applied Biosystems 3730 DNA Analyzer at Robarts Research Institute (University of Western Ontario, London, Ontario). I annotated allele sizes with reference to the internal size standard LIZ using GeneMapper 4.0 software to visualize and manually identify alleles. I used ARLEQUIN version 3.12 (Excoffier *et al.*, 2005) to determine observed (Hₒ) and expected heterozygosities (Hₑ), and to test for deviations from Hardy-Weinberg equilibrium. This analysis included allele randomizations within populations (1000 permutations) and over all populations (10 000 permutations). In these analyses populations of red crossbills included crossbills from the same vocal type that were also captured at the same location. I considered the vocal type by sampling location groupings as “putative populations”. There were 12 such “putative populations”, however I excluded three of these, either due to small sample size (vocal type 4 captured at Olympic peninsula (*n* = 1), and vocal type 4
captured at North California coast (n= 1)), or when all individuals in a particular grouping were missing microsatellite information at more than two loci (vocal type 3 at Teton mountain, (n= 4)), for a total of nine crossbill “putative populations” included in these analyses. I tested for violations of Hardy-Weinberg equilibrium at each putative population. Multiple tests for Hardy-Weinberg equilibrium were not controlled for with Bonferroni’s corrections. I tested for linkage disequilibrium between each locus included in analysis (seven in total). No loci showed significant departures from Hardy-Weinberg equilibrium (all p > 0.05), nor were any pairs of loci found to be in significant linkage disequilibrium (all p > 0.05).

2.2.3 Data analysis

I conducted an analysis of molecular variance (AMOVA) using ARLEQUIN version 3.12 (Excoffier et al., 2005), this included calculating population pairwise $F_{ST}$ values across the seven microsatellite loci and their probabilities (110 permutations). I then converted putative population pairwise $F_{ST}$ values to linearized $F_{ST}$ values using the formula: $F_{ST}/(1-F_{ST})$ (Rousset, 1996). I performed simple linear regressions in GraphPad Prism v.6.01 (2012), with geographic distance (ln km) as the independent variable and genetic distance ($F_{ST}/(1-F_{ST})$) as the dependent variable.

I used a partial Mantel correlation (Mantel, 1967), implemented the package ‘vegan’ (Oksansen et al., 2015) in R V.0.97.551 (2012), to determine whether vocal type explained a significant proportion of variation in pairwise genetic distance ($F_{ST}/(1-F_{ST})$) when controlling for geographic distance between sites. I also used the package ‘vegan’ to perform a simple Mantel correlation within crossbills of vocal type 3 captured at different sites, to determine whether genetic distance varied with geographic distance within a single vocal type, that is, to determine whether vocal type 3 shows isolation by distance.

I used Bayesian model-based clustering analysis, implemented in STRUCTURE 2.3.3 (Falush et al., 2003) to further compare genetic structuring among and within vocal types and among sample sites. I used the admixture and correlated allele frequency models, as recommended by the authors, to estimate the optimal number of clusters of individuals (K). Sampling location was used as prior information to assist clustering and maximize
my ability to detect subtle genetic structure (Hubisz et al., 2009). I tested models $K = 1$ through 9 to examine genetic differentiation between the nine vocal type by sampling location groupings. Running conditions included an initial burn-in period of 50,000 iterations followed by a run length of 100,000. I calculated the posterior probability for each value of $K$ using ln-likelihood scores (Falush et al., 2003). To confirm consistency between trials, I performed five replicate runs for each value of $K$. 


Figure 2.1. Map showing locations of the five trapping sites at which red crossbills of four vocal types were captured. OP = Olympic Peninsula, CR = Columbia River, CC = California Coast, WM = Warner Mountain, TM = Teton Mountain. Numbers within each box refer to vocal types captured at each site; all = all vocal types.
Table 2.1 Sites at which red crossbills (*n* = 437) were sampled. Sampling was conducted in Washington, Oregon, California, and Wyoming between 2003-2012 (Cornelius & Hahn, 2012).

<table>
<thead>
<tr>
<th>Code</th>
<th>Site Name</th>
<th>Geographic coordinates</th>
<th>Vocal type, (<em>n</em>)</th>
<th><em>N</em>_total</th>
</tr>
</thead>
<tbody>
<tr>
<td>OP</td>
<td>Olympic Peninsula</td>
<td>47°N, 123°W</td>
<td>3, (130)</td>
<td>131</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4, (1)</td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>Columbia River</td>
<td>46°N, 123°W</td>
<td>3, (131)</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4, (10)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>Northern California Coast</td>
<td>41°N, 124°W</td>
<td>3, (24)</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4, (1)</td>
<td></td>
</tr>
<tr>
<td>WM</td>
<td>Warner Mountain</td>
<td>42°N, 120°W</td>
<td>2, (25)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5, (5)</td>
<td></td>
</tr>
<tr>
<td>TM</td>
<td>Teton Mountain</td>
<td>43°N, 110°W</td>
<td>2, (3)</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3, (4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4, (34)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5, (69)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.2. Microsatellite loci at which red crossbills were genotyped.

<table>
<thead>
<tr>
<th>Name</th>
<th>Repeat motif</th>
<th>Species developed for</th>
<th>Size (bp)</th>
<th>Annealing Temperature (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOX1</td>
<td>(CTTT)_n</td>
<td>Scottish Crossbill</td>
<td>276 - 464</td>
<td>59</td>
<td>Piertney <em>et al.</em> (1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>(Loxia scotica)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOX3</td>
<td>(CTTT)_n</td>
<td>Scottish Crossbill</td>
<td>149 - 473</td>
<td>59</td>
<td>Piertney <em>et al.</em> (1998)</td>
</tr>
<tr>
<td>LOX4</td>
<td>(CTTT)_n</td>
<td>Scottish Crossbill</td>
<td>172 - 286</td>
<td>60</td>
<td>Piertney <em>et al.</em> (1998)</td>
</tr>
<tr>
<td>LOX6</td>
<td>(CTTT)_n</td>
<td>Scottish Crossbill</td>
<td>188 - 394</td>
<td>58</td>
<td>Piertney <em>et al.</em> (1998)</td>
</tr>
<tr>
<td>LOX7</td>
<td>(CTTT)_n</td>
<td>Scottish Crossbill</td>
<td>126 - 194</td>
<td>58</td>
<td>Piertney <em>et al.</em> (1998)</td>
</tr>
<tr>
<td>LOX8</td>
<td>(CTTT)_n ,</td>
<td>Scottish Crossbill</td>
<td>282 - 412</td>
<td>58</td>
<td>Piertney <em>et al.</em> (1998)</td>
</tr>
<tr>
<td></td>
<td>(CCTT)_n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CtB23</td>
<td>(ATG)_n</td>
<td>American goldfinch</td>
<td>92 -167</td>
<td>58</td>
<td>Tarvin (2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>(Carduelis tristis)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CtC16</td>
<td>(ATC)_n</td>
<td>American goldfinch</td>
<td>175 - 335</td>
<td>58</td>
<td>Tarvin (2006)</td>
</tr>
<tr>
<td>CtC105</td>
<td>(CATC)_n</td>
<td>American goldfinch</td>
<td>256 - 328</td>
<td>59</td>
<td>Tarvin (2006)</td>
</tr>
<tr>
<td>CtA8</td>
<td>(GA)_n</td>
<td>American goldfinch</td>
<td>254 - 476</td>
<td>59</td>
<td>Tarvin (2006)</td>
</tr>
</tbody>
</table>
2.3 Results

