INTERSPECIFIC INTERACTIONS BETWEEN MOUNTAIN AND BLACK-CAPPED CHICKADEES WITHIN A CONTACT ZONE

by

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ABSTRACT

Hybridization occurs at least occasionally in more than 9% of bird species. Within the Paridae (chickadees and titmice), hybridization is documented both among the Old World species and North American species. Europe has a larger number of sympatric tit species than does North America, but appears to have less hybridization. These overlapping species may have developed isolating mechanisms to decrease hybridization and as a result maintain distinct species. In North America, little research has focused on potential hybridization between the two closely related species that occasionally occur in sympatry and anecdotal evidence suggests they occasionally hybridize: black-capped (*Poecile atricapillus*) and mountain (*P. gambelli*) chickadees. My PhD research focuses on the interspecific relationships and potential hybridization between black-capped chickadees and mountain chickadees at the John Prince Research Forest (JPRF) in northern British Columbia. I focused my work on behavioural mechanisms that may reinforce species isolation and genetic analysis to determine the amount of intermixing that is occurring between species.

Through nuclear and mitochondrial DNA analyses, I found evidence of hybridization. All but one of these hybrids were phenotypically mountain chickadees that had black-capped DNA in their genome. Further, all hybrids that were found amongst nestlings occurred in mountain chickadee nests showing genetic patterns indicating extra-pair copulations between female mountain chickadees and males black-capped chickadees.

As dominance status is known to affect mate choice in black-capped chickadees, interspecific social hierarchies have the potential to interfere with interspecific interactions and create the potential for hybridization. My field observations and aviary experiments showed that black-capped chickadees are dominant over mountain chickadees in almost all circumstances. In aviary trials comparing responsiveness of either species to flock-rallying *chick-a-dee* calls, I found that mountain chickadees respond equivalently to calls of either species, whereas black-capped chickadee reply significantly more to their own species call than to heterospecific call. This suggests these two closely-related species may differ in their perception of the relative threat associated with intra versus interspecific competition.

During the breeding season, I compared dawn singing behaviour (a mating display given by males during the female fertility period) recorded from both the JPRF and allopatric populations of either species. Mountain chickadees within the contact zone alter both their vocalization patterns and structure to a greater extent than black-capped chickadees; compared to allopatric populations, sympatric mountain chickadees use *chick-a-dee* calls to a greater extent during the chorus, and alter the frequency structure of their whistled songs, shifting these away from the structure of black-capped songs. Such a situation illustrates a character displacement scenario from the subordinate species where closely related species occur in sympatry. As females appear to use the dawn chorus to make extra-pair mating decisions, character displacement might constitute a species isolation mechanism.

Though breeding territories overlap, I found black-capped and mountain chickadees segregate by microhabitat during the breeding season. Black-capped chickadees are more likely to use deciduous trees and mountain chickadees use more coniferous trees for nesting. Habitat around the nest tree also differs between species in vegetation composition. Despite these differences in microhabitat, both species have similar reproductive success: the subordinate mountain chickadees are not pushed into less preferred habitat. Mountain chickadees, however, breed on average six days later than black-capped chickadees, which might aide in reducing competition for prey during peak periods of nestling demand.

RESUMÉ

L’hybridation se produit au moins occasionnellement chez plus de 9% des espèces d'oiseaux. Au sein des Paridés (les mésanges), l'hybridation a été reportée aussi bien parmi les espèces de l'Ancien Monde qu’en Amérique du nord. Bien qu'en Europe on retrouve un plus grand nombre d'espèces de mésanges au sein du même habitat, l`hybridation est moins fréquente : ces espèces peuvent avoir développé des mécanismes de renforcement spécifiques afin de maintenir des espèces distinctes. En Amérique du Nord, peu de recherches ont porté sur le potentiel d'hybridation entre les deux espèces génétiquement les plus proches: la mésange à tête noire (*Poecile atricapillus*) et la mésange des montagnes (*P. gambelli*). Mon doctorat porte sur les relations interspécifiques et l'hybridation potentielle entre ces deux espèces au nord de la Colombie-Britannique au Canada. Je me suis concentrée sur les mécanismes comportementaux qui peuvent limiter la reproduction entre espèces.

Grâce à des analyses d'ADN nucléaire et mitochondrial, j'ai mis en évidence la présence d`hybrides (adultes et oisillons) entre la mésange à tête noire et la mésange des montagnes au sein de mon site d’étude. Tous sauf un de ces hybrides présentaient le phenotype ‘mésanges des montagnes’ avec de l’ADN de mésange à tête noire dans leur génome. Aussi, les oisillons hybrides ont été trouvés uniquement dans des nids de mésange des montagnes, résultant d’un phénomène de cocufiage : les femelles de mésanges des montagnes se sont reproduites avec des males de mésange à tête noire.

Le statut social influençant le choix du partenaire chez les mésanges, la hiérarchie interspécifique peut interférer avec les interactions interspécifiques et éventuellement promouvoir l'hybridation. A partir d’observations sur le terrain et d’expériences en volières, j’ai montré que les mésanges à tête noire sont dominantes sur les mésanges des montagnes.

De plus, lors d’expériences en volière j’ai démontré que les mésanges des montagnes répondent de manière équivalente aux cris des deux espèces, tandis que les mésanges à tête noire répondent plus aux cris de leur propre espèce qu’aux cris des mésanges des montagnes. Ainsi la mésange à tête noire semble ignorer la mésange des montagnes, alors que la mésange des montagnes perçoit les oiseaux des deux espèces comme d’éventuel compétiteur.

Pendant la saison de reproduction, j'ai comparé le chant de l'aube (parade donné par les mâles au moment ou les femelles sont fertiles) au sein de la zone de contact avec le chant de l’aube de populations allopatriques. Les mésanges des montagnes au sein de la zone de contact modifient à la fois leur type de vocalisation et la structure de leur chant par rapport à des populations allopatriques : elles utilisent plus de cris que de chants et la fréquence de leur chant est différente de celle des mésanges à tête noire uniquement lorsqu’elles se trouvent en présence de mésanges à tête noire. Comme les femelles semblent utiliser le chant de l’aube pour évaluer la qualité des males et choisir leur partenaire, cette modification du chant de l’aube pourrait constituer un mécanisme de renforcement spécifique.

Enfin, bien que les territoires de reproduction se chevauchent, j'ai montré que les mésanges des montagnes et les mésanges à tête noires utilisent différents microhabitats. Les mésanges à tête noire utilisent des feuillus alors que les mésanges des montagnes utilisent plutôt des conifères pour la nidification. L`habitat autour du nid diffère également en terme de végétation. En dépit de ces différences entre microhabitats, les deux espèces ont le mêmes succès reproducteur, indiquant que les mésanges des montagnes ne sont pas poussées dans des habitats de moindre qualité du fait de leur subordination. Les mésanges des montagnes, cependant, se reproduisent en moyenne six jours plus tard que les mésanges à tête noire, ce qui pourrait réduire la compétition pendant la période de nourrissage des oisillons.

CO-AUTHORSHIP

For all chapters in this thesis, I was the primary investigator, leading: the design of studies; collection of data; and, analysis of data. I wrote the initial drafts of all manuscripts and was responsible for incorporating comments and feedback on previous drafts into the final versions seen in this thesis. However, despite the use of first-person singular in writing the thesis, I would like to acknowledge that this work was not conducted in isolation. Thibault Grava assisted in all aspects of field work for this study, and without his assistance this work could not have been conducted; to acknowledge his contribution, he is included in authorship on all publications that stem from my work. In addition, genetic analysis associated with chapter 2 of this thesis was reliant on contributions and the expertise of Theresa Burg and several students in her lab at the University of Lethbridge. All these persons are included as co-authors in publication of Chapter 2. In addition, Regis Didier allowed me to use frass data he was collecting for an independent study, as well as assisted with chickadee recording, nest finding and monitoring that were integral for my research. He is listed as a co-author on several chapters for his contributions to my work. Finally, my supervisor, Ken Otter, contributed to experimental design, data analysis and writing on all studies included in this thesis, and is included in authorship on all resulting manuscripts.

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1- GENERAL INTRODUCTION

The functional definition of ‘species” has long been debated among botanists, invertebrate zoologists and vertebrate zoologists (Wheeler & Meier 2000) and is known as “the species problem”. Mayden (1997) listed 22 different species concepts within the literature. Most vertebrate zoologists utilize the *Biological Species Concept* defined by Mayr (1942), whereby a species is defined as a group of actually or potentially interbreeding natural populations that are reproductively isolated from other such groups. This definition has mainly been criticized for failing to define species among asexually-reproducing organisms; such organisms form clonal groups, rather than populations of individuals, and there is little potential for interbreeding. Similarly, the *Biological Species Concept* is difficult to apply to fossil organisms, where the potential of divergent forms to interbreed is impossible to determine. Yet, despite these misgivings, the *Biological Species Concept* is applicable to vertebrates, and most scientists who work on these organisms agree that species can be defined on the basis of the potential for two populations to interbreed and produce viable offspring.

Emergence of new species is a gradual phenomenon; in nature, populations are constantly changing and, without genetic exchange with other populations, could begin to diverge from other populations and become separate species. While there are several possible mechanisms that cause species divergence (speciation), three processes - allopatric, parapatric and sympatric speciation - have received the most theoretical and empirical work. Allopatric speciation occurs when an ancestral population becomes segregated by a physical barrier, resulting in two or more geographically-isolated subpopulations. Sympatric speciation occurs when radical changes in the genome of one or more subpopulations reproductively isolate these groups from the parent population, despite overlapping distributions. Parapatric speciation occurs when a part of the population occupies a new niche and becomes reproductively isolated from the rest of the population. Each mechanism relies upon isolation of breeding units, followed by continued divergence of breeding units from one another that further enhances the separation. This eventually leads to incompatibility between groups, such that they fail to interbreed even if given opportunity to do so.

Divergence leading to the level of complete speciation typically exceeds the observational time-frame of human studies. However, during the early 20th century, biologists such as Jordan (1905) and Mayr (1942) inferred speciation indirectly by observing geographical variation among closely related species occurring on either side of geographically-adjacent regions, but which were separated by a dispersal barrier. Divergence of form in either location was explained by slightly different selective pressures in each region, which would eventually lead to enough accumulated change to establish behavioural or physiological barriers to interbreeding. Such observations shed light on understanding allopatric speciation, but do not fully explain how sympatric nor parapatric speciation occur. The difficulty in determining the causal mechanisms of speciation events is that we usually observe only the outcome of the process, not the common ancestor or the intermediate forms.

1.1 Ring Species and Speciation

One situation constitutes an illustration in space of a speciation phenomenon that can be used to infer variation in time: ring species (Irwin et al. 2001). Ring species (Cain 1954) are a connected series of neighbouring, interbreeding populations whose distribution expands, but also diverges, around some type of physical barrier. Thus, the population distribution occurs in a ring around some uninhabitable central area, with the two outer arms of the ring coming into secondary contact at the far end of their distribution. The two terminal populations in the series, however, have diverged sufficiently during these separate range expansions that they are now too distantly related to interbreed. Such terminal populations can either coexist or be geographically isolated by the series of intermediate populations. Around the ring, the traits of one of the end species change gradually through each intermediate population into the traits associated with the population at the opposite end of the distribution. Throughout this continuous cline, the neighbouring populations are fully able to interbreed. It is only at the two terminus populations that the full extent of divergence is evident, and results in failure of the populations to interbreed.

Two ring species have been well studied and described since the 1930s. The Asian greenish warbler group (*Phylloscopus trochiloises*) were first described by Ticehurst (1938); ancestors of this complex spread from south of the Himalayas east and west around the Tibetan Plateau, the centre of which is uninhabitable to the warblers. North of this plateau, the two terminal populations come into secondary contact – the two forms coexist but are reproductively isolated, primarily through divergent songs that function in mate recognition (Irwin et al. 2001). The Ensatina salamanders group (*Ensatina eschscholtzii*) in northern California expand southward along two fronts (Sierra Nevada mountains and the coastal mountains). Where those two fronts meet in southern California, the two terminal populations have distinct colour patterns and are reproductively isolated (Stebbins 1949). Complementary genetic studies have now confirmed these associations between geographical variation and genetic relationships for both the salamander group (e.g. Wake & Yanev 1986, Wake & Schneider 1998) and the warbler group (Irwin et al. 2001).

The greenish warblers' song or the Ensatina salamanders' colour pattern are characteristics that seem to hinder interbreeding between the two closely related species. Such factors that are known to maintain species isolation following a speciation event are called species isolating mechanisms (Mayr 1970, Mallet 1998). Reproductive isolation can be pre-zygotic, preventing breeding from occurring or fertilization from ensuing – these include segregation in range distribution between populations that prevent the distinct groups from encountering one another, or species-specific mating displays that encourage mate-choice within groups. Alternately, reproductive isolating mechanisms can be post-zygotic, such as hybrid sterility or reduced viability of hybrid offspring. While hybrid pairings can produce fertile offspring (Grant & Grant 1997), hybrids may suffer reduced viability and reproductive success if they are morphologically and ecologically intermediate to parental species, especially in circumstances where these differ in niche ecology. Pre and post-zygotic species isolation mechanisms can play a role at specific, critical periods or occur year round, preventing hybrid matings. Niche segregation will prevent interspecific mating by keeping two species isolated during both the breeding season and the non-breeding season. Further, differences in secondary sexual characters, such as plumage in birds, are likely to prevent interspecific interactions (i.e., mate choice) particularly during the breeding season. Sexual selection can then promote reproductive isolation: if hybrids suffer from reduced fitness, then individuals that choose mates exhibiting characteristics of their own species will have greater reproductive success (Dobzhansky 1951, Howard 1993). Such reinforcement of these characteristics during mate choice increases species discrimination and hinders maladaptive interspecific mating. Selection decreases the potential for hybridization by selecting individuals that are able to recognized conspecifics, and preferentially choosing these over heterospecific as mates. This differential mate selection is presumed to be strongest in areas where both species overlap. Noor (1999) reviewed examples of reinforcement across taxa, such as in the contact zone between pied (*Ficedula hypoleuca*) and collared flycatchers (*F. Alibicollis*). When pied flycatchers are sympatric with collared flycatchers, their plumage is brown; whereas, in allopatric populations, their plumage is contrasting black and white. This color change is associated with differences in female pied flycatcher preferences between areas of allopatry vs sympatry (Saetre et al. 1997).

These species-isolating mechanisms allow reproductive isolation between populations in sympatric and parapatric speciation, or if secondary contact occurs following allopatric speciation. Among the greenish warbler group, differences in song provide a pre-zygotic species isolation mechanism. However, if such closely related species come into secondary contact, and isolating mechanisms are not sufficiently well developed, there is potential for hybridization, which might in turn affect species integrity.

1.2 Hybridization

Hybridization is fairly common in many taxa, with vascular plants being more likely to hybridize than animals (Mayr 1963). However, within the kingdom Animalia, few groups are known to hybridize as frequently as the vascular plants where hybridization occurs between up to 25% of the species (Mallet 2005). Hybridization rate for the passion flower butterflies (*Heliconius* sp.) is known to be between 25 and 28% (Mallet et al. 1998). Hybridization in birds worldwide occurs occasionally in more than 9% of species (Grant &

Grant 1992), and in more than 75% of the species within some groups such as the duck species (Anatinae) in Britain (reviewed in Mallet 2005).

Physiological and behavioural differences that emerge when species are isolated in allopatric speciation may be reinforced should the species distributions again become sympatric, preventing hybridization. Emlen et al. (1975) reported that indigo and lazuli buntings (*Passerina cyanea* and *P. amoena*) respond aggressively to heterospecific song in sympatry but not in allopatry. They attributed this behaviour with a learned response to an ecological competitor in these overlapping populations. These two species do occasionally hybridize, so heterospecific males may also be viewed as competitors for mates (Emlen et al. 1975).

There appear to be examples among closely related species where behavioural isolating mechanisms prevent hybrid matings, even when fertile offspring may result. For example, common chaffinches (*Fringilla coelebs*) and blue chaffinches (*F. teydea*) do not respond to heterospecific songs when they occur in sympatry and do not hybridize, possibly because the two species use different ecological resources (Lynch and Baker 1990). However, if the isolating mechanisms are not sufficiently strong so as to hinder hybridization, and hybrid viability is not compromised, distinct species may integrate and reduce overall biodiversity. Such consequences may occur naturally, but of greater concern are anthropocentrically-generated situations that create sympatry and species-integration. For example, blue-winged (*Verminora pinus)* and golden-winged (*V. chrysophera*) warblers became sympatric due to land-use practices in the eastern US: abandonment of fields and/or reforestation has induced a northward shift of the blue-winged warblers range into the golden-winged range. The population of the latter species has since declined (Gill 1980, 1997). These two species hybridize frequently, the hybrids are fertile, and backcrossing has been observed. The exact causes of the decline of the golden-winged population remain unknown; it may be the result of direct competition, habitat perturbation, hybridization or a combination of these factors (Confer 1998).