2.3.1 Crossbill genetic structure

I found no evidence that crossbills captured farther away were more genetically differentiated than crossbills captured closer together, regardless of vocal type (similar vocal type: $R^2 = 0.008$, $F_{1,4} = 0.032$, $P = 0.87$; different vocal type: $R^2 = 0.025$, $F_{1,28} = 0.71$, $P = 0.41$; Figure 2.2; Table 2.3). From the partial Mantel correlation, crossbill vocal types were not genetically differentiated when controlling for geographic distance (Mantel $R = -8.2 \times 10^{-11}$, $P = 0.46$, 999 permutations, $n = 66$). In addition, the program STRUCTURE estimated a $K$ of 1 (range of posterior probabilities for $K=1$ was -8402.4 to -8345.9) for all crossbill vocal types, further indicating a lack of genetic differentiation among vocal types and among different trapping locations.

In crossbills belonging to vocal type 3, however, there was a significant positive relationship between genetic distance and geographic distance ($R^2 = 0.80$, $F_{1,4} = 15.1$, $P = 0.018$, Figure 2.3). However, from the Mantel correlation, geographic distance and genetic distance between crossbills of vocal type 3 were not correlated (Mantel $R = 0.8$, $P = 0.08$, 999 permutations, $n = 6$).
Table 2.3. Pairwise matrix of geographic distance (km; above diagonal) and genetic differentiation ($F_{ST}$; below diagonal) between crossbill vocal type (2,3,4, and 5) and trapping location (five trapping sites from Washington, Oregon, California, and Wyoming). No pairwise values of $F_{ST}$ were significantly different from zero.

<table>
<thead>
<tr>
<th>Vocal type - Site</th>
<th>2 - TM</th>
<th>2 - WM</th>
<th>3 - CC</th>
<th>3 - CR</th>
<th>3 - OP</th>
<th>4 - TM</th>
<th>4 - CR</th>
<th>5 - TM</th>
<th>5 - WM</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 - TM</td>
<td>-</td>
<td>827</td>
<td>1177</td>
<td>1082</td>
<td>1113</td>
<td>0.00</td>
<td>1082</td>
<td>0.00</td>
<td>827</td>
</tr>
<tr>
<td>2 - WM</td>
<td>-0.061</td>
<td>-</td>
<td>351</td>
<td>505</td>
<td>605</td>
<td>827</td>
<td>505</td>
<td>827</td>
<td>0.00</td>
</tr>
<tr>
<td>3 - CC</td>
<td>0.004</td>
<td>-0.084</td>
<td>-</td>
<td>562</td>
<td>672</td>
<td>1177</td>
<td>562</td>
<td>1177</td>
<td>351</td>
</tr>
<tr>
<td>3 - CR</td>
<td>-0.019</td>
<td>-0.105</td>
<td>-0.005</td>
<td>-</td>
<td>111</td>
<td>1082</td>
<td>0.00</td>
<td>1082</td>
<td>505</td>
</tr>
<tr>
<td>3 - OP</td>
<td>-0.105</td>
<td>-0.154</td>
<td>-0.030</td>
<td>-0.078</td>
<td>-</td>
<td>1113</td>
<td>111</td>
<td>1113</td>
<td>605</td>
</tr>
<tr>
<td>4 - TM</td>
<td>-0.028</td>
<td>-0.072</td>
<td>0.002</td>
<td>-0.005</td>
<td>-0.022</td>
<td>-</td>
<td>1082</td>
<td>0.00</td>
<td>827</td>
</tr>
<tr>
<td>4 - CR</td>
<td>-0.031</td>
<td>-0.034</td>
<td>-0.118</td>
<td>-0.102</td>
<td>-0.284</td>
<td>-0.119</td>
<td>-</td>
<td>1082</td>
<td>505</td>
</tr>
<tr>
<td>5 - TM</td>
<td>0.022</td>
<td>-0.078</td>
<td>-0.034</td>
<td>-0.028</td>
<td>-0.181</td>
<td>-0.059</td>
<td>-0.007</td>
<td>-</td>
<td>827</td>
</tr>
<tr>
<td>5 - WM</td>
<td>-0.014</td>
<td>-0.383</td>
<td>-0.073</td>
<td>-0.067</td>
<td>-0.307</td>
<td>-0.114</td>
<td>-0.219</td>
<td>-0.014</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 2.2. Genetic distance ($F_{ST}/1-F_{ST}$) as a function of geographic distance (ln km) between putative populations of crossbills with similar and dissimilar vocal types (closed circles and open circle respectively).
Figure 2.3. Pairwise genetic distance ($F_{ST}/1-F_{ST}$) increased with geographic distance (ln km) in vocal type 3 crossbills captured at five locations in Washington, Oregon, California, and Wyoming. Dashed line indicates a simple linear regression.
2.4 Discussion

In contrast to my prediction that ecological and behavioural differences between different crossbill vocal types would result in genetic differentiation among vocal types, I found that red crossbill vocal types 2, 3, 4 and 5 were not genetically distinct from one another. Moreover, when considering all vocal types together, I found no effect of geographic distance on genetic distance among groups of birds captured at different sites. Taken together, these findings suggest that neither geography nor ecology are important drivers of population differentiation in this species. The high mobility of red crossbills may facilitate gene flow over large geographic distances, relative to non-nomadic species, and ecological and behavioural differences among vocal types may be less important than previously thought in reducing gene flow. However, and also in contrast to my original expectations, crossbills of vocal type 3 did show isolation by distance suggesting some effect of geographic distance on gene flow in at least some crossbill vocal types. The effect of isolation by distance of vocal type 3 crossbills is possibly driven by one pairwise comparison, and therefore caution should be taken when considering this relationship.

The lack of genetic differentiation I found among red crossbill vocal types contrasts with previous findings for this species (Parchman et al., 2006). However, that study had relatively low sample sizes (9 – 41 individuals per vocal type, in contrast to 28 – 285 individuals per vocal type in this study). Greater sample sizes in this study should provide a more reliable indicator of genetic structure among red crossbill vocal types. Assuming that my findings are correct, the observed lack of genetic differentiation among crossbill vocal types suggests that crossbill vocal types, at least the ones examined here, do not mate completely assortatively. Crossbills are characterized by temporally opportunistic breeding behaviour (Hahn, 1998), and this opportunism might also extend to mate choice, particularly if assortative mating based on vocal type is not possible. Findings of assortative mating in red crossbills are based on studies performed in laboratory settings (Snowberg & Benkman, 2007). Mate choice in the wild may be influenced by other factors beyond vocal type, resulting in discrepancies between preferences expressed in captivity and patterns found in free-living animals. For example, female song sparrows (Melospiza melodia) strongly preferred males with larger song repertoires when in
captivity, but repertoire size did not influence female choice of mates in the wild (Searcy, 1984).

Another factor that may have contributed to the difference between my results and those of Parchman et al., 2006 is the choice of genetic marker. Parchman et al., 2006 used amplified fragment length polymorphism (AFLP) loci, and in contrast, I used hypervariable microsatellite markers to infer genetic differentiation and population structuring within and among vocal types. The utility of microsatellites for inferring genetic differentiation has been criticized (Goldstein et al., 1995). First, microsatellite mutations are thought to result from strand slippage during DNA replication, resulting in a difference in the number of repeat units such that new alleles are not completely independent of their ancestral alleles. The infinite alleles model, on which much population genetic theory is based, may not accurately describe this mutational process (Goldstein et al., 1995). Moreover, because the mutation rate of microsatellites is relatively high, homoplasy (shared alleles at a locus due to similar mutations rather than to recent shared ancestry) may be relatively common in these markers (Rousset, 1996). Therefore, calculations of population differentiation based on microsatellite markers using measures such as $F_{ST}$ may not accurately reflect the true population structure.

Another potential contributor to the lack of genetic differentiation I observed between crossbill vocal types is that the program STRUCTURE (Falush et al., 2003) has low power to detect subtle genetic differentiation (Hubiz et al., 2009). STRUCTURE attempts to sort individuals into Hardy-Weinberg populations, ideally resulting in groups of individuals with similar allele frequencies. However, when the program is used to group individuals from many putative populations into a relatively smaller number of groups (and thus uncover underlying genetic structure), the program often places individuals into too few clusters (Kalinowski, 2011), particularly when populations differ in sample size. In my study, there was variation in sample size among groupings of vocal type by geographic capture locations, and this may have led STRUCTURE to underestimate the actual number of genetic clusters in this system. As an alternative, an unrooted neighbor-joining tree constructed from an unbiased genetic distance may be better suited for describing population structure (Kalinowski, 2011).
Previous genetic studies involving red crossbills representative of vocal type 3 had very low sample sizes \((n=10)\) (Parchman et al., 2006), thus my study is the first, to my knowledge, to adequately assess the population genetic structure of this vocal type. I found significant isolation by distance in this vocal type, in contrast to the lack of geographic isolation detected in the sample as a whole. The genetic structure detected for vocal type 3 may reflect a relatively high sample size for this type, but may also indicate relatively high levels of site fidelity within this group to core breeding areas. Crossbills can travel up to 3000 km in a year, but evidence from common crossbills (Loxia complex) suggest that individuals return to core breeding areas of their original population when local food sources are replenished (Knox, 1992). Although no individuals were recaptured during the decade long sampling effort of crossbills used in this study (Cornelius & Hahn, 2012), capture locations were chosen based on high crossbill abundance and may thus represent core breeding areas for vocal type 3 crossbills. The long (multi-year) sampling period could have included multiple conifer cone regeneration events, and thus multiple return trips by vocal type 3 individuals. As tracking technology improves our ability to track the movements of individual birds, following individuals of nomadic species such as crossbills will dramatically increase our understanding of the movements of nomadic birds at the population and even individual level. However, it should be noted that this relationship is possibly driven by one pairwise comparison, represented by a single data point. To further investigate and confirm the isolation by distance relationship in this vocal type, more sampling of vocal type 3 crossbills from intermediate distances should be performed.
2.5 References


3 Red crossbills and their bloodborne parasites

3.1 Introduction

Parasites reduce several fitness components of host individuals. In various species of birds, infection with bloodborne parasites is associated with delayed arrival at the breeding grounds (e.g., in Parulidae wood warblers; DeGroote & Rodewald, 2010), delayed onset of breeding (e.g., in great tits Parus major; Allander & Bennett, 1995), reduced clutch size (e.g., in great tits; Norte et al., 2009) and fledging success (e.g., in aquatic warblers Acrocephalus paludicola; Dyrcz et al., 2005), and reduced survival (e.g., in great tits; Krams et al., 2013). Beyond their effects on the fitness of host individuals, parasites can also affect the evolutionary trajectories of host populations. In particular, if host populations coevolve with and become adapted to local parasite strains, parasite-mediated selection may represent a major cost of natal dispersal (Fincher & Thornhill, 2008; Sarquis-Adamson & MacDougall-Shackleton, in prep). Host individuals that disperse are likely to encounter unfamiliar parasite strains to which they may have reduced immunity. If so, parasite-mediated selection may reduce gene flow among host subpopulations and eventually promote host diversification.