1.3 Interspecific Interactions and Hybridization within the Paridae Family

Hybridization occurs in the Paridae (chickadees and titmice) both in North America and Europe (Curry 2005) involving some 25% of the species (Mallet 2005). The Paridae family is composed of ~51 species present across the northern hemisphere and Africa (Gill et al. 2005). Phylogenetic studies (Gill et al. 1993, Gill et al. 2005) have confirmed the old world center of origin as Mayr & Short (1970) suggested. Based on *cytochrome-b* gene sequence and DNA hybridization data, Gill et al. (2005) showed that new world species originated from two successive invasions: 4 million years ago (mya) the ancestor of the European crested tits (subgenus *Lophophanes*) gave rise to the North American crested titmice (subgenus *Baeolophus*) and 3.5 mya North American chickadees (subgenus *Poecile*) radiated from a common ancestor to two Eurasian species, the willow tit (*Poecile montanus*) and the marsh tit (*P. palustris*).

Europe has a larger number of sympatric Paridae species than North America. These overlapping species may have developed isolating mechanisms to decrease hybridization and as a result maintain distinct species. Several studies have been conducted on the interspecific interaction among sympatric populations of tits in Europe such as between blue tit (*Cyanistes caeruleus*) and great tit (*Parus major*) (e.g. Dhondt 1980, Erokene et al. 1998, Doutrelant et al. 2000 a&b, Hansen & Slagsvold 2004) or between willow tit (*Poecile montanus*) and crested tits (*Lophophanes cristatus*) (e.g. Alatalo 1982). Dhondt (1989) reviewed interspecific competition amongst the Paridae, mainly between the old world species. In North America, there is less multi-species overlap; distribution ranges tend to be more parapatric than sympatric (Dhondt 2007), which may mean that mechanisms to prevent hybridization within a contact zone have had less opportunity to evolve (Curry 2005).

In the chickadee family, black-capped and Carolina chickadee (*Poecile atricapillus* and *P. carolinensis*) hybridization is well studied (e.g. Brewer 1963, Braun & Robbins 1986, Curry 2005). Both species are widely distributed, with black-capped chickadees being more northerly and Carolina's more southerly in their ranges across North America. The distribution includes a large sympatric contact zone across the north-eastern US, and several studies show both genetic and behavioural evidence of hybridization (e.g. Bronson et al. 2003 a&b, Curry 2005, Reudink et al. 2006, Reudink et al. 2007). However, these two species are not the most closely related species within the chickadee genus (Gill et al. 2005). Little research, has focused on potential hybridization in more genetically-similar sister-species, such as the black-crested and tufted titmouse (*B. atricristatus* and *bicolour*) (Banks et al. 2002) and the black-capped and mountain chickadee (*P. gambelli*) (Minock 1972, Hill & Lein 1988, Hill & Lein 1989 a&b).

Despite their genetic and behavioural similarity, black-capped and mountain chickadees do not appear to frequently hybridize. Black-capped chickadees are found in a variety of habitats from the Atlantic to Pacific Coasts in the southern part of Canada and northern half of the United States (fig 1.1; Foote et al. 2010). The mountain chickadee has a more restricted distribution and is found only west of the Rocky Mountains, where they occur in higher elevation areas and more coniferous forests than those favoured by black-capped chickadees (fig 1.1; McCallum et al. 1999).

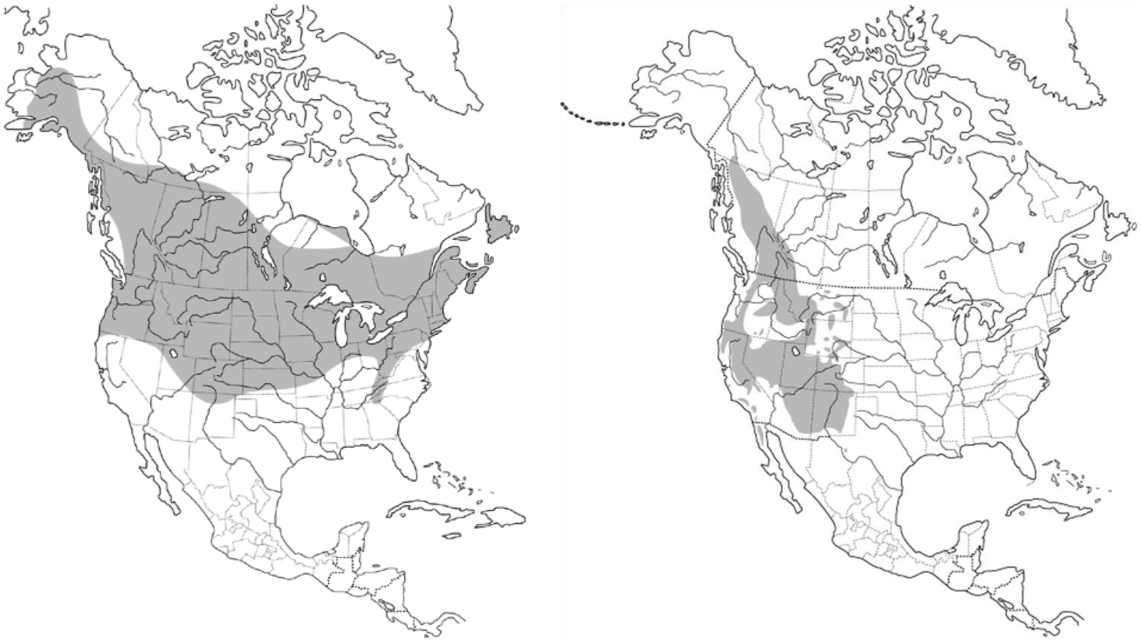

Figure 1.1: Distribution ranges of Black-capped chickadees (left – from Foote et al. 2010) and mountain chickadees (right – from McCallum et al. 1999). Reprinted with permission from "Birds of North America Online" http://bna.birds.cornell.edu/bna

Although their geographic ranges overlap, populations of either species are typically allopatric at local scales, with mountain chickadees occupying dry conifer forests, whereas black-capped chickadees are found in mixed forests with much higher deciduous component (McCallum et al. 1999, Foote et al. 2010). Therefore, a primary isolating mechanisms seems to rely upon ecological segregation. However, the behaviour of the two species seems to be relatively similar even if there is much less research on mountain chickadees than on black-capped chickadees. Anecdotally, hybridization is known to occur in situations where the two species' distribution overlaps (Braun & Robbins 1986, McCallum et al. 1999). However, such reported hybrids are still rare, likely because sympatry between these two groups is less common. Such sympatry does occur, however, and circumstances that induce this overlapping may have anthropogenic roots.

1.4 Study Site

The John Prince Research Forest (hereafter JPRF) in central British Columbia (N54° 40'- W124° 24'), Canada constitutes a contact zone between black-capped and mountain chickadees. This research forest is a mid-elevation site (~800 to 850m above sea level) consisting of a mosaic of habitats resulting from different levels of commercial forestry over the past century. The 13000 ha research forest contains patches composed of both coniferous species (Douglas fir *Pseudotsuga menziesii*, hybrid white spruce *Picea glauca* X *engelmannii*, lodgepole pine *Pinus contorta* var. *latifolia*) and deciduous trees (black cottonwood *Populus balsamifera*, paper birch *Betula papyrifera*, trembling aspen *Populus tremuloides*).

Over the last century, timber harvesting in the sub-boreal forest has created this patchwork of divergent habitat types (Bernsohn 1981). In the JPRF, lumber operations in the 1940s were focused on harvesting Douglas-fir trees used for building permanent structures associated with mining activities. Following this period, all available tree species were harvested as fuel for the mine, with a preference for the Douglas-fir (MacGregor 2002). The resulting regeneration was biased towards deciduous species, inducing an increase of black-capped-preferred habitat amongst the mountain-preferred coniferous forests. This variation in vegetation distribution may have induced a greater invasion of black-capped chickadee into areas traditionally populated by mountain chickadees. Since the early 1970s, around 1400ha of clear cutting has occurred in the JPRF with openings ranging in size from 7 to 280ha and averaging 100ha (Grainger 2002). Over the last 60 years, forty one percent of the research forest has been harvested using a variety of silviculture management (MacGregor 2002). This small-scale clear cutting spread over the landscape has created a mosaic patchwork of habitat with areas typical of mountain chickadee abutting black-capped chickadee-preferred habitat, creating circumstances where the two species come into contact.

Neighbouring stands in this working forest have populations of either black-capped chickadees (mainly in stands of mature deciduous forest of trembling aspen and paper birch), or mountain chickadees (predominantly Douglas fir or hybrid white spruce forests). Other stands have overlapping populations of both species of chickadee, especially in areas with both coniferous and deciduous components (pers obs). A complete transition in the dominant species within a forest patch can occur over as little as 500m. It has long been hypothesized, that in such overlapping populations mountain chickadees and black-capped chickadees would hybridize, similar to the extensively-studied hybridization between black-capped chickadees and Carolina chickadees in the US (Curry 2005). However, there are few visual clues in the birds that have been banded at the research forest in the past few years to indicate that hybrids are common.

I studied a banded overlapping population of mountain and black-capped chickadees within the JPRF between 2007-2010. I compared the behavioural patterns of mountain and black-capped chickadees in this sympatric population with behaviours among two allopatric populations – one population of mountain chickadees and one of black-capped chickadees (fig 1.2). Both populations were subjects of long-term studies for at least a five year period (Martin & Norris 2007, Otter et al. 2007) and these two populations were considered as pure populations as 95% of the chickadees were from one species only, with few incidental occurrences of the other species. The pure black-capped chickadee population is situated in Prince George (130km from JPRF), Canada (N53°53’- W122°48’). It is a mixed forest with higher deciduous component (paper birch, trembling aspen) and some coniferous trees (mainly lodgepole pine and hybrid white spruce). Elevation is between 700 and 750 m above sea level. The pure mountain chickadee population is located at Riske Creek (300km from JPRF), Canada (N51°57- W122°30) and is composed of forest stands (mainly Douglas-fir, lodgepole pine, and few trembling aspen) surrounded by grassland. Elevation is between 900-950m above sea level in the studied areas.

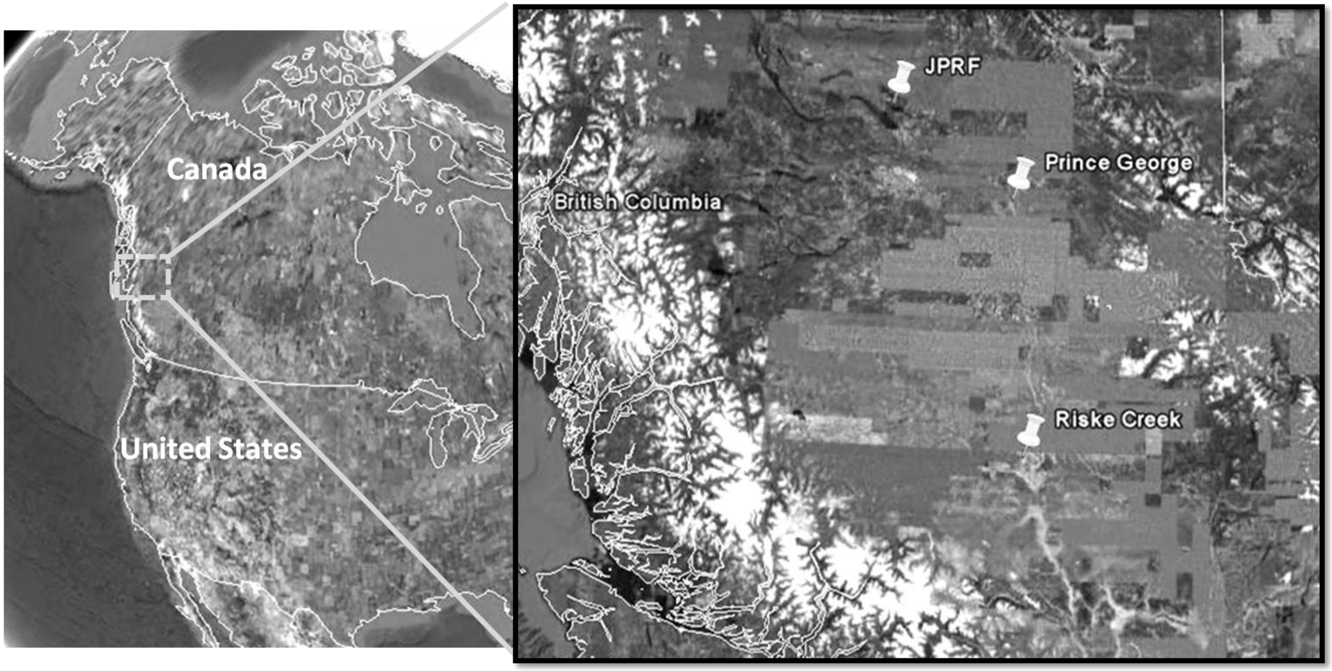

Figure 1.2: Map representing the study area where the two species come into contact (JPRF), the pure black-capped chickadee population (Prince George) and the pure mountain chickadee population (Riske Creek). (images from Google Earth)

1.5 Outline of Thesis and Objectives

My PhD research focuses on the interspecific relationships and potential hybridization between black-capped chickadees and mountain chickadees in forest plots at the John Prince Research Forest (JPRF). I focused my work on potential for hybridization (Chapter 2) and the impact of behavioural mechanisms that may reinforce species isolation, such as interspecific dominance-interactions (Chapter 2) and singing-behaviour (Chapter 3 & 4). As these two species are thought to have diverged about 2.5 mya (Gill et al. 1993), species isolation mechanisms might not be strong enough to hinder hybridization when black-capped and mountain chickadee distributions overlap. Using genetic analysis (mtDNA and microsatellite markers), I determined the amount of intermixing that is occurring between species, and identified whether this genetic mixing results from inter-pair matings or from extra-pair copulations (Chapter 2). By observing birds during both the breeding and the non-breeding seasons, I was able to identify which behaviours, if any, might be responsible to maintain species integrity and to which extent these behaviours might interfere with reproduction. Dominance status and dawn chorus behaviour are known to influence mate choice and reproductive success in black-capped chickadees (e.g. Mennill & Otter 2007, Ratcliff et al. 2007). If similar mechanisms of mate choice have evolved in mountain chickadees, overlap between the two species may result in occasional mis-extension of these mating preferences across species. To determine whether there is asymmetry in competitive abilities between these two species, I observed interspecific interactions and the resulting social hierarchies both in winter flocks and in aviary trials (Chapter 2). I conducted experiments in aviaries to test black-capped and mountain chickadees responsiveness to conspecific and heterospecific calls (Chapter 3) to assess whether or not either species perceive heterospecifics as competitors for resources. I then focused on the breeding season, comparing dawn chorus behaviour between species in both the overlapping zone and with two pure populations of either species (Chapter 4) to determine whether there was evidence of character displacement in signals. Finally, I monitored the extent of niche partitioning vs direct competition for resources in breeding territories and nest sites between these species (Chapter 5). I determined breeding timing, reproductive success and provisioning rate for both species in the contact zone to determine whether interspecific interactions affected either species' reproductive performance, or whether niche partitioning during the breeding season reduced this aspect of interspecific competition (Chapter 5).

By comparing black-capped chickadee and mountain chickadee behaviour at key intervals throughout the winter and spring, my goal was to understand the level of interspecific interactions that were occurring between these two closely related species and how this related to potential for competition both ecologically and reproductively. Through genetic analysis, I determined whether such interactions could be leading to hybridization, especially where species mate choice behaviour (through selection for similar sexually-selected traits) may transcend species. I then looked for evidence of character displacement and niche partitioning that might be under selection to reduce the potential for hybridization.

2- INTERSPECIFIC DOMINANCE AND HYBRIDIZATION BETWEEN BLACK-CAPPED CHICKADEE AND MOUNTAIN CHICKADEE

Abstract- Black-capped chickadees (*Poecile atricapillus*) and mountain chickadees (*P. gambeli*) are ecologically segregated due to differences in habitat preference. However, forestry practices in north-western Canada have created a mosaic of coniferous (mountain chickadee habitat) and deciduous forest patches (black-capped habitat), which might explain the observed regional sympatry between these two closely related species. In *Poecile* species, social hierarchies amongst conspecific individuals influence life-history parameters such as mate-choice. As a result, interspecific social hierarchy might drive hybridization between these two closely related species. By conducting field observations and aviary experiments, I demonstrated that black-capped chickadees are dominant over mountain chickadees. Using a combination of species-specific phenotypes (plumage), mtDNA to assess maternal genotype and microsatellite markers, I confirmed that genetic mixing occurs within our contact zone, but that the pattern of parentage appears directional. All but one of the adult hybrids was phenotypically identified as mountain chickadee and had mountain chickadee mtDNA. Further, all nestlings where microsatellites detected mixed-species ancestry were from mountain chickadee nests with both attending parents having mountain phenotypes. All mtDNA from these nestlings was mountain chickadee except for one individual, and in all cases these nestlings showed genetic patterns of having arisen through extra-pair copulations between female mountain and male black-capped chickadees. Our results suggest that hybridization may result from males of the mountain chickadees having lower expression of a preferred trait (dominance) than the black-capped chickadees.

2.1 Introduction

Within species of the family Paridae (chickadees and titmice), dominance rank in winter flocks is known to drive mate choice (Otter et al. 1998, Mennill et al. 2004), breeding success (Otter et al. 1999), overwinter survival and access to resources (Desrochers 1989, Ficken et al. 1990). Females paired with dominant males may also benefit from more secured and undisturbed foraging from other flock members under the protection provided by the female's mate (Hogstad 1988, Hogstad 1992, Lemmon et al. 1997). These benefits to females, both in winter resources and nesting success, may partially explain female preference within-species for high-ranking males as social mate and/or extra-pair partners (Otter & Ratcliffe 1996, Ratcliffe et al. 2007). Yet the benefits from relative dominance relationships may not be restricted to within-species (intraspecific) interactions.

In Europe, up to six different species within the Paridae family can live in sympatric populations (Dhondt 2007). Relative dominance relationships between these species exist within mixed flocks, and can result in not only competition over food but also nesting sites (Dhondt 1989). In contrast, many North American parids are parapatric and it is rare to have more than two chickadee species (*Poecile* spp.*)* overlapping in the same zone (Dhondt 2007). North American parids form winter flocks where the intraspecific dominance relationships are both stable and linear (Ekman 1989), and this stability can also extend to interspecific relationships in regions where overlap occurs. For example, within the contact zone between Carolina chickadees (*Poecile carolinensis*) and black-capped chickadees (*P. atricapillus*) in eastern North America, aviary experiments showed that Carolina chickadees tend to be dominant over the black-capped chickadees (Bronson et al. 2003b). In addition to potential competition for food and nesting resources, interspecific hierarchies may influence mate choice; Bronson et al. (2003 b) found female Carolina and black-capped chickadees tend to preferentially associate with the dominant males in aviary trials regardless of their species relative to the female.

Even though some clarifications are still needed, most studies suggest that mountain chickadees (*P. gambeli*) and black-capped chickadees are sister species within the black-headed chickadee clade (Gill et al. 1993, Gill et al. 2005). They are typically allopatric at local scales due to ecological segregation: mountain chickadees prefer high elevation, dry conifer forests, whereas black-capped chickadees are associated with lower elevations and mixed forests with much higher deciduous component (Foote et al. 2010, McCallum et al. 1999). As a result, areas of sympatric contact along an altitudinal gradient do occur between these two species in western North America. Within species, both mountain chickadees and black-capped chickadees share a similar social hierarchy organization. They overwinter in flocks with stable and linear hierarchies: males are dominant over females and juveniles are typically subordinate to adult birds within these sex-classes (Minock 1972, Ekman 1989; Smith 1991). Previous studies in contact zones suggested that black-capped chickadees are dominant to mountain chickadees (Minock 1972, Hill & Lein 1989b). Minock (1972) found that while black-capped chickadees typically dominate mountain chickadees at winter feeding stations, there were a number of cases where mountain chickadees dominated interactions (about 20% of observed encounters). Both studies were unable to control for the effect of age and sex of interactants, factors known to influence dominance relationships in several parids (Ekman 1989, Smith 1991, McCallum et al. 1999). If a stable and linear interspecific hierarchy does exist among these two sister species, the general preference of females for dominant males may extend to heterospecifics (Bronson et al. 2003b), and could drive hybridization through cross-species mate choice.

Our objective was to observe interactions between wintering mountain and black-capped chickadees in a contact zone in northern British Columbia, Canada, to determine relative interspecific hierarchies within mixed winter flocks. I observed natural encounters at temporary winter feeders of both intraspecific and interspecific interactions among individually banded birds for which age and sex had previously been determined. Third-party effects, such as audience of observers or the presence of a dominant mate in the vicinity can influence the outcome of natural encounters: Hogstad (1992) showed that the female mated to the alpha male experienced less aggression from the other flocks members and had both increased foraging time and decreased vigilance rates when her mate was close by (less than 5 m). As a result, I also paired birds in aviaries to confirm the assessment of relative interspecific dominance relationships.

I then used plumage, mtDNA and microsatellite analyses to distinguish between mountain and black-capped genotypes and phenotypes of both adults and nestlings from the study site, in comparison to two single-species control populations, to determine the amount of hybridization in our contact zone. As mtDNA is maternally inherited, it allowed me to determine the maternal genotype for all individuals tested, which I compared to individual’s plumage patterns, as these two species are dimorphic for plumage patterns. Individuals with plumage phenotype of one species, but mtDNA of the other species, would indicate introgression. Using genetic differences between the two species, microsatellite analysis allowed us to detect mixed-species parentage in adults. Further, as all nests in our population had conspecific social pairs, microsatellite analysis allowed us to determine whether nestlings from these nests showed mixed-species parentage (arising from extra-pair behaviour).

2.2 Methods

2.2.1. Study species and study site

In the fall and early winter of 2007-2008 and 2008-2009, I collected blood and banded birds with a unique combination of one Canadian Wildlife Service numbered aluminium band and three plastic coloured bands at the JPRF, in northern British Columbia, Canada (54° 40’ N-124° 24’ W). Black-capped chickadees and mountain chickadees are easily distinguished based on plumage patterns, the main differences being the presence of a white supercilliary line in the mountain chickadee, which is absent in the black-capped chickadee. Further, black-capped chickadees have prominent white edges to the secondary feathers that are lacking on mountain chickadees. I classified birds as phenotypically mountain chickadee or black-capped chickadee based on species-characteristic plumage. I determined the sex of the birds by using a combination of body measurements (wing chord, tail, tarsal length and weight), males being larger than female in both species (McCallum et al. 1999, Foote et al. 2010), and confirmed these assessments during the breeding season with sex-specific behaviour (e.g. male feeding its mate). Age was determined using the shape and the colour pattern of the outermost rectrix (Pyle 1997). I also used long-term data sets for aging; as most birds at the study sites are banded in their first fall/winter, multi-year banding records allow us to identify adults from juvenile birds in both species.

2.2.2. Field observations

Temporary feeding stations were set up in March - April 2008 and January - February 2009. I observed birds interacting at these feeders for periods lasting from 0.5 to 2 hours, depending on the number of birds and/or number of interactions (observations were longer when more birds were present, or more interactions were occurring). I recorded both interspecific and intraspecific interactions to determine the social hierarchy within species and across species. I used four different behaviours to determine the relative rank of two interacting birds: (1) chase - the focal bird chases away its opponent, (2) supplant - the focal bird supplants its opponent, (3) submissive posture - the focal bird gives a display that elicits a submissive posture from an opponent, and (4) wait - the opponent waits for the focal bird to leave before approaching the feeder. These behaviours are often associated with each other (e.g. submissive postures often follow being supplanted) and the focal bird was considered dominant over its opponent if any of these behaviours were witnessed (Ficken et al 1990, Otter et al. 1998, Ratcliffe et al. 2007). I also recorded the number of birds of each species and every visit to the feeder to control for frequency of interactions in relation to differential use of feeders by either species.

2.2.3. Aviary experiment

I conducted aviary experiments in late winter (February and March 2009) to determine the interspecific social hierarchy. I paired the birds by sex and age to control for likely effects of these two parameters. One bird of each species was caught in its flock territory (using mist nets or potter traps) during the day and immediately transported to and released into the aviary. To ensure that birds had no previous contact with each other, I paired birds caught from territories at least 3 km apart. I kept the birds overnight to let them acclimate to the aviary conditions and ran the experiment the next morning.

The aviary was divided into three different compartments. Each outside compartment was provided with unlimited food (sunflower seeds) and shelters (tree and nestbox). The central compartment was used to run the experiment; sliding walls allowed us to open the two outside compartments housing either bird to allow them access to the central compartment, and create visual contact between the two individuals. A feeder was set up in the middle of the central compartment prior to starting trials, with a mesh divider (1 cm x 1 cm plastic garden mesh) centred on the feeder to allow visual contact and close proximity over the resource, but preventing physical contact. The food source from the outer compartments was removed 1 hour before the trials started. To start the trials, I opened the sliding barriers allowing the birds to interact around the central feeder. Each trial lasted one hour and I recorded the number of visits to the feeder and agonistic interactions (chases, supplants, waits and submissive postures). After 1 hour of observations, the birds were isolated in their respective compartment, caught and released into their flock’s territory (no bird was held more than 24 hours).

2.2.4. Genetic analysis

Due to difficulty of accessing the natural cavities in which the mountain chickadees nest, I assessed evidence and origin of mixed parentage in both adult and nestlings from the studied contact zone using a combination of expressed phenotype (plumage) versus maternal genotype (mtDNA) and microsatellites markers.

I sampled individuals from two pure populations to identify species-specific genetic patterns for both mountain chickadees (N=26 Riske Creek, BC, 51° 57’ N - 122° 30’ W) and black-capped chickadees (N=30 Prince George, BC, 53° 53’ N - 122° 48’ W). These two populations were considered as pure populations: 95% of the chickadees were from one species only, with few incidental occurrence of the other. These totals are based upon at least 5 years of population monitoring in either population (Otter et al. 2007, KM. Martin, pers comm.). For either reference population, there were no phenotypic indications of mixing between the species.

DNA was extracted from 5 μl of blood-ethanol mix using standard chelex extraction (Walsh et al. 1991). For each individual the mitochondrial control region was sequenced and genotypes were obtained for six microsatellite loci. MtDNA sequences were used to assess the maternal linage of each bird. In species where hybridization occurs, the phenotype does not always match the mtDNA. For example hybridization between hermit and Townsend’s warblers has resulted in phenotypically pure Townsend’s warblers outside of the hermit warbler range containing hermit warbler mitochondrial DNA (Rohwer et al. 2001). MtDNA for the control region was amplified using 2 μM each LbcchCR1 (CCA CCA CCC CAT AAT AAG GA) and HCRCbox (CCA CTT GTA TCT GTG ARG AGC) primer, 200 μM dNTPs, 2.5 mM $MgCl_2$ and 2.5 U *Taq* polymerase in *Promega Flexi* buffer. The thermal profile was 94 ^oC for 120 s, 50 ^oC for 45 s, 72^oC for one cycle, followed by 37 cycles of 94^oC for 30 s, 54^oC for 45 s and 72^oC for 60 s and a final step of 72^oC for 300 s and 4^oC for 20 s. Samples were sequenced on an ABI 3130 sequencer using a BigDye terminator kit following removal of unincorporated primers and dNTPs using Exo-SAP (exonuclease and shrimp alkaline phosphatase). Sequencing reactions were cleaned using sodium acetate precipitation prior to injection of the sequencing reaction.

Six avian microsatellites were used for genotyping: Ppi2 (Martinez et al. 1999), Titgata39 (Wang et al. 2005), Titgata02 (Wang et al. 2005), Pdo5 (Griffith et al. 1999), Escu6 (Hanotte et al. 1994), and Pat 14 (Otter et al. 1998). PCR cocktail contained 0.05 µM of a fluorescently labelled M13 primer (700 or 800 nm wavelength), 2 µM of the forward and reverse primer, 200 µM dNTP, $MgCl_2$, 0.5 U of *Taq* polymerase in a 1x PCR buffer. A 2 mM $MgCl_2$ concentration was used for four loci, the exceptions being Ppi2 (1.5 mM) and Escu6 (1 mM). The 5' end of each forward primer was modified with the addition of M13 sequence (CAC GAC GTT GTA AAA CGA C) to allow for direct incorporation of a fluorescently labelled M13 primer (Burg et al. 2006). Three loci (Titgata39, Escu6 and Ppi2) were amplified using a two-step annealing procedure: one cycle for 2 min at 94°C, 45 s at 50°C, 60 s at 72°C; seven cycles of 60 s at 94°C, 30 s at 50°C, 45 s at 72°C; 31 cycles of 30 s at 94°C, 30 s at 52°C, 45 s at 72°C; and one final cycle of 300 s at 72°C. The other three loci (Titgata02, Pdo5 and Pat14) were amplified using a similar two-step annealing process with seven cycles at 50°C and 25 cycles at 52°C. PCR products were run on a 6% acrylamide gel on a Licor 4300 (Licor Inc.). Individuals of known allele sizes, negative controls and a 50-350 bp size standard were included on each load/channel to ensure that alleles were sized consistently between gels. As alleles covered a range of sizes and alleles were sized using the size standard and a set of positive controls. All gels were scored manually by two different people.

2.2.5. Analyses

For the social hierarchy data, I conducted a combination of binomial tests and Fisher exact tests to compare numbers of observed interactions won by the different species and in different circumstances.

MtDNA sequences were visually aligned using MEGA4 (Tamura et al. 2007) and assigned as either mountain chickadee or black-capped chickadee based on sequence similarity to birds from the pure populations. The mountain and black-capped chickadee sequences were highly divergent and easily assigned to one of the two species (table 2.1). GenAlEx (Peakall & Smouse 2001) was used to test for deviations from Hardy-Weinberg and linkage equilibrium and to estimate standard diversity measurements for the microsatellite markers. STRUCTURE 2.3.3 (Falush et al. 2003, Pritchard et al. 2000) was used to estimate proportion membership of each individual to black-capped or mountain chickadee clusters (K=2). STRUCTURE uses genetic data to assign individuals to clusters based on their genotype and determine the probability of recent ancestry from each cluster. I used prior sampling information (phenotype) for the birds from ‘pure’ populations. For mixed populations and nestlings, I did not include any prior information. STRUCTURE uses this sampling information to help it assign individuals to each cluster, but the final assignment (i.e. ancestry coefficient) is based on the genetic data.

Table 2.1: Variable sites in the mitochondrial control region of black-capped (BC) and mountain chickadees (MO). Numbers represent the individual samples, and samples of both black-capped and mountain chickadees are derived from pure populations where >95% of birds in the area over multiple years of study were of one species only. A subset of samples are presented here to represent the general differences and not all of the variable sites are contained within these 12 individuals. Sites are numbers on the H strand relative to the position of the sequencing primer. Nucleotide similarity to the reference sample BC _08 is indicated by a ".".

	variable sites				
	11111	1222222223	3333333344	4444556666	67
	3468834669	9366667880	1222244513	3457161137	70
	9136838470	5723456091	1013779124	5975130373	69
BC_08	CCCAGCTCCG	TACTGGGTGT	CCTACTCATA	ATTGGCCTAA	TT
BC_09	....	....	....	....	..
BC_10	....	C	T.CG......	....	..
BC_11	....	....	....	....	..
BC_12	....A.....	C	T.CG......	....	.C
BC_13	....	...C.....C	T.CG......	....	..
MO_102	TTTGATCTTA	.GTCC.ACA.	.TCGTCG.AG	.AAAAGTCGG	CC
MO_103	TTTGATCTTA	.GTCC.ACA.	.TCGTCG.AG	.AAAAGTCGG	CC
MO_104	TTTGATCTTA	.GTCCAACA.	.TCGTCG.AG	.AAAAGTCGG	CC
MO_105	TTTGATCTTA	.GTCC.ACA.	.TCGTCG.AG	.AAAAGTCGG	CC
MO_106	TTTGATCTTA	.GTCCAACA.	.TCGTCGGAG	.AAAAGTCGG	CC
MO_107	TTTGATCTTA	C.TCCAAC..	.TCGTCG.AG	GAAAAGTCGG	CC

Only individuals with three or more genotypes were included and most individuals (85%) had genotypes for four or more loci. As STRUCTURE is sensitive to the inclusion of kin groups, separate runs were done for nest mates. For each dataset all of the adults and a maximum of one nestling from each nest were run using 20000 burnin, 50000 Markov Chain Monte Carlo runs, correlated allele frequencies and admixture. An additional set of runs was done using an adult only dataset. A total of 16 datasets were created as the maximum number of siblings was 16. Each dataset was run three times and results from all runs were averaged. As adults were run for each of the datasets, the ancestry coefficients (Q values) from all 51 runs were averaged. Using the ancestry coefficients from the pure populations, I determined a conservative threshold value for mixed ancestry. Black-capped chickadees from pure populations had an average Q value of 0.91 (range of 0.74-0.96) whereas mountain chickadees from the pure population had an average of 0.98 (0.97-0.99). The Q values of individuals in the dataset were bimodal in distribution, with individuals having either >0.74 or <0.62 values. As the lowest value for an individual in a pure population was 0.74, I set the threshold for inclusion halfway between the minimum value of the upper and maximum of the lower distributions; any individual with Q value of less than 0.68 assignment to one or the other species was investigated as being of mixed-species ancestry.

2.3. Results

2.3.1. Field observations

At temporary winter feeding stations, black-capped chickadees were generally dominant over their mountain chickadees counterparts (159 of 190 interactions - 83.7%;

binomial test p<0.0001) even though I observed some mountain chickadees dominating black-capped chickadees (31 interactions - 16.3%). To control for the effect of sex and age on interspecific encounters, I compared only those interactions between birds of known age and sex (table 2.2). Black-capped chickadees were dominant over their mountain chickadee counterparts when birds were matched by sex in 33 instances out of 37 (binomial test p<0.0005), by age in 26 out of 27 encounters (binomial test p<0.0005) and both sex and age in all cases (binomial test p<0.0005, n=15) (table 2.1). Further, when interactions contravened the typical patterns in chickadees, i.e. females dominating males (n=22) or juveniles dominating adults bird (n=33), the dominant female (binomial test p<0.0005) or the dominant juvenile (binomial test p<0.0005) was a black-capped chickadee outranking a mountain chickadee competitor in all but one instance (table 2.2).