Haemosporidian (bloodborne) parasites (phylum Apicomplexa) are geographically widespread, infecting vertebrate species around the globe (Atkinson & Van Riper, 1991). In birds, for example, about 68 % of species are infected by haemosporidian parasites (Atkinson & van Riper, 1991), which are transmitted between host individuals by blood-sucking Dipteran insect vectors. Evolutionary relationships within this group have been resolved based on their mitochondrial cytochrome b sequences (Hellgren et al., 2008), indicating that avian Haemosporidia include three genera: Leucocytozoon, and sister taxa Plasmodium and Haemoproteus. Their widespread distribution, and parallels to human bloodborne parasites have made avian haemosporidia an attractive model with which to explore host-parasite interactions.
Often, parasites show low pathogenicity to their hosts, suggesting that host-parasite interactions may have resulted in mutual adaptation between parasites and hosts. Hawai‘i ‘Amakihi (*Hemignathus virens*) from areas that were naturally exposed to an avian malarial parasite (*Plasmodium relictum*) had overall lower mortality and cleared infections better when experimentally infected with *P. relictum* than did non-local birds that had presumably not encountered the parasite (Atkinson *et al.*, 2013). Chronic levels of avian malaria infection (*Plasmodium relictum*) in native Hawai‘i ‘Amakihi did not reduce reproductive success (Kilpatrick *et al.*, 2006). Examples from this system suggest the evolution of host tolerance to their parasite. However, differences in pathogenicity and parasite-mediated effects on hosts make it difficult to generalize about host-parasite interactions in wild populations.

Most studies of interactions between birds and their bloodborne parasites have used bird species which either do not migrate (Kilpatrick *et al.*, 2006) or that have predictable seasonal migratory movements. Far less is known about host-parasite interactions in species with unpredictable movement patterns, such as nomadic migrants. Nomadism as a life-history strategy appears to have evolved in response to food sources that occur unpredictably through time and are heterogeneously distributed across the landscape. Nomadic species move long distances in search of a new area rich in nutrients to exploit (Senar *et al.*, 1992). It is important to consider how hosts with unique life histories such as nomadism may interact with their parasites.

Red crossbills (*Loxia curvirostra*) are known for their irruptive and nomadic movements in response to conifer cone masting events. As reviewed in chapter 1, red crossbills are distributed across North America from southern Yukon to southern Mexico (Groth, 1988), but can be categorized into ten behaviourally, morphologically and ecologically distinct vocal types, each primarily associated with different conifer species (Groth, 1993). The differences in ecology of the conifer species associated with different crossbill vocal types may influence associated insect communities, and may thus influence the vectors and hence the bloodborne parasites to which crossbill vocal types are exposed.
In this study, I used molecular techniques to characterize haematozoan parasite communities in red crossbills of four different vocal types, captured at multiple locations in the western United States. My primary objective was to determine whether different vocal types are subject to different parasite communities. Because vocally distinct red crossbill types are also ecologically distinct (Benkman, 2003) and differ in prevalence of ectoparasite infestation (scaly leg mites; Edelaar & van Eerde, 2011), I predicted that different vocal types would also be subject to different haematozoan parasite communities. My secondary objective was to determine the relative importance of geographic distance and ecological difference in predicting parasite community similarity among red crossbill vocal types. I predicted that red crossbills of the same vocal type would have similar parasite communities despite the geographic distances (up to 1200 km) between trapping locations.
3.2 Methods

3.2.1 Study animals and sampling

All red crossbill samples were collected in five locations throughout Washington, Oregon, California, and Wyoming from 2003-2012 (Cornelius & Hahn, 2012). The samples include crossbills from four vocal types (types 2, 3, 4 and 5; Groth, 1993). I only included crossbills that had been identified to vocal type (437 birds). Capture locations, and vocal types of birds captured at each site, are summarized in Figure 2.1 and Table 2.1. Because multiple vocal types were captured at each of the five capture locations, I identified 12 “putative populations” of crossbills. Individuals captured at the same location and belonging to the same vocal type were considered to belong to the same “putative population”, whereas individuals belonging to different vocal types and/or captured at different locations were considered to belong to different “putative populations”.

Upon capturing each crossbill, a small blood sample was taken from each bird through brachial venipuncture. A portion of blood was blotted onto high wet-strength filter paper, saturated with 0.5 M Na-EDTA (pH 8.0) then allowed to air-dry, for subsequent genetic analysis. Another drop of whole blood was placed onto a clean glass microscope slide, and gently pulled across the slide using a second slide, to make a thin-film blood smear. Smears were air-dried, fixed in absolute methanol, stained with a Hema 3 manual staining system (Fisher Scientific, Pittsburgh, PA, USA), and examined under oil immersion using a light microscope at 1000x magnification. They examined 10,000 erythrocytes for each smear, and using morphological characteristics to identify parasites, noted cells infected with Plasmodium spp. and/or Haemoproteus spp. (Cornelius et al., 2014). No infections by Leucocytozoon spp. were detected via examination of blood smears (Cornelius et al., 2014). Individuals were categorized as infected or uninfected with Plasmodium and/or Haemoproteus based on whether or not at least one parasite was detected in the scan of 10,000 erythrocytes (Cornelius et al., 2014).

3.2.3 Genetic analysis of parasite prevalence

Microscopic examination of thin-film blood smears can sometimes fail to detect low-level (chronic) infections, and does not allow for fine-scale resolution of parasites below the
genus level. Thus, as a complementary analysis, I used molecular techniques to detect infected individuals and identify parasite lineages. As outlined in chapter 2, I extracted genomic DNA (comprising both host DNA and DNA of bloodborne parasites) from dried blood, using an ammonium-acetate based salting-out protocol modified from Laitinen et al., (1994).

I used a two-step, nested polymerase chain reaction (PCR) and parasite-specific primers to amplify and sequence a portion of the parasite mitochondrial cytochrome b gene (Bensch et al., 2000; Hellgren et al., 2004), Figure 3.1). In the first round of PCR, primers HaemNFI and HaemNR3 (Hellgren et al., 2004) were used to amplify a 617 bp fragment (including primers) of haemosporidian mtDNA. These first-round primers amplify cytochrome b of Haemoproteus, Plasmodium, and Leucocytozoon spp. (Hellgren et al., 2004). PCR reactions were conducted in a final volume of 25 µL and included 50 ng of template DNA, 1.25 mM of each nucleotide, 1.5 mM MgCl₂, 1x PCR buffer without MgCl₂ (Sigma-Aldrich: 10 mM Tris-HCl, pH 8.3 at 25 °C, 50 mM KCl, 0.001% gelatin), 0.6 mM of each primer and 0.5 U Taq DNA polymerase (Fisher Scientific). The thermocycling profile included an initial denaturing step of 94 °C for 3 min; followed by 20 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s; then a final extension step at 72 °C for 10 min (Hellgren et al., 2004). In each batch of PCRs (approximately 20 crossbill samples) I included one negative control, made with 1 µL of sterile water in place of template DNA, and one positive control, made with 50 ng of template DNA from a song sparrow (Melospiza melodia) previously screened and found to be co-infected with Leucocytozoon, Plasmodium and Haemoproteus.