Table 2.2: Interspecific interactions in natural environment between black-capped chickadee (BC) and mountain chickadee (M); when birds were 1- of the same sex, 2- the same age, 3- matched both by sex and age, 4- female fighting off a male and 5- a juvenile (second year, or SY) bird dominant over an adult (after-second year - ASY) bird (only interactions with birds of known sex and/or age are included in each comparison).

		BC dominant	M dominant
1-	paired by sex	33	4
	- ♂ vs ♂	18	0
	- ♀ vs ♀	15	4
2-	paired by age (adult birds only)	26	1
3-	paired by sex and age	15	0
4-	♀ dominant to ♂	21	1
5-	juvenile dominant to adult	33	0

2.3.2. Aviary experiment

Dyadic interactions in aviaries clearly revealed black-capped chickadees as the dominant species: out of 11 dyads, black-capped chickadees were dominant over mountain chickadees in all 11 cases (winning a combined total of 81 of 82 interactions observed during the 11 dyadic trials, binomial test, p=0.02, n=11). Only one overt dominance display given by a mountain chickadee was observed in all trials. However, the black-capped chickadee involved in this dyad responded with both a chase and supplant less than one minute after this event, and it dominated the paired mountain chickadee in all additional interactions (n=5) witnessed during this one-hour trial.

2.3.3. Genetic analysis

I obtained 734 bp of sequence from the mtDNA control region containing 73 variable sites of which 31 - 6 transversions and 25 transitions - were fixed differences between the two species. All of the sampled individuals from either reference population (mountain chickadees – Riske Creek; black-capped chickadees – Prince George) had mtDNA matching their species phenotype. Further, all microsatellite loci were in HWE and none showed evidence of linkage. The six loci were highly variable (table 2.3) and each species contained unique alleles.

Table 2.3: Allelic variation at the six microsatellite markers (locus as named in original reference, see text). Size ranges for microsatellite alleles are given (size in base pairs) along with the number of alleles (# alleles), and observed (Ho) and expected (He) heterozygosities. Private alleles are number of species specific alleles found in one of the two species.

locus		black-capped	mountain
Ppi2	size	308-536	318-544
	# alleles	37	24
	Ho	0.83	0.78
	He	0.93	0.92
	private alleles	22	10
Titgata39	size	224-260	220-252
	# alleles	10	10
	Ho	0.80	0.85
	He	0.76	0.86
	private alleles	1	1
Titgata02	size	216-272	220-260
	# alleles	14	10
	Ho	0.85	0.80
	He	0.86	0.80
	private alleles	6	1
Pdo5	size	250-336	240-290
	# alleles	16	19
	Ho	0.71	0.76
	He	0.81	0.83
	private alleles	8	9
Escu6	size	120-162	124-154
	# alleles	19	16
	Ho	0.89	0.91
	He	0.91	0.86
	private alleles	6	2
Pat14	size	137-165	135-169
	# alleles	15	16
	Ho	0.80	0.88
	He	0.84	0.88
	private alleles	10	5

Within the overlap zone, all but one individual had mtDNA matching their species phenotype and nestlings from the same nest contained the same mtDNA haplotype. The single exception was one mountain chickadee nestling (N-09-75) that had a mixed mtDNA profile containing both black-capped and mountain chickadee sequences (i.e., possibly heteroplasmy). This sample was re-extracted, re-amplified and sequenced a second time. In both analyses, the sample had allelic pattern consistent with only a single individual; it had maximum two alleles per locus for the microsatellites analysis. Thus, the pattern did not appear to arise from erroneous contamination of the sample from a second individual.

A total of 15 individuals (n=264) in the overlap zone showed evidence of mixed-species ancestry in microsatellite analysis (fig. 2.1). Only one of the 65 adult black-capped chickadees from the mixed area had less than 68% assignment to black-capped chickadee via microsatellites, but had black-capped mtDNA (table 2.4). The remaining 14 birds with evidence of mixed-species ancestry were all phenotypically mountain chickadees (n=97) and had mountain mtDNA (except nestling N-09-75 mentioned above), but black-capped chickadee ancestry based on nuclear microsatellites ranged from 32.3 to 90.6% (table 2.4). Seven of those birds were adult mountain chickadee (n=63 mountain chickadee adults sampled in the contact zone) and seven were nestlings (n=34 mountain chickadee nestlings sampled). One nestling had inconclusive mtDNA (N-09-75 mentioned above) and also had mixed nuclear DNA (56.7% mountain and 43.3% black-capped ancestry). Genetic analysis for the social mother from this nest was not available but none of the six other nestlings from that nest showed evidence of heteroplasmy. However, one other nestling from that same nest showed evidence of mixed-species ancestry through microsatellite analysis, indicating that mixed mtDNA for nestlings N-09-75 might be due to a rare phenomenon of paternal leakage (Kvist et al. 2003).

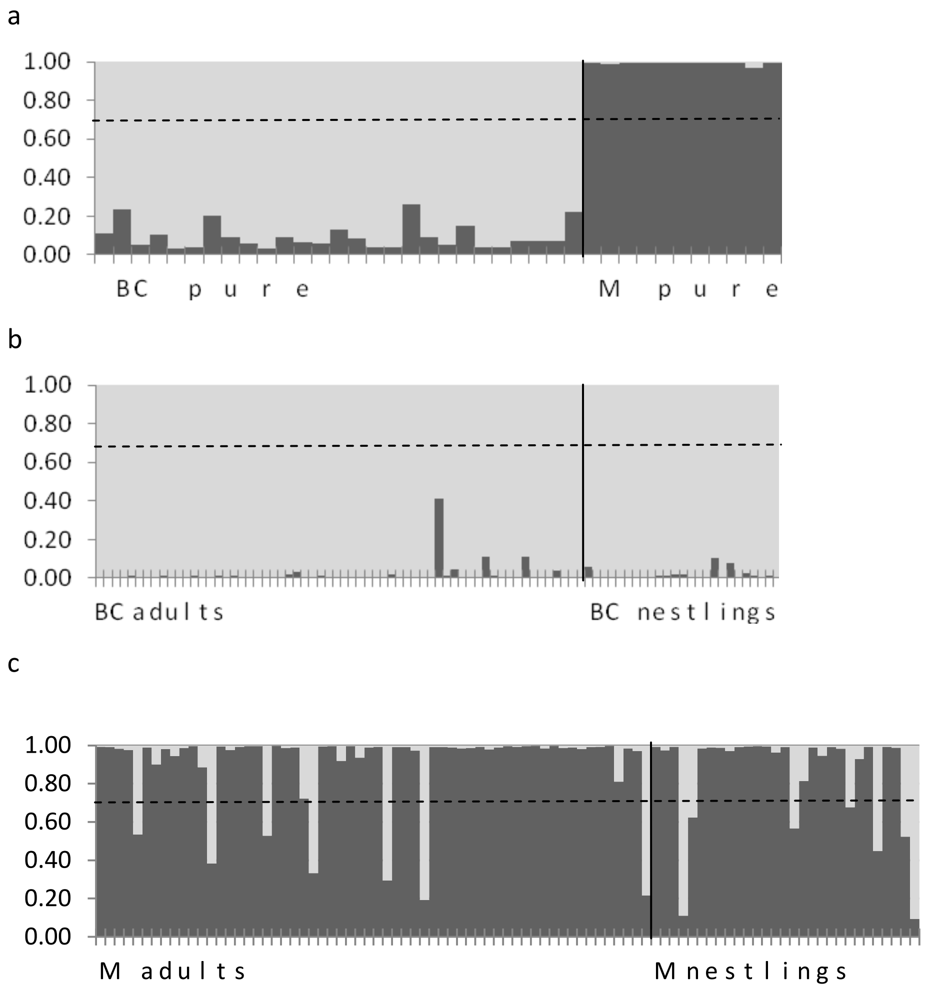

Figure 2.1: a. Q value from the pure black-capped chickadee (BC) population in Prince George and the pure mountain chickadee (M) population in Riske creek (top) in comparison with the STRUCTURE output for the hybrid index scores for both: b. adults and nestlings black-capped (n=167); and, c. mountain (n=97) chickadee sampled at JPRF. Vertical axis indicates the probability of having mountain chickadee microsatellites markers. Dash line at Q=0.68.

Table 2.4: Number of individuals sampled at the JPRF: adults phenotypically mountain chickadee and nestlings sampled in a nest where both parents were phenotypically mountain chickadee are classified as “M”. Similarly black-capped chickadee adults and nestlings are classified as “BC”. Genotyping was done using microsatellite analysis. All hybrids are from the area of overlap.

	classified as M	classified as BC
Adults non-hybrids	56	64
Genetically-determined hybrids	7	1
Nestlings non-hybrids	27	102
Genetically-determined hybrids	7	0

Among the six mountain chickadee nests tested, 9/34 nestlings were determined to have genotypes consistent with being extra-pair (e.g. being half siblings to the remaining nestlings within the same brood). Extra-pair young were found in four of the six nests tested. Of the nine EP nestlings, seven were also classified by STRUCTURE as having DNA from both mountain and black-capped chickadees (Q values less than 0.68 assignment to either species), and were thus classified as hybrids. At least three of the seven nestlings from mountain chickadee nests contained private alleles found only in black-capped chickadees, and the remaining four had genetic profiles for paternal alleles more consistent with black-capped than mountain chickadees in this mixed population. These hybrids were found in all four nests with extra-pair young; in two nests all extra-pair nestlings were hybrids, and in the remaining two nests there was one hybrid and one within-species extra-pair nestling. In all but one of the nine extra-pair young, the mtDNA was mountain chickadee, suggesting that the attending mountain chickadee female at the nest was the genetic mother, and the extra-pair sire was a black-capped male. The remaining case was the nestling classified as a hybrid based on microsatellite loci, and showing heteroplasmy in the mtDNA (above).

In contrast, 10 of 16 black-capped chickadee nests contained evidence of extra-pair paternity, but in no instances did any of the black-capped nestlings (n=102) have evidence of mixed-species ancestry. The combined phenotype, mtDNA and microsatellite data suggest all hybridization detected among nestlings results from female mountain chickadees seeking extra-pair copulations from male black-capped chickadees.

2.4. Discussion

Black-capped chickadees were the clear dominant species in the contact zone. Not only was there a significant bias in dominance relationships between birds matched for age and sex in field observations, but all aviary dyads were won by black-capped chickadees. Within conspecific chickadee flocks, males typically dominate females and adults dominate juveniles (McCallum et al. 1999, Ratcliffe et al. 2007). In this study I observed that female black-capped chickadees were consistently dominant over male mountain chickadees with whom they interacted. Further, adult mountain chickadees were subordinate to juvenile black-capped chickadees in almost all interspecific interactions where the age of competitors was known. This social hierarchy would tend to increase the linearity effect across species: black-capped chickadees are always dominant to their mountain chickadee counterparts regardless of the sex and/or the age.

This linearity effect seems to be particularly strong between these two closely-related species when compared to other parids. Within the contact zone between black-capped chickadee and Carolina chickadee, an aviary study by Bronson et al. (2003 b) showed male Carolina chickadees were generally dominant to black-capped chickadees, but they did observe some reversals. Similarly, studies in Europe on tits showed a two-way doubly asymmetric interaction: the great tit (*Parus major*) is dominant over the blue tit (*Cyanistes caeruleus*) during the non-breeding season (Haftorn 1993) and the smaller blue tit is competitively dominant during the breeding season (Dhondt 1989).

Hybridization in Paridae has been reported within both New World and Old World species (reviewed by Curry 2005, Curry et al. 2007). As a result the genetic analyses showing evidence of genetic mixing between black-capped and mountain chickadees within this contact zone isn't surprising. As hybrid nestlings were only found in mountain chickadee nests that also showed evidence of extra-pair paternity, the clear asymmetry in the relative dominance between these species in our contact zone may influence female choice and extra-pair copulation. Indeed, black-capped chickadees tend to initiate extra-pair matings with males of higher rank than their social mate (Smith 1988, Otter et al. 1994, Otter et al. 1998, Mennill et al. 2004). Similarly, if the social hierarchy with Carolina chickadees being dominant over black-capped chickadees in aviary trials (Bronson et al. 2003 b) held in the field, it might explain the asymmetry in mating patterns between these two species. Indeed, Reudink et al. (2006) found that individuals that were more black-capped-like tended to lose more paternity in their nest than did the Carolina-like males (Reudink et al. 2006), and that extra-pair sires were more Carolina-like than the social male.

Randler (2002) proposed three different hypotheses that might cause females to mate with a heterospecific male: (1) one of the two species involved is less abundant, resulting in females breeding with a heterospecific partner rather than not breeding at all, (2) females fail to recognize conspecifics versus heterospecifics, and/or (3) heterospecific males may have subnormal, or lower, expression of sexually-selected signals than do conspecific males. Even though mountain chickadees are less common than black-capped chickadees in our study area (pers. obs.), both species are relatively abundant in this overlap zone. Also mountain chickadees and black-capped chickadees are easily distinguishable phenotypically through both plumage and vocal cues, so it is unlikely females are unable to distinguish between species. As all the social partners chosen by either species are conspecific it also suggests that females do discriminate to species level. Even if assortative mating by species is the frequent mode of reproduction for both species, hybridization may arise through extra-pair matings if: 1. females base decisions about engaging in extra-pair behaviour on a signal that is common to both species, and 2. there is an asymmetry between males of either species in expression of those signals (Hartman et al. 2011). If females of either species in our study area tend to seek extra-pair copulations from dominant males, mountain chickadee females might be more likely to engage in mixed-species mating than black-capped chickadee females. As a result, hybridization would be expected to result from extension of extra-pair behaviour across species. Our genetic data on nestlings confirm that such directional extra-pair copulations may be driving hybridization.

I did observe adults that had evidence of mixed species ancestry, and all but one was phenotypically mountain chickadee. However none of these hybrids bred within our study site, which indicates that black-capped/mountain chickadee hybrids are viable but whether or not they are fertile remains to be addressed.

3- DIFFERENTIAL RESPONSE TO INTERSPECIFIC AND INTRASPECIFIC SIGNALS AMONGST CHICKADEES

Abstract- Black-capped chickadees (*Poecile atricapillus*) and mountain chickadees (*P. gambeli*) have a similar vocal repertoire and share many other life history traits, yet black-capped chickadees are socially dominant to mountain chickadees where populations overlap. Previous research suggested that in contact zones, both species respond weakly to heterospecific songs during the breeding season, and has suggested minimal interspecific competition. However, both black-capped and mountain chickadees discriminate between conspecific and heterospecific *chick-a-dee* calls, suggesting attention is paid to interspecific signals. I compared the responses of both black-capped and mountain chickadees to conspecific and heterospecific *chick-a-dee* calls during the winter, when both species compete for the same food resources. I conducted an aviary playback experiment exposing both species to playback composed of heterospecific and conspecific *chick-a-dee* calls, which had been recorded in the context of finding food sources. Responses from the tested birds were measured by recording vocalisations and behaviour. Black-capped chickadees responded significantly more to conspecific than to heterospecific stimuli, whereas the subordinate mountain chickadees responded to both mountain and black-capped chickadee calls. Based upon the reactions to playbacks, our results suggest these two closely-related species may differ in their perception of the relative threat associated with intra versus interspecific competition.

3.1. Introduction

Closely related species that live in sympatry often occupy different ecological niches, thus reducing the potential for interspecific competition over limiting resources (Dhondt 1989). In Europe, where it is common to have more than one species within the family Paridae (titmice and chickadees) occurring in sympatry (Dhondt 2007), studies suggest that interspecific competition may be prevalent (Dhondt 2011). Alatalo et al. (1985, 1987) showed that willow tits (*Poecile montanus*), crested tits (*Lophophanes cristatus*) and coal tits (*Periparus ater*) compete over foraging sites in winter. By experimentally removing one of the species, they observed that the other species often shifted to occupy parts of the trees vacated by the removed species. This shift suggested that the niche segregation among co-occuring species may function to reduce interspecific competition. Within the sympatric zone of five *Paridae* species in England, Lister (1980) observed that tits minimise niche overlap and increase aggressive interactions between species when food is scarce. Similarly, in North American members of the family *Sittidae*, white-breasted nuthatches (*Sitta carolinensis*) and red-breasted nuthatches *(S. canadensis)* forage on different parts of the trees when they co-occur, but white-breasted nuthatches will expand their foraging area into red-breasted nuthatch feeding locations when the latter species is absent (Stallcup 1968).

In North America, the distribution of the *Paridae* species tends to be more allopatric than that in Europe; sympatric zones often occur on the intersection between species' range limits, but often involve only two overlapping species (Dhondt 2007). In these contact zones, interspecific competition between chickadees and titmice is likely to occur. Such is the case between Carolina chickadees (*Poecile carolinensis*) and tufted titmice (*Baeolophus bicolor*) that form mix-species flocks in the winter, in which the tufted titmouse is socially dominant to the Carolina chickadee (Waite & Grubb 1988). When Cimprich & Grubb (1994) experimentally removed the dominant titmice, Carolina chickadees spent more time foraging on the ground, the microsites typically preferred by the titmice.

Black-capped chickadees (*Poecile atricapillus*) and mountain chickadees (*P. gambeli*) are considered sister-species (Gill et al. 1993, 2005) that diverged approximately 3.5 mya (Gill et al. 2005) and still share similar social organisation and vocal repertoire (McCallum et al.1999, Foote et al. 2010). These two species typically segregate by both habitat and altitude within their geographic range, and populations of either species are often allopatric at the local scale. However, contact zones do occur where ecological and altitudinal ranges intersect. Hill & Lein (1988, 1989 a) found little evidence that either chickadee species perceive heterospecifics as competitors during the breeding season. However, interactions during the non-breeding season have received less study. In our study site, black-capped and mountain chickadees form mixed-species winter flocks, in which black-capped chickadees are socially dominant to mountain chickadees (chapter 2) at both temporary winter feeding stations and in aviary trials. During these interactions at feeders between both individuals and flocks, chickadees often vocalize, using *chick-a-dee* calls, suggesting that these calls may have interspecific functions in addition to their intraspecific role.

The *chick-a-dee* call has been extensively studied in chickadees (Hailman 1989, Hailman & Ficken 1996, McCallum et al. 1999, Foote et al. 2010). All species within the genus *Poecile* use this complex vocal signal, which can convey information in a variety of contexts. *Chick-a-dee* calls of both species are composed of 4 note types (referred to as A, B, C, and D), sung in a fixed order, A→B→C→D. While this syntax is maintained, the number of repetitions of each note type can vary, and not all note types are included in all renditions of the call, resulting in an infinite number of possible combinations of this call type (Hailman 1989, Lucas & Freeburg 2007). Mountain chickadees also produce two additional variants (or hybrids) of these note types (Bloomfield et al. 2004). These consist of an intermediate note between note A and B (denoted A/B) and a variation of the D note referred to as Dh. While Dh notes were also recorded from black-capped chickadees in our study population, no recordings of *chick-a-dee* calls from black-capped chickadees in our study area contained *A/B* notes. C*hick-a-dee* calls are used in several different contexts, and the syllable structure of the call appears to vary with this context (Lucas & Freeberg 2007, Freeberg 2008). The one apparent commonality in function across contexts in which is it used is that the call rallies other individuals (mates or flockmates) to the signaller (Freeberg 2008, Foote et al. 2010) either in defense of a resource or in mobbing against a perceived threat. *Chick-a-dees* are often given during within and between-flock interactions (e.g. Ficken et al. 1978). Birds also use *chick-a-dee* calls when encountering a new food sources (Freeberg 2008) and may convey this information as a means of gathering mates or flockmates to the resource (Mahurin & Freeberg 2009). These calls are also commonly given during interflock disputes (Ficken et al. 1978, Nowicki 1983, pers obs), and may rally flockmates to aid in contests with other flocks. *Chick-a-dees* used in the context of mobbing stationary avian predators may contain larger numbers of, and harsher renditions of, D notes than are associated with the use of the call in other contexts (Gaddis 1985, Templeton et al. 2005, Soard & Ritchison 2009, Courter & Ritchison 2010), although some studies suggest that flying avian predators can elicit *chick-a-dees* with large numbers of A notes (Freeberg 2008). The structure of the notes themselves may also convey information used by receivers. Dawson et al. (2006) demonstrated that all notes within both black-capped and mountain *chick-a-dee* calls contain species-specific acoustics characteristics, and Charrier & Sturdy (2005) showed that black-capped chickadees are able to recognize their own species' c*hick-a-dee* calls. Within species, individuals can identify flock membership (Nowicki 1983) and specific individuals (Charrier et al. 2004) using c*hick-a-dee* call features. However, none of these studies have addressed if and how sympatric black-capped and mountain chickadees utilise information encoded in both their own species' vs heterospecifics' calls.

Because black-capped and mountain chickadees have overlapping ecological and environmental requirements in our northern study area, they might perceive both conspecifics and heterospecifics as competitors during the winter when food is scarce. I investigated whether birds differentially respond to conspecific and heterospecific *chick-a-dee* calls. Stimuli used in the study were recorded from single birds upon locating a temporary food source (bird feeder containing sunflower seeds) with few to no other birds in the immediate vicinity. Thus, the context appears to be identification of a food and/or intent to utilize a food resource. If birds respond similarly to calls of either species, it might indicate that every individual is viewed as a potential competitor for resources, regardless of species: in other words, interspecific competition is perceived to be as strong as intraspecific competition.

3.2. Methods

3.2.1. Study area and study species

Both black-capped chickadee and mountain chickadee from the contact population at the JPRF were caught within their flocks' territories (using mist nets or potter trap) and immediately transported to an aviary (fig 3.1) at the centrally-located field station within the research forest. All birds were captured within 6km of the field station. To reduce any effect of stress associated with capture, I allowed the birds to habituate for an hour in the aviary before I ran the experiment. Twelve black-capped and 13 mountain chickadees were tested between 13 – 22 March 2010.

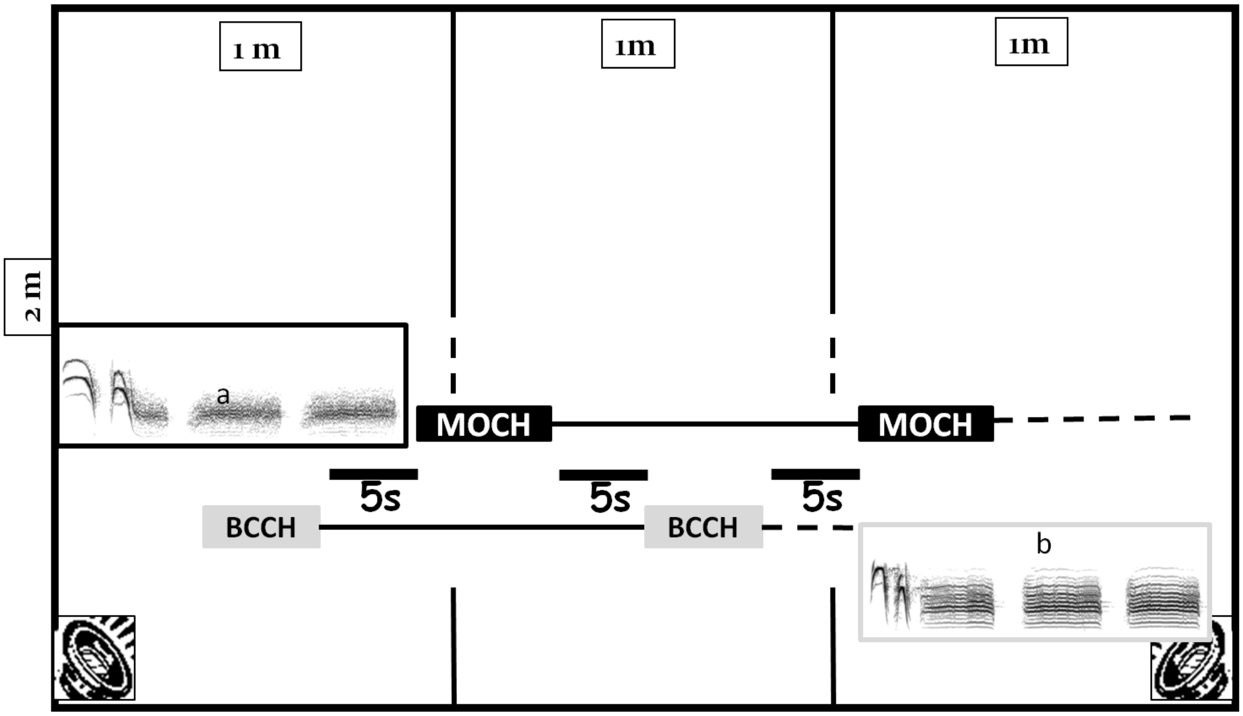

Figure 3.1: Aviary design and playback stimuli illustration with “a” the spectrogram representation of the mountain (MOCH) *chick-a-dee* call and “b” the spectrogram representation of the black-capped (BCCH) *chick-a-dee* call .

3.2.2. Stimuli description

Both male and female black-capped and mountain chickadees produce *chick-a-dee* calls all year round (Hailman & Ficken 1996). The calls used were recorded at different temporary winter feeding station in early winter 2010 (December 2009 and January 2010), using a Marantz PMD671 digital recorder with either a Sennheiser ME67 with a K6 power supply or a Sennheiser MKH70 with an MZA14 power supply. To control for the context, recordings were made in the absence of predators, and were produced by a single bird when first arriving at the food source. The birds were spatially isolated from other flockmates when calls were produced, and recordings were made prior to the breakup of winter flocks and separation of pairs onto breeding territories, which occurs in late April to early May in our study area. As a result I can largely exclude the contexts of predator identification/response, sexual display or even immediate agonistic interactions from the stimuli recordings. Rather, the *chick-a-dee* calls used in the study would be contextually associated with location of a winter food source, and perhaps attracting mates/flockmates to aid in defending this resource (Mahurin and Freeberg 2009). As there is no discernable difference between male and female *chick-a-dee* calls (e.g. Bloomfield et al. 2004, Charrier et al. 2004) I did not control for sex of bird recorded for playback stimuli.

To avoid any effect of note syntax, I selected calls from both species and paired each mountain *chick-a-dee* call with a black-capped *chick-a-dee* call of similar note composition. I used a single *chick-a-dee* stimulus from 10 individuals of each species, then paired these (one mountain and one black-capped chickadee stimulus) to create 10 different playback dyads. Because A , A/B, and B notes represent a continuous gradient of syllables (Bloomfield et al. 2004) in mountain *chick-a-dee* calls, I grouped those three note types into a single category (A-B). To be consistent, I also classified black-capped *chick-a-dee* call note type A and B as one note type: A-B. Note type C was particularly rare (in less than 1% of the calls) in the call I recorded at the temporary feeding station, as a result I did not include any note C in my playbacks. All calls used were composed of one, two or four note A-B, one Dh-note and two D-note (4 to 7 notes in total for an average call length of 1.14s). Using Avisoft SASLab-Pro software, each call was filtered to erase background noise: low-pass filter at 1kHz and high-pass filter at 10kHz.

The stimuli broadcast to individual birds were recorded at least 5km from the subject bird's territory to avoid previous contact between subjects and source of stimulus. I mounted two speakers, one on each side of the aviary (fig 3.1). I broadcasted playback in the aviary using a stereo file composed of one species' call type on the right channel alternating with the other species' call type on the left channel. This design resulted in *chick-a-dees* of one stimulus type (mountain vs black-capped chickadee) being broadcast from one speaker, with the calls of the other stimulus type broadcast from the opposing speaker. This inter-speaker distance would not be atypical for the spacing of individuals of different flocks having found a common food source. Stimuli were presented in alternating fashion; one stimulus (e.g. mountain chickadee call) was broadcast from one speaker, followed by five sec of silence, then followed by a broadcast of the other stimulus (e.g. black-capped chickadee) from the other speaker, followed by five sec silence. This sequence was repeated 8 times for a total of nine identical mountain *chick-a-dee* calls alternating with nine identical black-capped *chick-a-dee* calls (fig 3.1). I block-randomized the species of the first broadcast stimulus (black-capped versus mountain), and from which channel each species' call was broadcast, to create an equal number of all combinations. The volume of each playback was between 74 and 78dB measured at 1.5m (Goldline SPL 120 sound pressure level meter). I used 10 different stimuli pairs to test 13 mountain chickadees and 12 black-capped chickadees. Each playback was used a maximum of two times with conspecific birds and maximum three times in total (either to test two black-capped and one mountain chickadee or one black-capped and two mountain chickadees).

3.2.3. Playback experiment

Birds were placed into the central compartment of the aviary, and sliding doors were used to isolate the bird from the outer two compartments containing the playback speakers. I allowed one hour for habituation to the aviary prior to starting the experiment. At the start of the experiment, the two doors isolating the outer compartments were opened, allowing the bird access into these areas that contained feeders and the broadcast speakers. The feeder in the central compartment was removed. Once the compartments were opened, I began two minutes of pre-playback observations. I then began the broadcasts and conducted observations during the playback, and then continued observations for two minutes following the end of the broadcast. I recorded the birds' behaviour, spatial location within the aviary, and vocalisations during these 3 periods - pre-playback, during-playback, post-playback – using a Marantz PMD671 digital recorder with a Sennheiser MKH70 with an MZA14 power supply. Spatial position of the bird was dictated onto these tapes by an observer at a blind 5m from the aviary.

3.2.4. Birds' movement, acoustic and statistical analysis

I split response during trials into response towards heterospecific call and toward conspecific calls. Approaches were defined as entering the outer sides of the partitioned aviary in which the stimuli were being broadcast. Vocal responses directed towards a particular stimulus were defined as the bird orienting its body towards and vocalizing in the direction of one or the other speaker. Responses measured were: response latency (sec) during the 5 seconds following each stimuli for a bird to respond with either vocalizations or movement directed towards the speaker (response latency was measured between “0” -when the bird react while the call was still playing- to “5” -if the bird did not respond); time spent within the same outer compartment of the aviary as the stimuli; and, number of vocalizations given in the 5 second silence period that followed each stimuli. The response of subjects to heterospecific and conspecific stimuli was measured as the cumulative number of vocalizations or movements directed towards these stimuli types across the trial.

Recordings were analysed using Avisoft SASLab-Pro software. I counted not only the number of vocalisations (e.g: *chick-a-dee* calls, contact calls) from the tested bird during the four experimental periods, but also the note composition (A-B, C, Dh, D) of each *chick-a-dee* call. Statistical analysis was conducted on STATISTICA 8 software. I used only non-parametric tests due to small sample size. I compared measures of response within-subjects across the pre-playback, playback and post-playback periods using Friedman ANOVAs, and then Wilcoxon Signed-Rank Tests for post-hoc comparisons. I compared response measures within each period of the trials, and cumulative behaviour across trials, between subjects (mountain chickadees vs black-capped chickadees) using Mann-Whitney U tests.

3.3. Results

3.3.1. Spatial movement

a- Movement - latency of response

I did not find any differences between tested species (black-capped vs mountain chickadees) in their latency to approach the first broadcast stimuli (regardless of stimulus class) (Mann-Whitney U test, p=0.13, N=25). Also, neither subject species showed a difference in latency to respond to either conspecific versus heterospecific stimulus (black-capped chickadees - Wilcoxon test, p=0.81, N=12; mountain chickadees - Wilcoxon test, p=0.88, N=13).

b- Spatial location response

I did not find significant differences in the time spent in the conspecific compartment of the aviary during the PRE, playback and POST periods for either mountain chickadees (Friedman ANOVA, N=13, df=2, p=0.25) or black-capped chickadees (Friedman ANOVA, N=12, df=2, p=0.92). I further did not find significant differences for the time spent in the heterospecific compartment during the three experimental periods for either black-capped (Friedman ANOVA, N=12, df=2, p>0.9) or mountain chickadees (Friedman ANOVA, N=13, df=2, p>0.1).

When considering only the playback period, there was no significant difference in the time that the subject birds spent in each compartment (conspecific, central, vs heterospecific) among either the tested black-capped chickadees (Friedman ANOVA, N=12, df=2, p=0.66) or mountain chickadees (Friedman ANOVA, N=13, df=2, p=0.24).

Finally, the time each test species spent in the conspecific compartment during the playback period does not differ significantly when comparing the responses of tested black-capped vs. mountain chickadees (Mann Whitney U test, N=25, p=0.76). Similarly, I found no significant differences for the time each test species spent in the heterospecific compartment during the playback (Mann Whitney U test, N=25, p=0.85).

3.3.2. Vocal response

a- Vocal response latency

There was no difference in the latency of the first vocal response to the first stimuli presented (regardless of stimulus type) between the tested black-capped and mountain chickadees (Mann-Whitney U test, p=0.13, N=25). Further, I found no difference in how rapidly mountain chickadees responded with vocalizations to either conspecific or heterospecific stimulus (Wilcoxon test on the average response latency to each presented stimuli throughout the playback, p=0.28, N=13). However, black-capped chickadees had a lower latency when responding to conspecific stimuli compared to heterospecific stimuli, (Wilcoxon test, p=0.03, N=12, fig 3.2).