Amplification products from the first-round PCR reactions were used as the templates for second-round PCR reactions, following Hellgren et al., (2004). Second-round PCRs used primers specific for Leucocytozoon (HaemFL and HaemR2L; Hellgren et al., 2004) or for Haemoproteus/Plasmodium (HaemF and HaemR2; Bensch et al., 2000) and were nested within the first-round amplification product (Figure 3.1), to amplify a fragment of 480 bp excluding primers (Leucocytozoon) or 478 bp excluding primers (Haemoproteus/Plasmodium). All birds were screened with both sets of primers. Thus, for each crossbill screened I conducted a first-round PCR, a second-round PCR to detect
Leucocytozoon infection, and another second-round PCR to detect Haemoproteus or Plasmodium infection. Conditions for second-round PCR conditions were identical to first-round conditions, except that I used 2 µL of first-round PCR product as the template, parasite-specific primers were used as described above, and 35 rather than 20 cycles were performed.

To identify infected birds, I electrophoresed 5 µL of second-round PCR product on a 2% agarose gel stained with RedSafe™ and visualized the gel under UV light. On each gel I ran a 1000 bp DNA ladder (Fisher Scientific) to allow me to compare product sizes with expected sizes (Hellgren et al., 2004). For individuals showing a band of the expected product size (478 - 480 bp) I removed excess primers and dNTPs from the second-round PCR product by digesting with Exonuclease I and FastAP Thermosensitive Alkaline Phosphate (Fisher Scientific Waltham, Massachusetts). These products were then sent for Sanger sequencing on an Applied Biosystems 3730 DNA Analyzer at the Robarts Research Institute (University of Western Ontario, London, Ontario). I compared the resultant nucleotide sequences to published sequences (as of November 2015) using the BLAST (Basic Local Alignment Search Tool) (Altschul et al., 1990) tool from GenBank, available at http://www.ncbi.nlm.nih.gov/blast/Blast.cgi, as well as the MalAvi database (http://mbio-serv2.mbioekol.lu.se/Malavi/blast.html; Zhang et al., 2000). I then determined, for each crossbill I found to be infected, the genus (Plasmodium, Haemoproteus or Leucocytozoon) of the parasite based on sequence similarity to previously published lineages. Within each genus I categorized the infection according to lineage, defined as sequences with 99 - 100% sequence similarity.

Sequences from four birds showed double chromatogram peaks at multiple sites within second round PCR products of Haemoproteus/Plasmodium PCR (Figure 3.2), suggesting that these birds were simultaneously infected by more than one parasite lineage. To resolve this, I cloned each of these potentially co-infected samples using the pGEM® - T Easy Vector Systems kit (Promega Corporation, Madison, WI, USA), using amplification products from the second-round parasite PCR (as described above, HaemF and HaemR2; Bensch et al., 2000). I followed the ligation and transformation protocol as outlined by the manufacturer (Promega Corporation). To grow the bacteria after transformation, I
made SOC broth with SOC medium (0.034 g/mL) and distilled water. To plate the bacteria I made lysogeny broth (LB) agar plates (0.04 g/mL of LB agar), and added ampicillin (0.00005 g/mL) to the LB mixture. I selected eight colonies from each plate (one plate per bird) and suspended each in 50 µL of 1xTE. Nested parasite-specific PCRs for *Haemoproteus/Plasmodium* were performed as outlined above, using colony suspensions as the template DNA. To confirm amplification, I electrophoresed 5 µL of second-round PCR product under the conditions outlined above. I then removed excess primers and dNTPs from second-round PCR product as outlined above, and sent products for Sanger sequencing at the Robarts Research Institute as outlined above.

3.2.4 Data analysis

Infections detected by molecular analysis allowed me to score prevalence of each parasite lineage in each putative population. For the infections detected through molecular analysis I counted the number of individuals infected with that lineage through molecular analysis. Infections detected by microscopy only allowed me to score prevalence of each parasite genus in each putative population. I counted the number of individuals found to be infected by at least one cell of that genus when screening 10,000 RBC (data taken from Cornelius *et al.*, 2014). When I combined data from both molecular and microscopy techniques, I was again limited to genus by genus comparisons. I counted the number of individuals found to be infected by that parasite genus by molecular analysis, and/or through microscopy.

I used the prevalence of each parasite lineage observed within each putative population to calculate pairwise Bray-Curtis community dissimilarity indices between all putative populations. Bray-Curtis dissimilarity was calculated using the formula:

\[
D = 1 - 2 \frac{\sum_{i=1}^{S} \min(a_i, c_i)}{\sum_{i=1}^{S} (a_i + c_i)}
\]

The abundances of the species of site ‘A’ are indicated by \(a_i\), and the abundances of the species of site ‘C’ are indicated by \(c_i\).
This index of dissimilarity gives greater weight to more common lineages (Krebs, 1999), and is preferable to alternate indices of community similarity/dissimilarity because Bray-Curtis is influenced by differences in species abundance between sites (putative populations), whereas the Jaccard index and Sorensen distance only incorporate the presence or absence of a species. I calculated all Bray-Curtis indices based on relative parasite abundance data instead of raw count data because the number of crossbills sampled at each location was not equal.

In addition to the pairwise matrix of Bray-Curtis distance, I generated a pairwise matrix of geographic distance between putative populations, as described in chapter 2, and a matrix of vocal type dissimilarity (same type = 0, different types = 1) for all such combinations. I performed simple linear regressions in GraphPad Prism v.6.01 (2012), with geographic distance (km) as the independent variable and Bray-Curtis dissimilarity indices as the dependent variable. Because I used multiple complementary approaches to detect infections, I conducted separate calculations of Bray-Curtis dissimilarity and thus separate regressions for parasite infections identified by molecular methods, microscopy, or a combined approach as described above.