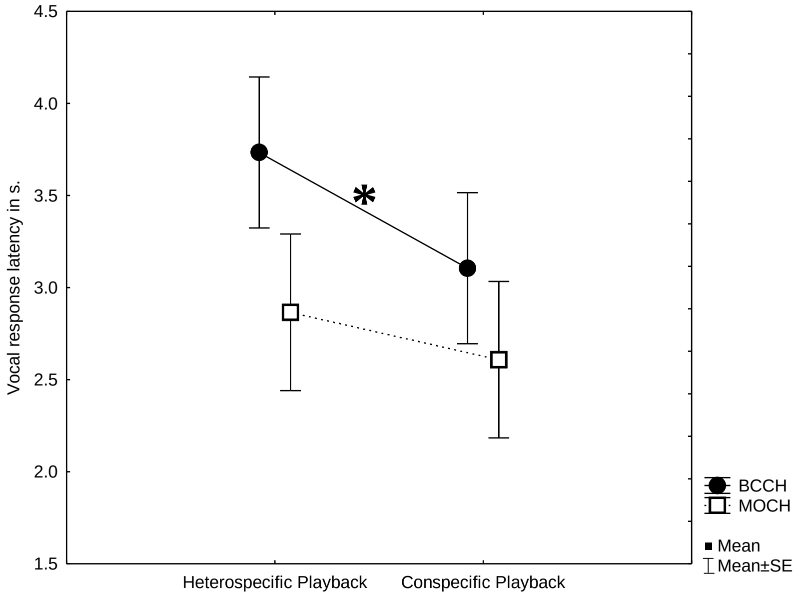

Figure 3.2: Vocal response latency in seconds with respective standard errors bars of both black-capped chickadee (BCCH, N=12) and mountain chickadee (MOCH, N=13) to heterospecific stimulus and conspecific stimulus. * indicates significant differences.

b- Vocalization rates and composition

Tested birds of either species had higher vocalization rates during the playback period than during the pre- and post-playback periods (Friedman ANOVA: black-capped chickadees - N=12, df=3 p=0.02; mountain chickadees - N=13, df=3, p<0.001). I also compared vocal activity between the two study species during the different phases of the experiment (fig 3.3). I found that the black-capped chickadees had higher overall vocal activity than did the mountain chickadees during the non-playback periods, but it was significant only between species for the pre-playback phase (Mann Whitney U test, p=0.04, N=25, fig 3.3). While the playbacks were broadcasted, I found that mountain chickadees were more vocal than black-capped chickadees, especially during the heterospecific playback period (Mann Whitney U test, p=0.05, N=25, fig 3.3).

During the playback period, I compared vocalization rates given in response to either stimulus class. This rate was defined as the total vocalizations given during the broadcast of a stimulus and the silence period following it until the opposing stimulus was broadcast from the opposing speaker. I combined the total number of vocalizations in these periods for either stimulus type (heterospecific vs conspecific) across the trial. Black-capped chickadees had higher vocalisation rates per minute in response to conspecific playback than to the heterospecific playback (Wilcoxon test, p=0.005, N=12, fig 3.3). However, there was no difference in the response of mountain chickadee subjects to either conspecific or heterospecific stimuli (Wilcoxon test, p=0.28, N=13, fig 3.3).

I found a parallel effect when I separate the response measures by call types (contact calls vs *chick-a-dee* calls) given in response to stimuli. Black-capped chickadees produced more contact calls (Wilcoxon test, p=0.03, N=12) and more *chick-a-dee* calls (Wilcoxon test, p=0.01, N=12) to conspecific stimulus than to heterospecific stimuli during the playback period. Conversely, there was no difference in contact calls (Wilcoxon test, p=0.13, N=13) nor in *chick-a-dee* calls (Wilcoxon test, p=0.87, N=13) given in response to heterospecific vs conspecific stimuli by tested mountain chickadees. However, mountain chickadees produced significantly more contact calls than *chick-a-dee* calls in their response to both conspecific stimuli (Wilcoxon test, p=0.001, N=13) and heterospecific stimuli (Wilcoxon test, p=0.002, N=13). There was no such difference in the black-capped chickadees' response to conspecific playback (Wilcoxon test, p=0.07, N=12) or heterospecific playback (Wilcoxon test, p=0.16, N=12).

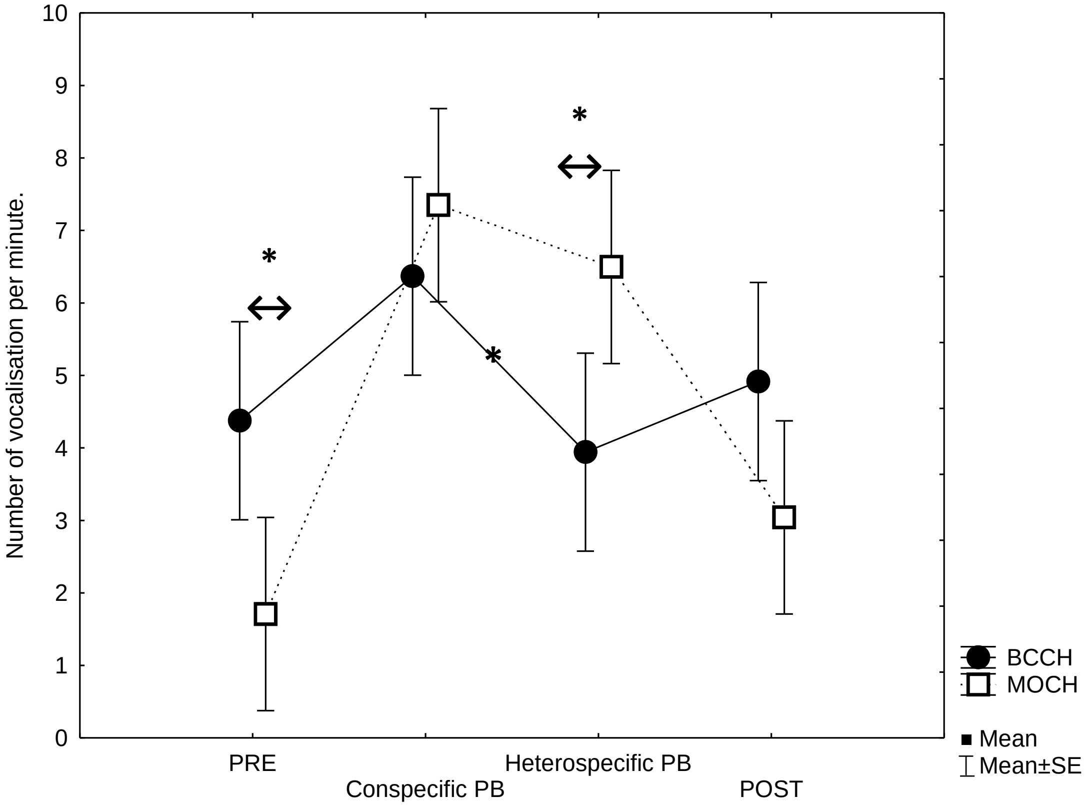

Figure 3.3: Vocalisation rate per minute during the 4 experimental phases with corresponding error bars (PRE: 2min period before the playback was broadcasted, Conspecific PB: conspecific playback was broadcasted, Heterospecific PB: heterospecific playback was broadcasted, POST: 2 min after the playback was broadcasted) for both black-capped chickadee (BCCH, N=12) and mountain chickadee (MOCH, N=13).
* over a double arrow indicates significant differences during a broadcast period between species (Mann-Whitney U test, $p<0.05$). * over a line indicates significant differences between comparison stimuli within species (Wilcoxon test, $P<0.05$)

Six mountain chickadees (out of 13) and 10 black-capped chickadees (out of 12) responded to the conspecific playback by producing their own *chick-a-dee* calls. Six mountain chickadees and 4 black-capped chickadees also produced *chick-a-dee* calls in response to the heterospecific playback (table 3.1). I observed that *chick-a-dee* calls given in response to the stimulus had significantly fewer notes than the playback stimulus for both mountain chickadee stimulus (Wilcoxon test, N=8, p=0.001) and black-capped chickadee stimulus (Wilcoxon test, N=10, p=0.02). Amongst the bird that use *chick-a-dee* calls in response to the stimulus, all mountain chickadee test subjects (n=6) and all but one black-capped chickadee test subjects (n=9) used D notes in their *chick-a-dee* calls in response to conspecific playback (table 3.1). Further, all but one mountain chickadee (n=5) and all but one black-capped chickadee test subject (n=3) used D notes in their *chick-a-dee* calls given in response to heterospecific playback (table 3.1). Calls given by mountain chickadee test-subjects in response to both conspecific and heterospecific stimulus had significantly fewer D notes than the playback call (Wilcoxon test, N=6, p=0.02). Tested black-capped chickadees also gave response calls with fewer D notes than the stimulus, but this is only significant in response to the conspecific playback (Wilcoxon test, N=10, p=0.01).

Table 3.1.: Average number of each note type (A, B, C and D) per chick-a-dee call given in response to conspecific and heterospecific stimulus by black-capped and mountain chickadees. Numbers in brackets indicate the number of test subjects that responded to stimuli with *chick-a-dee* calls out of the total number of birds tested (12 black-capped chickadees and 13 mountain chickadees).

	Black-capped chickadee		Mountain chickadee	
Number of note type/number of *chick-a-dee* call	Response to conspecific playback (n=10)	Response to heterospecific playback (n=4)	Response to conspecific playback (n=6)	Response to heterospecific playback (n=6)
note A/B	3.13	2.40	1.23	1.21
note C	0.44	0.14	0.037	0
note Dh	0.019	0.19	0.98	0.83
note D	1.71	1.40	0.39	0.61
All note	4.66	3.95	2.44	2.85

3.4. Discussion

Tested birds showed higher vocal activity during the stimulus phase of playback than before or after the playbacks were broadcasted, indicating that both black-capped and mountain chickadees reacted to the stimuli to which they were exposed. However, the increased vocal response of black-capped chickadees was confined to conspecific stimuli. Mountain chickadees, on the other hand, showed an increased vocal response to both heterospecific and conspecific stimuli, but did not appear to differentiate between these stimuli classes. This result suggests that black-capped chickadees show lower responsiveness to heterospecific than conspecific calls, but that mountain chickadee responded both strongly, and similarly, to both heterospecific and conspecific stimuli.

One possible explanation for this result is a failure of mountain chickadees to discriminate species-specific cues in calls and thus misdirected intended intraspecific signals, as has been suggested to occur among closely related species by different authors (e.g. Murray 1981). However, this interpretation is unlikely as both black-capped and mountain chickadees are capable of learning and discriminating between either species’ calls (Bloomfield and Sturdy 2008). Further, the differences between mountain and black-capped *chick-a-dee* calls are audible to humans, so it is unlikely the birds would fail to learn these differences in sympatric zones. Thus, it is more parsimonious to suggest that mountain chickadees may perceive conspecific and heterospecific signals as requiring similar levels of response.

The playbacks used in this experiment were recordings from unfamiliar birds during winter months when birds are foraging in flocks. Consequently, tested birds may perceive these stimuli as constituting a non-flock mate that has located the same food sources as being used by the subject bird. Nowicki (1983) found that chickadees do not typically respond to calls of flockmates by increasing their own vocal rates; in contrast, *chick-a-dee* stimuli from other flock mates, which would be perceived as competitors, elicted increased calling rates, similar to the results in our study. As flocks routinely dispute access to these feeders, the response calls of the subject birds may represent attempts to rally its own flockmates to aide it in contesting the resource from a perceived competitor individual/flock. My previous research in this area found that black-capped chickadees dominate mountain chickadees in these winter flocks (chapter 2). As such, the subordinate mountain chickadee may not represent a threat to the dominant black-capped chickadee, as even female and juvenile black-capped chickadees can displace adult, male mountain chickadees from resources (chapter 2). For this reason, I might expect lower responsiveness of tested black-capped chickadees towards heterospecific vs conspecific stimuli, as seen in this study. Conversely, black-capped chickadee intruders might well constitute a perceived threat to contested resources to a mountain chickadee. Thus, I might expect that responsiveness of tested mountain chickadees towards heterospecific stimuli as being similar to that given to conspecific stimuli – it may benefit a subject mountain chickadee to rally flockmates to contest both mountain chickadee and black-capped chickadee competitors.

An alternate interpretation of my data is vocal parasitism, whereby the subordinate mountain chickadee uses information from the black-capped chickadee to locate their food. As calls used for the playbacks were recorded at a temporary feeding station, stimuli were composed of calls used in a food-finding context. The black-capped chickadee is a generalist species that occurs in a wide variety of habitats whereas mountain chickadees tend to be more affiliated with coniferous forests (Foote et al. 2010, McCallum et al. 1999). In our study area, forest practices have created a mosaic of habitat types and ages within the research forest (Grainger 2002). This mixed habitat might be more challenging for the mountain chickadees than it is for the black-capped chickadees and mountain chickadees might learn to parasitize black-capped chickadee vocalisations in this contact zone. However, I think this interpretation is unlikely, as such parasitism would predict that the response to stimuli would be associated with an approach without vocalizations ("stealth") to locate the advertised food. Vocalising in response to playback would presumably alert the more dominant black-capped chickadee to the intruder and could elicit unwanted aggression. In this study, I observed a vocal response to heterospecific stimuli from mountain chickadees, but not a taxis response. Many of these calls were contact calls used to alert others of the signallers location (Odum 1942, Gaddis 1985). Thus, it would not appear that the mountain chickadee subjects were intending to remain undetected. Further, the note types of the *chick-a-dees* given in response to stimuli were A-B and D notes. Such *chick-a-dee* calls are often given during interflock contests (Lucas & Freeberg 2007, pers obs), and, as such, it is likely that the vocalisations were intended for agonistic interactions.

Interestingly, while the response calls contained D notes, the number of D notes in the response calls of either species was consistently lower than the number that had been in the stimulus calls. If the vocal responsiveness of our tested subjects resulted from these birds perceiving the broadcast stimulus was advertising the presence of an avian predator (e.g. Templeton et al. 2005, Soard & Ritchison 2009, Courter & Ritchison 2010), I would have predicted both a positive taxis response to the playback (mobbing) and an increase in the number of D notes in the response calls used by the subject birds. Neither of these responses was seen among our test birds. Further, we would not necessarily have expected subject birds of either species to respond differentially to conspecific/heterospecific stimuli if they perceived the stimuli was advertising the presence of a predator, as this information conveys an equal threat to small birds regardless of species. C*hick-a-dee* calls recorded from black-capped chickadees in the context of predator mobbing attract both black-capped and mountain chickadees (pers obs), but also cause other small passerines to approach and mob the broadcast speaker (e.g. Templeton and Greene 2007). This suggests that the increased calling of subjects elicited during playback studies was unlikely to be associated with perceived predation threat, further supporting my alternate interpretation that responses to the playback are likely to reflect a perceived competitive context. Thus, I conclude that the difference in response to heterspecific vs conspecific stimuli that I observed between black-capped and mountain chickadees is most likely the result of differences among these species on the perceived risk of interspecific vs intraspecific competition.

4- CHARACTER DISPLACEMENT IN DAWN CHORUSING BEHAVIOUR OF SYMPATRIC BLACK-CAPPED AND MOUNTAIN CHICKADEES

Abstract- Closely related species tend to be more distinct when occurring in sympatry than when they are allopatric. Such differences allow species specific identification and avoid interspecific matings. However, within overlapping ranges of usually-allopatric populations, such differences might not be obvious. In chickadees, dawn chorus behaviour is known to impact female mate choice. Within a contact zone between black-capped and mountain chickadees, I previously found directional hybridization occurs through female mountain chickadees engaging in extra-pair behaviour with male black-capped chickadees. In this chapter, I compared the chorus behaviour from the contact zone with dawn chorus recordings from allopatric populations of either species. I found that mountain chickadees in sympatric populations with black-capped chickadees alter their chorus; they use more *chick-a-dee* calls than songs when they co-occur with black-capped chickadees whereas they use similar proportions of calls and songs in areas without black-capped chickadees. I also found differences in the fine structure of the song, both species typically have a descending first note in their song, but I found that mountain chickadees that share their habitat with black-capped chickadees used an ascending first note. These differences in chorus pattern illustrated possible character displacement from the subordinate mountain chickadees, providing the potential to reduce acoustic overlap with the dominant black-capped chickadees. Also, by making their chorus easily distinguishable from the black-capped chickadee, mountain chickadee might signal their identity to conspecific females and limit mis-leading signals that could promote hybridization.

4.1. Introduction

Brown & Wilson (1956) observed that populations of closely-related species are often easily distinguishable (e.g. morphologically, behaviourally) when they occur in sympatry, whereas allopatric populations of the same species might be harder to differentiate. When closely-related species co-occur in sympatry, failure of individuals to discriminate species-specific signals may both increase interspecific competition and potential for interspecific matings, which could in turn lower individual fitness (Grant 1994). Therefore, one would expect selection to favour the evolution of differences in species-specific signals when species coexist to enhance discrimination. This divergent character displacement (Grant 1972, Schluter 1994) in sympatric populations is not necessarily evident in allopatric populations of the same species (Brown & Wilson 1956, Loftus-Hill & Littlejohn 1992). However, this displacement of characters does not necessarily occur symmetrically among the two species involved; when competitive interactions between species are, themselves, asymmetric, character shifts are predicted to be greater in the subordinate species (Miller 1968, Grant 1972, Doutrelant et al. 2000b).

Such situations are common within the Family Paridae (titmice and chickadees), where several species often overlap in distribution and interact over common resources (Dhondt 1989; Curry 2005). Many of these overlapping species have asymmetries in their competitive abilities, which might result in expected asymmetries in character shifts (e.g. Doutrelant et al 2000b). Among North American species, black-capped (*Poecile atricapillus*) and mountain chickadees (*P. gambeli*) co-occur in western populations, and interspecific competition between these species has been noted (Hill & Lein 1989a, Martin & Norris 2007; chapter 2).

Black-capped and mountain chickadees are considered a sister-species clade amongst the North American chickadees (Gill et al. 1993, 2005). Although this relationship still awaits confirmation with nuclear DNA analysis, the two species are none-the-less considered closely related. These species have an allopatric distribution due to ecological segregation – overlap zones occur along an altitudinal gradient where deciduous patches (black-capped-like habitat) abut coniferous forests (mountain-like habitat). At the JPRF in central British Columbia, Canada, where black-capped and mountain chickadees co-occur, black-capped chickadees are the dominant species (chapter 2). Hybridization is known to occur in this population, resulting from female mountain chickadees seeking extra-pair copulations with male black-capped chickadees (chapter 2). As females often use songs and calls in species recognition and mate choice (Nowicki & Searcy 2005), one might expect greater divergence in vocal behaviour among sympatric compared to allopatric populations of these two chickadee species. Further, as hybridization appears directional, and competitive interactions are very asymmetric (chapter 2), I predict that the character displacement in songs will be greatest among mountain chickadees in these contact zones.

Dawn chorusing is common within the Paridae Family. Males vocalise before sunrise for between 15 to 90 minutes in the vicinity of their cavity during the female fertility period (Otter et al. 1993, 1997, Gammon 2004, Mennill & Otter 2007). Usually, males stop singing or calling when females leave the nest or their roosting spot (e.g. Smith 1991, McCallum et al. 1999, Gammon 2004). Females join the males and copulation usually occurs, at which point dawn chorus bouts typically end (e.g. Otter & Ratcliffe 1993, Gammon 2004, pers. obs.). In some species, it has been shown that females use songs to assess male quality and extra-pair partners (e.g. Hasselquist et al. 1996, Kempenaers et al. 1997, Otter et al. 1997, Mennill et al. 2003). Dawn chorus bouts are composed of songs, calls or a mix of both depending on the species. Amongst black-capped chickadees the dominant vocalisations during the chorus is the *fee-bee* song. Songs are remarkably invariant from across most of their range (Hailman 1989, Kroodsma et al. 1995, reviewed in Mennill & Otter 2007). Even though songs are very stereotyped, variation in the fine structure shows individual variation (Christie et al. 2004), which Phillimore et al. (2002) showed that black-capped chickadees can use to discriminate between different individuals' songs. Mountain chickadees use a mix of whistled songs and *chick-a-dee* calls during their dawn chorus bouts and McCallum et al. (1999) suggested that calls may be directed to females and song to rival males (McCallum et al. 1999). Few researchers, though, have addressed the fine details of dawn chorus behaviour in mountain chickadees.

To maintain species integrity, species isolation mechanisms might evolve to reduce hybridization. As dawn chorusing is suggested to play an important role in mate or extra-pair assessment, I focus this research on mountain chickadees' dawn chorus in the overlap zone in relation to dawn choruses from pure black-capped and pure mountain chickadee populations. The goal was to determine whether the structure of the mountain chickadee chorus, or the songs themselves, differ among birds that co-occur in sympatry with black-capped chickadees compared to allopatric populations of mountain chickadees. Such a character shift among male mountain chickadees might be expected as selection to combat the directional hybridization I observe in my study contact zone (chapter 2).

4.2. Methods

4.2.1. Study sites (see fig 1.2.)

I sampled both black-capped and mountain chickadees at the JPRF, hereafter referred respectively as the "mixed-BC" and "mixed-MO" populations. I sampled two populations (one for either species) that were occupied by either black-capped (Prince George, British Columbia, hereafter referred to as "pure-BC") or mountain chickadees (Riske creek, British Columbia, hereafter "pure-MO"): 95% of the chickadees were from one species only, with few incidental occurrences of the other species. These totals are based upon at least 5 years of population monitoring in either population (Otter et al. 2007, K. Martin, pers comm.).

4.2.2. Dawn chorus recording

Dawn chorusing occurs during the early breeding season, in late April early May depending on years, site and species. A complete recording of one morning chorus is enough to cover the size of an individual's repertoire (Doutrelant et al. 2000a; Mennill & Otter 2007). I recorded male choruses from the first vocalization (*chick-a-dee* call or song) until the bird stop vocalising for at least 5 consecutive minutes or until copulation happened. I used a Marantz PMD671 digital recorder with either a Sennheiser ME67 microphone/K6 power supply or a Sennheiser MKH70 microphone/MZA14 power supply to records the mountain chickadees from the mixed-MO (n=10) and from the pure-MO population (n=8) in 2010. Comparison choruses from black-capped chickadees were drawn randomly from recordings associated with other studies in the pure-BC population (van Oort et al. 2006) and mixed-BC population (Grava et al. 2009). A total of 12 choruses were randomly selected from the mixed-BC population evenly distributed from recordings made in 2006, 2008 and 2009. A total of 10 choruses from black-capped chickadees in the pure-BC population were randomly selected from recordings made in 2000, 2002 and 2003. These latter recordings were made with a Marantz PMD430 audiocassette recorders in conjunction with one of the following directional microphones: a Sennheiser ME 67 with a K6 power supply, a Sennheiser MKH 70 with a MZA14 powersupply or an Audio-Technica ATB-815a.

4.2.3. Song and call analysis

I transcribed dawn chorus recordings of black-capped and mountain chickadees to determine the proportion of time that an individual used calls versus songs. As chickadees vocalised continuously during the dawn chorus, I analysed choruses in one-minute segments. I calculated the proportion of time in each minute spent producing either songs or *chick-a-dee* calls (this included the time of the vocalization itself and the inter-vocalization space). Birds typically sing one vocalization or the other in string sequences (many songs with no *chick-a-dee* calls, followed by strings of *chick-a-dee* calls with no songs). Then, I randomly isolated 9 songs from the recordings evenly distributed across the chorus for detailed spectral analysis, using the methodology similar to Christie et al. (2004). Using the sound analysis software Seewave (Sueur et al.2008) and R 2.8.1, I extracted frequency values at the start and at the end of each notes within each of the nine songs.

I conducted detailed acoustic analyses of songs by comparing the frequency at the end of the first note and at the end of the second note for each of the four study populations (fig.4.1). Previous research (e.g. Christie et al 2004) used the “internote ratio” (ratio between the frequency at the end of the first note and the frequency at the start of the second note) and the glissando (ratio between the frequency at the start and the frequency at the end of the first note) to describe black-capped chickadee dawn chorus acoustic structure. I calculated the glissando of the first note for both mountain and black-capped chickadee. Because the second note of the mountain chickadee has a highly-variable ascending start (pers. obs.), I used the ratio between the frequency at the end of the first note and the frequency at the end of second note to calculate the "internote ratio" (fig. 4.1).

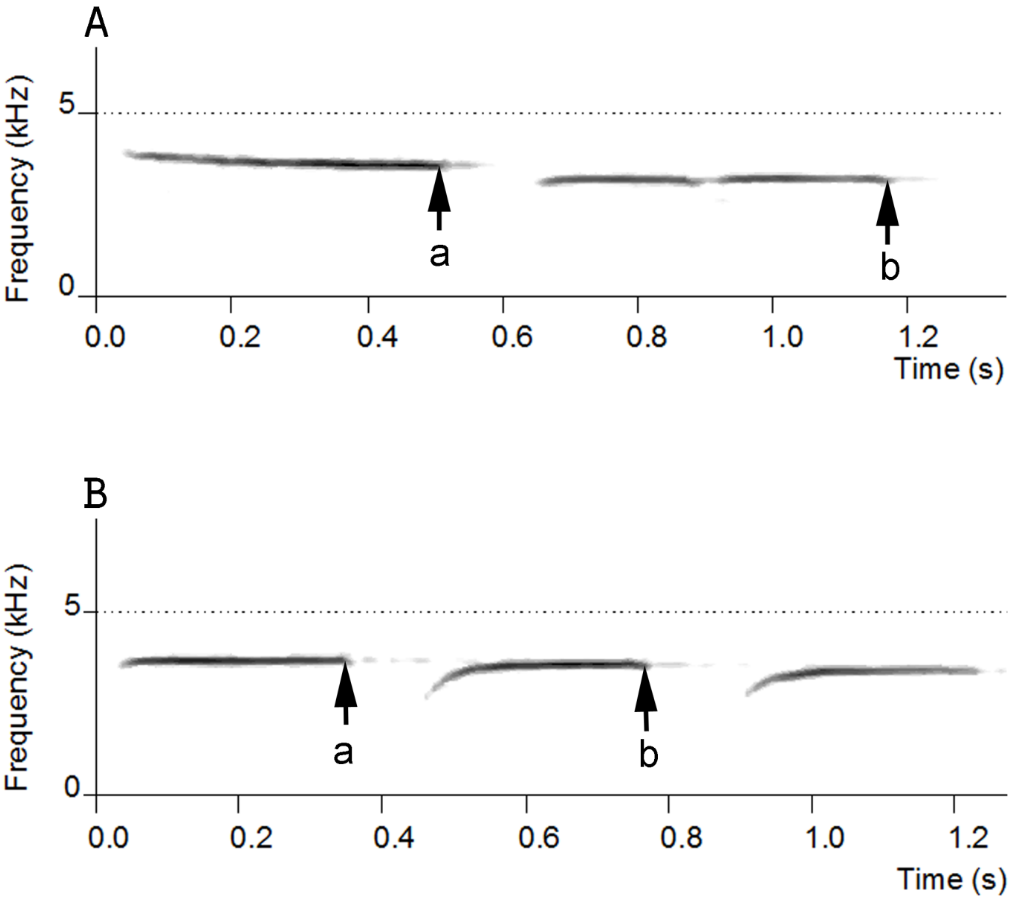

Figure 4.1.: Song sonogram from the mixed species site. A- black-capped chickadee song sonogram, B- mountain chickadee song sonogram. a is the frequency at the end of the first note and b the frequency at the end of the second note. I used the ratio a/b (internote ratio) for my detailed acoustic analysis.

I also conducted fine analysis of the mountain chickadee dawn choruses, as these have not been previously described in detail. I extracted 20 songs and 30 calls, evenly distributed across the chorus, to determine song and call note composition. Analysis was conducted with Avisoft SASLab-Pro software. Mountain chickadee call note types have been describe by Bloomfield et al. (2004). They differentiated 6 notes type: A, A/B, B, C, Dh and D (fig. 4.2). Because there is a constant gradient from note A to A/B to B, I classified those three note types as being note A-B. Statistical analysis was conducted on STATISTICA (version 8.0, StatSoft, Inc.). Nonparametric analyses were used where data was proportional (e.g. proportion of chorus spent producing songs vs calls) and General Linear Models were used where the data was continuous and met assumptions of normality.

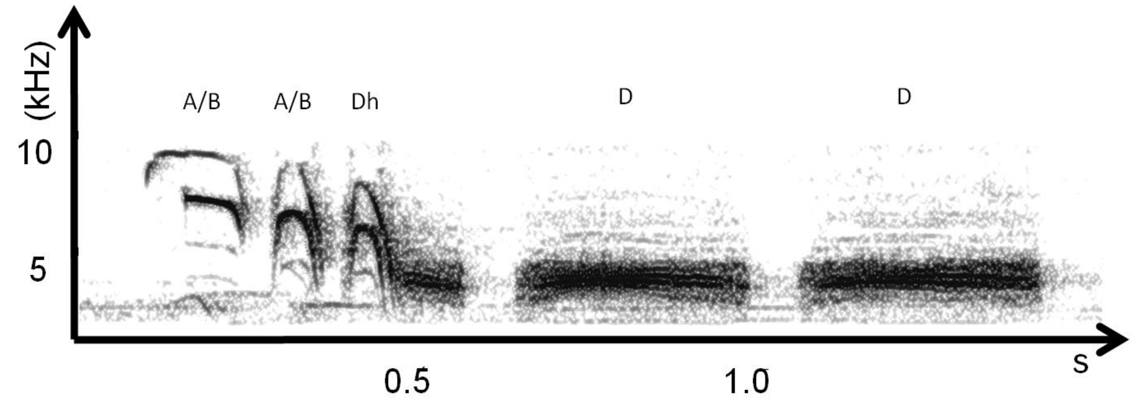

Figure 4.2.: Mountain chickadee *chick-a-dee* call sonogram. 95% of the calls used during the chorus were composed of note type A-B, Dh and D.

4.3. Results

4.3.1. General chorus pattern

Black-capped chickadee dawn choruses are composed almost exclusively of song, both for the sympatric and allopatric population. During an entire chorus, black-capped chickadees from mixed-BC population use songs 94% of the time during vocalization (Wilcoxon test on the time spent singing versus calling, p=0.002, n=12, power of analysis=100%) and in pure-BC they sing 92% of the time (Wilcoxon test on the time spent singing versus calling, p=0.005, n=10, power of analysis= 100%). The pure-BC and the mixed-BC populations did not significantly differ in their proportion of the total minutes that birds were singing during the dawn chorus (Mann Whitney U test p=0.89, n=22, effect size=0.12).

The dawn chorus of the mountain chickadee is a mix of songs and *chick-a-dee* calls. In pure-MO, there was no significant difference between the proportion of time spent producing songs (45% of the time) versus *chick-a-dee* calls (55%) during the chorus (Wilcoxon test p= 1, n=8, effect size=0.21); whereas in the contact zone, birds from the mixed-MO population spent significantly more time producing *chick-a-dee* calls (80% of the time) than songs (20% of the time) (Wilcoxon test p= 0.01, n=9, power of analysis=99.9%)(fig. 4.3). Birds from the mixed-MO use significantly more *chick-a-dee* calls than individuals from the pure-MO population (Mann Whitney U test p=0.05, n=17, power of analysis=100%).

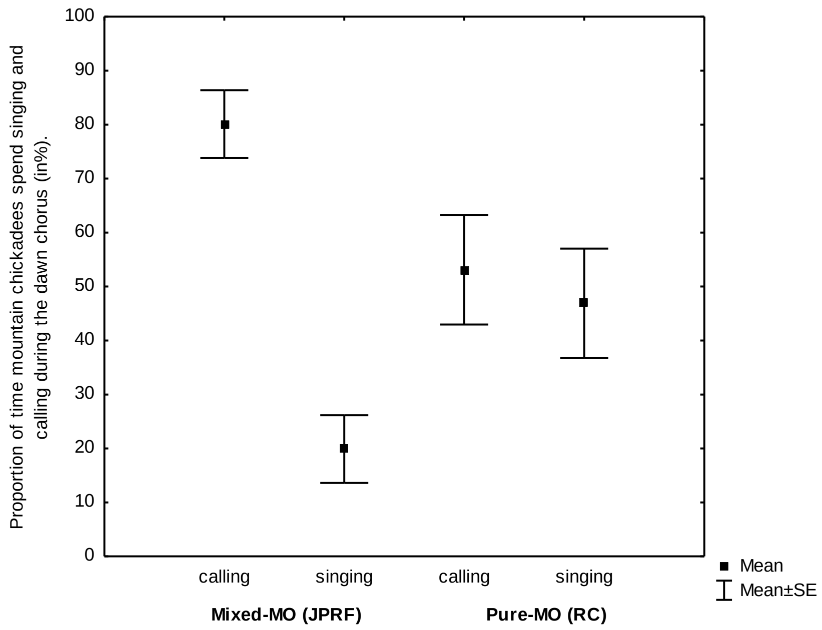

Figure 4.3: Proportion of time mountain chickadees from the mixed-MO population (JPRF, Wilcoxon test p= 0.01) and pure-MO population (RC, Wilcoxon test p= 1) spend calling and/or singing during the dawn chorus.

4.3.2. Chick-a-dee call analysis

Chick-a-dee calls used by mountain chickadees during the dawn chorus are highly stereotyped: amongst the 30 calls per bird I randomly extracted from the chorus, 95% were composed of note type A-B ; Dh and D (fig 4.2). Half of the calls were either A-B A-B A-B Dh D D (21% at mixed-MO population, 30% at pure-MO population) or A-B A-B Dh D D (29% at mixed-MO population, 20% at pure-MO population).

4.3.3. Song analysis

At both the pure-MO population and the mixed-MO population, mountain chickadees use songs that are primarily composed of three notes. I observed a higher amount of 2, 4 and

5-note songs among the mixed-MO population (32%), even though 3-note songs are still the most common (68%). By comparison, the pure-MO population used 3-note songs more consistently (92% of all songs). However, this difference in number of notes per song was not significantly different between sites (Mann-Whitney U test, p=0.16, n=14, effect size=0.44).