Some infections (n= 79) detected through microscopy by Cornelius et al., (2014) were not detected by my molecular methods, suggesting that molecular data on parasite infection likely included false negatives (i.e., individuals that were infected but not detected through PCR). To be conservative in data analysis, I chose to exclude all Bray-Curtis scores between two sites in complete ‘mismatch’ when comparing parasite data generated only from molecular methods. When comparing ecological abundance data, a complete ‘mismatch’ occurs when both sites in the comparison do not have any of the same species present (see hypothetical example in Table 3.1). Thus, I identified a complete ‘mismatch’ between a pair of putative populations when no overlap was detected in haemosporidian lineages observed in each, due to the possibility that such complete mismatches could reflect false negatives and not true mismatches, I excluded them from analyses.
I included Bray-Curtis scores generated from complete mismatches when comparing parasite abundances generated from microscopy, and combined data from both methods, on the assumption that these approaches were more likely to reflect true differences in parasite communities.
Figure 3.1. Schematic illustration of the directions and combinations of the different parasite- specific primers. Dashed lines represent primers for first-round amplification in the nested PCR. Solid lines represent primer combination for amplification of *Leucocytozoon* (HaemFL/HaemR2L) and of *Haemoproteus* and *Plasmodium* (HaemF/HaemR2). Modified from Hellgren et al., (2004).
Figure 3.2. Comparison of two chromatograms analyzed with MEGA 6 (Tamura et al., 2013) depicting: a) crossbill infected with one confirmed *Haemoproteus/Plasmodium* lineage (single chromatogram peaks at each nucleotide site), and b) crossbill suspected to have a co-infection (double chromatogram peaks at multiple nucleotide sites) and was later confirmed with vector cloning. Base pair (bp) length is shown along the top of each frame. Identified nucleotide base pairs are indicated (A,G,C, or T) and non-resolved nucleotide base pairs are indicated by ‘N’.
Table 3.1. Example of a complete ‘mismatch’ between relative parasite abundance data from captures captured at location A and location B. These complete ‘mismatches’ were excluded from analyses to calculate Bray-Curtis dissimilarity scores when using haematozoan parasite lineages detected from PCR-based methods.

<table>
<thead>
<tr>
<th>Parasite lineage</th>
<th>Location A</th>
<th>Location B</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Haemoproteus</em> 1</td>
<td>0.47</td>
<td>0</td>
</tr>
<tr>
<td><em>Haemoproteus</em> 2</td>
<td>0.13</td>
<td>0</td>
</tr>
<tr>
<td><em>Plasmodium</em> 1</td>
<td>0.13</td>
<td>0</td>
</tr>
<tr>
<td><em>Plasmodium</em> 2</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td><em>Leucocytozoon</em> 1</td>
<td>0.06</td>
<td>0</td>
</tr>
<tr>
<td><em>Leucocytozoon</em> 2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Leucocytozoon</em> 3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Site total:** 1.0          1.0
3.3 Results

Of the 437 birds screened using molecular methods, four *Plasmodium* infections, 28 *Haemoproteus* infections, and five *Leucocytozoon* infections were identified, for a total of 38 identified infections (including birds with co-infections). By contrast, based on microscope analysis, there were six positive *Plasmodium* scores, and 111 positive *Haemoproteus* scores reported by Cornelius *et al.*, (2014), for a total of 117 identified infections (including birds with co-infections). No infections by *Leucocytozoon* spp. were detected via examination of blood smears (Cornelius *et al.*, 2014).

The 38 infections detected through molecular methods comprised seven distinct lineages (defined as a group of sequences with less than 2.0% cytochrome *b* sequence divergence). Specifically, I detected two lineages of *Haemoproteus*, each with 100% sequence identity to a previously-published *Haemoproteus* lineage (SISKIN1, Genbank accession number KR049263, and PYERY01, Genbank accession number GQ395658); three lineages of *Leucocytozoon*, each with 100% sequence identity to a previously-published *Leucocytozoon* lineage (CB1, Genbank accession number FJ168564; ROF17, Genbank accession number JX556911; ROF16, Genbank accession number JX556910); and two *Plasmodium* lineages. Of the two *Plasmodium* lineages detected, one showed 100% identity to a previously published *Plasmodium* lineage (JA01, Genbank accession number KM598212), and the other (RECR1) is reported here for the first time.

*Haemoproteus* infections accounted for 89.7% of all haematozoan infections when data were pooled from both methods (Figure 3.3c), and of this genus the most common lineage, SISKIN1, accounted for 79% of all parasite infections detected by PCR-based methods (Figure 3.5a). *Plasmodium* infections accounted for 7.1% of all infections (data pooled from both methods; Figure 3.3c), and *Leucocytozoon* spp. infections were responsible for 3.2% of all detected infections (PCR-based methods only; Figure 3.3a).

Pairwise values of Bray-Curtis dissimilarity ranged between putative populations. Geographic distance between putative populations also did not explain a significant proportion of variation in Bray-Curtis dissimilarity of haematozoan parasite communities. When Bray-Curtis dissimilarity was calculated based on molecular methods, geographic
distance did not predict Bray-Curtis dissimilarity of parasite communities (linear regression: dissimilar vocal type: $R^2 = 0.036$, $F_{1,6} = 0.22$, $P=0.65$, Figure 3.4a). This analysis did not include any pairs of putative populations of the same vocal type. Similarly, when Bray-Curtis dissimilarity was calculated based on microscopic analysis of parasites, geographic distance did not predict Bray-Curtis dissimilarity of parasite communities (similar vocal type: $R^2=0.29$, $F_{1,5}=2.1$, $P=0.21$; dissimilar vocal type: $R^2=0.014$, $F_{1,19}=0.27$, $P=0.61$; Figure 3.4b). Finally, when parasite prevalence was determined using a combination of both approaches (molecular and microscopy), geographic distance did not predict Bray-Curtis dissimilarity of parasite communities (similar vocal type: $R^2=0.039$, $F_{1,7}=0.29$, $P=0.60$; dissimilar vocal type: $R^2=0.005$, $F_{1,25}=0.12$, $P=0.71$; Figure 3.4c).

Within vocal type 3, the class for which the most data on parasite prevalence was available, birds sampled at different sites were not characterized by significantly different parasite communities. That is, I found no relationship between pairwise geographic distance and Bray-Curtis dissimilarity of parasite communities (calculated based on molecular and microscopy methods combined; linear regression: $R^2=0.035$, $F_{1,4}=0.15$, $P=0.72$; Figure 3.5).
Figure 3.3. Pie charts representing the percentage of haematozoan parasites from *Haemoproteus* spp., *Leucocytozoon* spp., and *Plasmodium* spp. detected in red crossbills (representing vocal types 2, 3, 4, and 5) using: a) PCR-based methods, b) blood-smear analysis, and c) data pooled from both PCR-based and blood-smear analysis methods. Total number of parasite infections (co-infections included) recovered by each method are identified below each pie chart. Percentages are based on raw parasite counts.
Figure 3.4. Bray-Curtis dissimilarity scores from crossbill parasite infections detected by: a) parasite-specific PCR, b) blood-smear analysis, and c) combined PCR and blood-smear analysis data, as a function of geographic distance (km). Solid circles represent pairwise comparisons between putative populations of the same vocal type, and open circles represent pairwise comparisons between putative populations of different vocal types.
Figure 3.5. Bray-Curtis dissimilarity in haemosporidian parasite communities as a function of geographic distance (km) between pairwise comparisons of putative populations of vocal type 3 red crossbills.
Figure 3.6. Pie charts representing the percentage of haematozoan parasite infections (*Haemoproteus* spp., *Leucocytozoon* spp., and *Plasmodium* spp.) detected in red crossbills from: a) vocal type 2, b) vocal type 3, c) vocal type 4, and d) vocal type 5. Total number of parasite infections (co-infections included) recovered from crossbills representing each vocal type are identified below each pie chart (Total= n). Percentages are based on raw parasite counts (pooled data from both PCR-based and blood smear-analysis) from each crossbill vocal type.
3.4 Discussion

In contrast to my prediction that crossbill vocal types that differ in behaviour and ecology would also differ in their haematozoan parasite communities, vocal type did not explain a significant proportion of differences in parasite communities. Nor did geographic distance appear to contribute to differences in parasite communities, either within all vocal types or within vocal type 3. Overall, the molecular methods used here to detect parasitic infections appeared to be less sensitive than traditional microscopy, as 79 infections were detectable through microscopy but not through PCR. Of the infections detected by molecular techniques, I observed two lineages of *Haemoproteus*, two of *Plasmodium*, and three of *Leucocytozoon*, with *Haemoproteus* spp. representing 74% of infections.

Perhaps surprisingly, ecological differences among crossbill vocal types did not predict differences in haemosporidian parasite communities. The behavioural and ecological differences among crossbill vocal types may be too subtle to influence host choice by vectors or parasites. In a survey of 152 Afrotropical bird species, habitat was not found to be an important predictor of haemosporidian infection, but flocking behaviour, host nest type, and host nest location were explanatory (Lutz et al., 2015). All red crossbills, regardless of vocal type, are highly social and flock in large numbers. Perhaps the shared behaviours among crossbill vocal types are important factors in determining haemosporidian parasite communities, and may help explain the lack of differences in parasite communities detected in this study.

In the case of vector-mediated parasites, such as avian haematozoa, host specificity is highly influenced by the specificity of insect vectors. Crossbills can travel 3000 km in a particular year in response to changes in conifer crop abundance (Newton, 2006), and the timing of these movements is difficult to predict. The lack of differentiation in bloodborne parasite communities among crossbill vocal types may reflect their irruptive movements. From the perspective of the parasite, it would be maladaptive to specialize on a host that is unpredictable through space and time.

One reason for my failure to detect as many parasite infections as Cornelius et al. (2014) did in the same dataset could be degradation of DNA over time. Crossbill blood samples
included in this study were collected over ten years (2003-2012; Cornelius & Hahn, 2012), and despite preservation with Na-EDTA, samples may have degraded over time. This degradation is less problematic when dealing with host DNA (chapter 2) but when sampling blood from birds infected with bloodborne parasites, the resultant sample contains far more host DNA than parasite DNA. This, combined with the age of the samples, may have contributed to low ability of parasite-specific PCR to detect actual infections. Other studies using fresh bird blood in place of dried blood infections as the template in the PCR had very good agreement between infections detected by microscopy and molecular techniques (Rooney, unpublished). Ideally, fresh blood should be used to increase the efficacy of the parasite-specific PCR in identifying haematozoan infections.

The reduced ability of molecular methods to detect infections compared to traditional microscopic analysis may also be due in part to PCR primers selectively amplifying certain lineages. Although the primers, constructed from a highly conserved region of the mitochondrial DNA, were designed (Hellgren et al., 2004) to amplify all *Plasmodium* spp. and *Haemoproteus* spp. lineages, this may not be true. Supporting this, I was only able to isolate *Plasmodium* spp. lineages after cloning individual amplicons, suggesting that the primers selectively amplify *Haemoproteus* lineages. Indeed, the primers designed to amplify *Plasmodium* spp. and *Haemoproteus* spp. lineages have been found to be highly selective, especially in co-infections (Alarcon et al., 2006). Moreover, different lineages of *Haemoproteus* were isolated with vector cloning, suggesting that the primers may also be biased within *Haemoproteus* lineages. As more published sequences become available, I would advise that separate primers be designed for both *Haemoproteus* and *Plasmodium* lineages, based on conserved regions within each genus. Until then, relying only on the PCR-based method of detection could lead to an underestimation of the real haemosporidian diversity in wild birds (Valkiūnas et al., 2006; Szöllősi et al., 2008)

Of the three parasite genera I examined, *Haemoproteus* spp. accounted for 78% of all infections detected by molecular techniques, and 90% of all infections that were detected by molecular and microscopy combined. These infections comprised only two lineages of *Haemoproteus*. HSISKIN1 (*Haemoproteus tartakovskyi*; Valkiūnas, 1986) was the most common parasite lineage at every location, accounting for 72% of all infections. *H.*
*tartakovskyi* was first identified in red crossbills captured in the Curonian Spit in the Baltic sea (Valkūnas, 1986), and has since been found to infect several other bird species including hawfinches (*Coccothraustes coccothraustes*) in Slovakia (Berthová et al., 2012), skylarks (*Alauda arvensis*) in the Netherlands and southern Italy (Zehtindjiev et al., 2012), and pine siskins (*Carduelis pinus*) and common redpolls (*Acanthis flammea*) in Alaska (Oakgrove et al., 2014).

Like red crossbills, pine siskins and common redpolls live in gregarious flocks and make irruptive, nomadic movements in response to seed crops (Dawson, 1997; Knox & Lowther, 2000; Adkisson, 1996). These ecological similarities among avian hosts of *H. tartakovskyi* species may suggest host specificity of the insect vector. In Slovakian populations, *H. tartakovskyi* is transmitted by the Highland midge (*Culicoides impunctatus*; Valkūnas & Liutkevičius, 2002). It is unclear if the same vector is responsible for North American infections, but another species of biting midge, *C. nubeculosus*, was experimentally infected with *H. tartakovskyi* and successfully passed the parasite to wild caught siskins (Ziegytė et al., 2015). This suggests that multiple species of biting midges are competent vectors for this parasite.

Among infected crossbills in my study, the relative prevalence of *H. tartakovskyi* (72% of all infections) was dramatically higher than in crossbills sampled in the Curonian Spit (9% of all infections; Valkūnas & Iezhova, 2001). It is difficult to explain the greater relative prevalence of this lineage in North American crossbills without knowing the vector species responsible for transmission, but such vectors may be more abundant or active for a greater portion of the year within North America.

I identified two *Plasmodium* lineages in the crossbills I sampled. One of these lineages (RECR1) has not been previously described. Of the *Leucocytozoon* lineages detected, one (CB1) had 99% sequence identity to *L. fringillarium* (Woodcock, 1910), also found in red crossbills captured near the Curonian Spit in the Baltic sea (Valkūnas & Iezhova, 2001). This parasite has been described in over 200 bird species, on every continent except Antarctica (Valkūnas, 2005). This widespread distribution and lack of host
specificity suggests that it is not surprising that the relative prevalence of this lineage did not differ among crossbill vocal types or with geographic distance.

In conclusion, Haemosporidian parasite communities did not differ among different crossbill vocal types or with geographic distance between putative host populations. These findings suggest that the unpredictable irruptive movements made by crossbills may select against host-specificity of haematozoan parasites and/or of the dipteran vectors that transmit them.
3.5 References


Kilpatrick, A.M., LaPointe, D. A., Atkinson, C. T., Woodworth, B. L., Lease, J. K.,


Chapter 4

4 General discussion

4.1 Lack of differences in genetic structure among red crossbill vocal types

My thesis, to my knowledge, is the first to assess population genetic structuring of red crossbills representing vocal type 3, and the most comprehensive study to investigate genetic structuring among vocal types 2, 3, 4, and 5. Red crossbill flexibility in breeding behaviour and timing may have implications for genetic structuring within this nomadic species. Specifically, there was no genetic structure found among four red crossbill vocal types examined in this thesis. These results do not support the idea of complete assortative mating within vocal types (Snowberg & Benkman, 2007). I found no effect of geographic distance on genetic distance among groups of crossbills captured at different locations. Red crossbills are highly mobile and this may facilitate gene flow over large geographic distances, relative to non-nomadic species. Moreover, ecological and behavioural differences among vocal types may be less important than previously thought in reducing gene flow. However, crossbills of vocal type 3 did show isolation by distance suggesting some effect of geographic distance on gene flow in at least some crossbill vocal types. It should be noted that this significant relationship might have been driven by a single pairwise comparison between two putative crossbill populations. Increased sampling effort of vocal type 3 crossbills from intermediate geographic distances is needed to further explore this relationship.

The potential flexibility in behaviour may have important implications for red crossbill populations in the future. In light of recent estimates of climate change the distribution of parasites is expected to increase with their associated vectors as global temperatures increase (Harvell et al., 2003). Wild populations may be exposed to not just a higher risk of parasitism, but also novel parasites as the ranges of their vectors expand. Therefore, understanding the genetic underpinnings of host populations and assessing their associated parasites, especially in hosts with unique distributions and life-history traits, is important.
4.2 Lack of differences in parasite communities

Current widely used genetic approaches to determine haemosporidian infections in avian populations may not accurately determine the true amount or diversity of these parasites. A high amount of false negatives from my study suggests that these genetic tools should be used in tandem with other methods, such as blood-smear analysis, if fresh blood samples are not available. Other studies using freshly preserved blood (opposed to dried blood-blots used in this thesis) reported complimentary results between molecular and microscopic techniques for identifying haemosporidian infections (Rooney, unpublished). Furthermore, these genetic techniques may be especially poor at identifying co-infections, as suggested by Szöllősi et al. (2008). Reliance on only one method may result in an underestimation of the diversity of haemosporidian infections in natural avian populations.

Parasite lineages recovered in crossbill samples in this study included representatives of three genera: *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*. Lineages with identified morphospecies represented parasites with different global distributions and levels of bird host specificity. These parasites have different ecologies and may exert different selection on their hosts. Moreover, different invertebrate vectors transmit these parasites and thus vector ecologies should be considered in these host-parasite interactions. Unfortunately, not enough is known about parasite-vector or avian host-vector dynamics in this system.

Prevalence of haematozoan infections in the red crossbill samples included in this study was similar to those described in other population in the Curonian Spit of the Baltic sea (Valkiūnas & Iezhova, 2001). This indicates that despite ecological and morphological differences between populations, conserved aspects of red crossbill biology may have a stronger influence on infection susceptibility. The unique breeding phenology may be an adaptive strategy in response to selective pressures exerted by vector-driven parasites, and may explain lack of co-evolution between crossbills and bloodborne parasites. Crossbills may evade infection by modulating the seasonality of their breeding behaviour; they escape infection by limiting vulnerability when abundance is at its peak.

The nomadic movements and opportunistic breeding strategy of red crossbills make them
a unique system to study host-parasite interactions. Prevalence of haemtaozoan infections found in North American crossbills sampled in this thesis were similar to that of crossbills sampled in the Palearctic (Valkiūnas & Iezhova, 2001), suggesting that the opportunistic breeding strategy observed among red crossbills sampled in both regions is important in determining parasite communities. Nomadism by the host might discourage specificity by vectors and parasites. The lack of differences in parasite communities among red crossbill vocal types sampled in this thesis suggests that the nomadic and irruptive movements of red crossbills might facilitate host-switching events by bloodborne parasites as described in other host-parasite systems with highly mobile hosts (Jenkins et al., 2011), and ultimately de-escalate coevolutionary arms races between parasites and hosts. Hosts that have unusual life histories can provide important insight to host-parasite interactions.

4.3 Conclusions

Parasites are an important aspect of the living environment, however, little is known about host-parasite interactions in nomadic species. Red crossbills have been used as a flagship example to illustrate ecological speciation in a natural system since the emergence of this concept 70 years ago (Mayr, 1947). Population genetic structure in these birds does not support the idea of complete isolation among crossbill vocal types, and the lack of differences in parasite communities suggests features of crossbill ecology are not completely isolated among vocal types.
4.4 References


Curriculum Vitae

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Society of Biology Graduate Students, The University of Western Ontario
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People's Choice for Best Talk: Ecology and Evolution
Society of Biology Graduate Students, The University of Western Ontario
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Western Research Day
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Poster presentation
Biology Graduate Research Forum
University of Western Ontario
London, Ontario, Canada

May 2014
Contributed talk
Strong Minds, Strong Bones foundation
Windermere On the Mount retirement home
London, Ontario, Canada
Volunteer Work:

Biodiversity Gallery Opening chair
Winter 2015

Society of Biology Graduate Students elected representative – seminar committee member
September 2014 – 2015

Let’s Talk Science volunteer
September 2014 – 2015

Society of Biology Graduate Students elected representative – social committee member
September 2013 – 2014