There was no significant difference in the frequency at the end of the first note between birds of either species across the single-species populations (pure-MO and pure-BC) and mixed-species population (mixed-BC and mixed-MO) (general linear model, p=0.43, F(3,32)=0.948). However, I found significant differences between populations for the frequency at the end of the second note (general linear model, p=0.03, F(3,32)=3.299, power of analysis=70%). Post hoc analysis showed this difference occurred between the two chickadees at the contact zone site with mixed-MO's second note being higher pitch than the mixed-BC's second note (Tukey HSD test p=0.01, df=32, n=36). There was no difference in the pitch of the second note between black-capped chickadees from the pure-BC and the mixed-BC population (p=0.40). I found that the absolute pitch of the second note did not differ between the songs of mountain chickadees in the pure-MO or mixed-MO population (p= 0.37). However, examination of the internote ratio revealed that the mountain chickadees from the pure-MO population have a significantly lower ratio between the frequency at the end of the first note and the frequency at the end of the second note than the three other studied populations (general linear model, p<0.001, F(3,32)=47.542, power of analysis=100%)(fig 4.4). Post hoc analysis show significant differences between the mixed-MO population and: 1) the pure-MO population (Tukey HSD test p<0.001, df=32, n=36), 2) the mixed-BC population (Tukey HSD test p<0.001, df=32, n=36), and, 3) the pure-BC population (Tukey HSD test p<0.001, df=32, n=36). There were no significant differences in internote frequency ratios between the other three populations (mixed-BC, pure-BC and pure-MO) (all p >0.1).

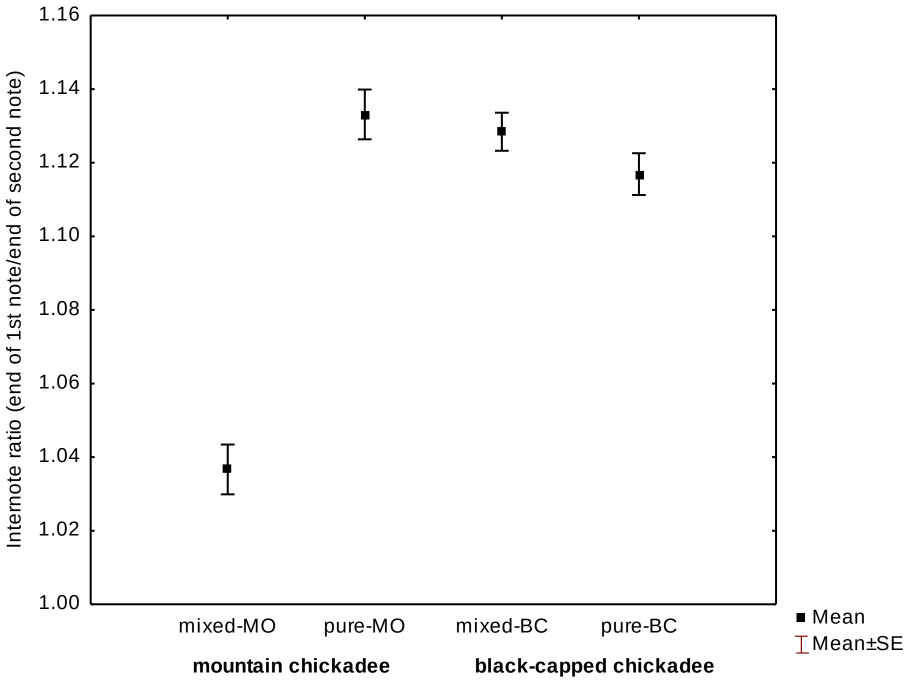

Figure 4.4: Internote ratio of sympatric and allopatric black-capped and mountain chickadees.

I also found that black-capped chickadees differ from mountain chickadees in the glissando of the first note (general linear model, p<0.001, F(3,32)=32.532, power of analysis=100%)(fig 4.5). Post hoc analysis revealed that the glissando of the first note is statistically different for the pure-MO population and both the mixed-BC (Tukey HSD test p<0.001, df=32, n=36) and the pure-BC population (Tukey HSD test p=0.005, df=32, n=36). The mixed-MO glissando also differs significantly from both pure-BC (Tukey HSD test p<0.001, df=32, n=36) and mixed-BC population (Tukey HSD test p<0.001, df=32, n=36).

Finally, I found that the glissando from the mixed-MO and the pure-MO population are significantly different (Tukey HSD test p=0.003, df=32, n=36). I also noticed that all studied populations except the mixed-MO had a glissando with a ratio>1. This indicates a descending first note whereas the mixed-MO glissando is less than one, illustrating a slightly ascending first note (fig 4.5).

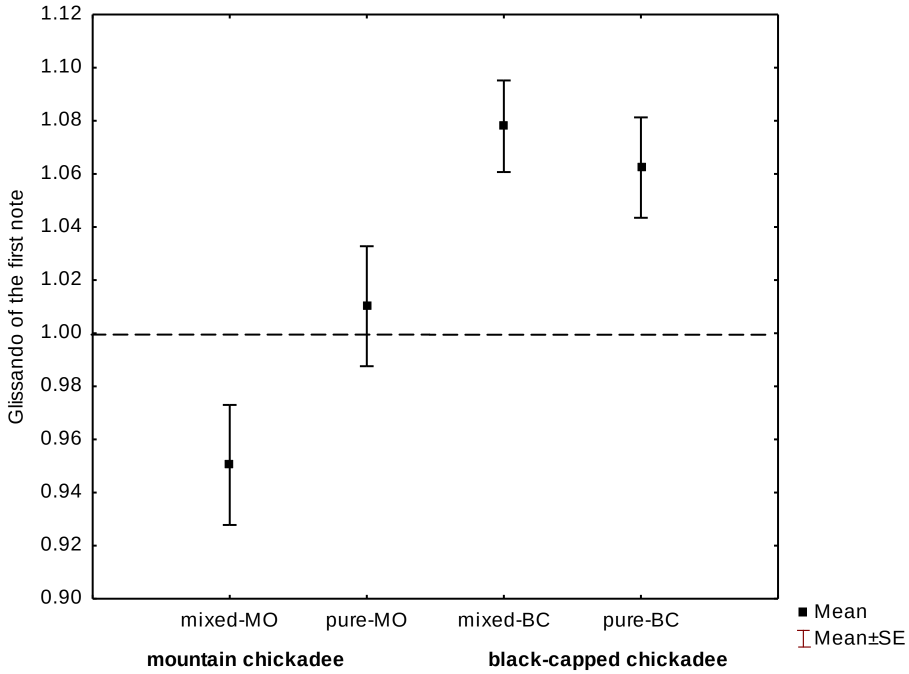

Figure 4.5: Glissando (ratio between the frequency at the start and at the end of the note) of the first note in the song for the four studied populations of chickadees. Only the mountain chickadee from the mixed-MO population has a glissando inferior to 1, indicating an ascending first note.

4.4. DISCUSSION

Mountain chickadee dawn chorus behaviour varied between our sampled populations that were either sympatric ("mixed-MO") or allopatric ("pure-MO") with black-capped chickadees. There was a significant increase in the proportion of *chick-a-dee* calls in the mixed-MO population compared to chorusing behaviour of mountain chickadees in a pure population. The more dominant black-capped chickadee, however, had no difference in chorusing behaviour between the pure-BC and mixed-BC populations, and was also consistent with dawn chorus patterns reported from across the majority of their distribution range (Gammon 2007).

Black-capped chickadees do not appear to modify their internote ratios (measured here as the frequency ratio between the end of the 1st syllable and the end of the second syllable) whether they co-occur with mountain chickadees (mixed-BC) or not (pure-BC). Interestingly, mountain chickadees in the pure-MO population have internote ratios similar to those of black-capped chickadees in either sampled population. The mountain chickadees from the mixed-MO population, however, had significantly lower internote ratios than any other measured groups, shifting the structure of their song away from the observed pattern of black-capped chickadee song.

The glissando (ratio of the frequency at the start and at the end of the first note) also varies between the four studied populations: mountain chickadees had significantly lower glissando in the first note of the song than did black-capped chickadees. While there was no differences in the glissando between the two black-capped chickadee populations, mountain chickadees from the mixed-MO population had significantly lower glissando ratios than mountain chickadees from the pure-MO population. As a result, mountain chickadees in our overlap zone have a first note that ascends slightly in frequency from start to end, whereas all other populations of either species have a first note that descended in frequency. Our results support the character shift hypothesis, as mountain but not black-capped chickadees, have altered songs when they co-occur with a closely related species. Similar results have been found between pied (*Ficedula hypoleuca*) and collared flycatcher (*F. alibicollis*) - differences between the song of either species are accentuated where their ranges overlap (Wallin 1986). Our results suggest, however, that this character shift is asymmetric. Black-capped chickadee song is highly stereotyped throughout the species range (Gammon 2007, Sturdy et al. 2007). The internal frequency structures, both within and between notes, are similar to those measured from mountain chickadee songs from our allopatric population (pure-MO) in Riske Creek. However, the mountain chickadees within the overlap zone (mixed-MO) at the JPRF show significant modification of these ratios away from those associated with black-capped chickadees. I demonstrated previously that black-capped chickadees are dominant to mountain chickadees within this contact zone (chapter 2). Thus, these results are also consistent with the greater character displacement occurring within the subordinate species (Miller 1968, Grant 1972, Doutrelant et al. 2000b).

Mountain chickadees in our mixed-MO population use a greater proportion of *chick-a-dee* calls during their chorus than do mountain chickadees in our pure-MO population. Previous research suggested that calls during the chorus of this species may be directed primarily towards females and song towards males (McCallum et al. 1999). Within our mixed species populations, I have found evidence of hybridization between mountain and black-capped chickadees, which appears to arise from directional extra-pair copulations, hybrid offspring occur only in mountain chickadee nests, and DNA analyses indicate these arise from female mountain chickadees having extra-pair copulations with black-capped chickadee males (chapter 2). Because of the lower social rank of mountain chickadees and hybridization through directional EPCs in my study sympatric zone, mountains chickadee males might increase their proportion of *chick-a-dee* calls in an attempt to limit interspecific extra-pair mating.

Dawn chorusing in black-capped chickadees appears to be at least partially directed towards females, including the focal birds' mate (Otter & Ratcliffe 1993). Sympatric male mountain chickadees might shift to signals during the choruses that are easily distinguishable (composed mainly of *chick-a-dee* calls rather than *fee-bee* songs) from the black-capped chickadees to avoid acoustic competition. This difference would be further accentuated by altering the note structure of those songs that are included during the chorus. Mountain chickadees in the sympatric zone have lower internote frequency ratios, and ascending rather than descending first notes, when compared to the mountain chickadees from the pure population and both populations of black-capped chickadees. The differences observed during the dawn chorus between the sympatric mountain chickadees and the three other studied populations (mixed-BC, pure-BC and pure-MO) might illustrate an attempt to avoid overlapping during the dawn chorus with the acoustic space used by the dominant black-capped chickadee. This change in both the structure of the song, as well as the use of fewer songs overall during the chorus, might also limit aggression from the dominant species; Gil & Gahr (2002) demonstrated that individuals expressing dominant signals are challenged more often. However, these differences in signalling are likely to increase differences in mate attraction, and thus may constitute a reproductive character displacement. If hybrids suffer reduced fitness, then character divergence might be selected through reinforcement against interspecific matings (e.g. Noor 1999). To confirm this interpretation, playback experiment will be needed to test black-capped chickadees' responsiveness to both sympatric and allopatric mountain chickadees' song.

In Europe, blue tit (*Cyanites caeruleus*) song repertoire varies depending on whether or not the birds co-occur with the more dominant great tit (*Parus major*). When great tits are present, blue tit repertoires are smaller (Doutrelant et al. 2000a) and they use more trilled songs (Doutrelant & Lambrecht 2001); this class of song types is most dissimilar to songs of great tits. Doutrelant et al. (2000b) also demonstrated that great tit responded less to trilled blue-tit songs, suggesting that trilled songs have evolved to avoid interspecific interactions, which is consistent with the character shift hypothesis. However, our results are surprising when compared with other data on chickadees hybrid zones. Curry et al. (2007) showed that males in the Carolina/black-capped chickadee hybrid zone are often bilingual; either species incorporates songs of the heterospecific species within its own repertoire instead of changing acoustic parameters of their own songs.

An alternate explanation for the observed acoustic variations between my four different populations might be based upon transmission differences due to the habitat structure. The habitat at the mixed species and the pure-BC site are similar (both sites are mature forests within the sub boreal spruce zone) but the pure-MO‘s site was more open with mature forest stands surrounded by grassland within the Interior Douglas fir biogeoclimatic zone. Marten & Marler (1977) found that sound transmission decreases with increasing vegetation density. As a result acoustic variations enhanced by habitat structure should lead individuals in less open areas (such as in the mixed-MO/mixed-BC site) to use more songs than calls, as pure tonal notes (such as *fee-bee* songs) tend to transmit better than vocalizations with broader frequency sweeps (such as *chick-a-dee* calls). In this study, I observed the opposite, which suggests that habitat structure is unlikely to be the cause of the observed differences. This conclusion helps reinforce that character displacement among closely-related species in sympatric populations may be the best explanator of the signals observed in this study.

5- MICRO-HABITAT SEGREGATION WITHIN OVERLAPPING RANGES

Abstract- Coexistence of species that share similar ecological requirements usually induces niche partitioning, which then limits interspecific competition. Microhabitat segregation can occur either year round, only during the breeding season, or only during the non-breeding season. In Europe, Paridae species are known to use different ecological niches where they co-occur. In North America, where sympatry between chickadee and titmice is less extensive, less research has addressed microhabitat use when closely-related species do co-occur. I examined a contact zone of mountain and black-capped chickadees in which both species occur in mixed-species winter flocks with frequent interspecific interactions. Hybridization in this population is both frequent and directed, with hybrids forming from extra-pair matings between female mountain chickadees and male mountain chickadees. In this study, I assessed patterns of microhabitat associations among birds during the breeding season. I found interspecific interactions are less frequent during the breeding season, and that each species seems to be associated with its specific microhabitat within overlapping territories - more coniferous areas for the mountain chickadees and deciduous for the black-capped chickadees. Reproductive success and provisioning rates were similar between the two species, suggesting that neither species was forced to use lower-quality habitat. I also observed differences in timing of breeding onset, which might also limit interspecific competition over resources during nestling provisioning.

5.1. Introduction

Lack (1954) contended that differentiation in foraging behaviour is one of the primary factors that allows for overlapping home ranges between species; competition over resources would be diminished if evolution favoured foraging-niche segregation among overlapping species. This would be especially true where the prey selected by both species overlaps, as might occur when species share phylogenetic history or a particular foraging guild. When such species have overlapping distributions, one would predict that they would become more specialized in their ecological niches than among non-overlapping populations of the same species. Such segregation may limit or eliminate interspecific competition. Stallcup (1968), for example, reported that among overlapping populations of wintering white-breasted (*Sitta carolinensis*), pygmy nuthatches (*S. pygmea*) and hairy woodpeckers (*Picoides villosus*), each species preferentially forages within a specific part of the habitat. While this niche segregation is diminished during the breeding season, previous research suggested that overlap in foraging niche during the summer months was the results of an increase in overall insect abundance, which reduced the potential for food competition (Colquhoun & Morley 1943, Hartley 1953).

Where there is asymmetry in the competitive abilities among overlapping species, a number of comparative studies and field experiments have demonstrated that competition over foraging sites will induce niche shifts (reviewed by Alatalo 1982, Alatalo et al. 1986). In such cases, the dominant species will typically occupy their preferred foraging niche; there is often no perceivable difference in foraging behaviour of the dominant species among populations that either do or do not overlap with competitors. Conversely, the subordinate species is likely to avoid sites preferred by the dominant competitor, and thus may occupy foraging sites that differ markedly from where this species occurs in isolation. Allopatric populations of yellow-headed blackbirds (*Xanthocephalus xanthocephalus*) and red-winged blackbirds (*Agelaius phoeniceus*) use similar ecological niches, but when they occur in sympatry the yellow-headed blackbird excludes the redwings from both preferred territories and nest sites (Miller 1968). Similarly competition for nest site among guilds of cavity-nesting birds often results in exclusion of subordinate species from preferred nest sites, with the latter group often relegated to cavities that are either unused or unavailable to the more dominant competitors (e.g. Martin et al. 2004, Martin & Norris 2007).

In Europe, species within the family Paridae (chickadees and titmice) often occur in sympatry with up to six species coexisting (Sturman 1968, Lack 1969). Within this group, there is ample evidence of divergence in foraging niches (e.g. Lack 1969) and even in nest-site segregation (e.g. Dhondt 1989). Conversely, North American species within the Paridae are typically more allopatric, with often only one or two species overlapping in distribution. Some authors suggest that this non-overlapping distribution has been enhanced by interspecific competition, suggesting that these species segregate ecologically (Lack 1969). Where chickadee species overlap in geographic range, they often segregate by forest types or microhabitat preferences within the same forests, such as occurs between boreal chickadees (*Poecile hudsonicus*) and black-capped chickadees (Dixon 1961) and between chestnut-backed chickadees (*P. rufescens*) and black-capped chickadees (Smith 1967, Sturman 1968). Among some species, though, both overlap and similarity in ecological preferences occurs.

Black-capped and mountain chickadees (*P. atricapillus* and *P. gambeli*) are typically segregated by microhabitat, but sympatry does occur in areas where a mosaic of habitat preferred by either species co-occurs (Hill & Lein 1988). Within an overlapping population in the Canadian Rockies, Hill & Lein (1988) demonstrated that both species had distinct foraging behaviour - black-capped chickadees forage lower and more often in deciduous trees than do mountain chickadees. They also suggested that black-capped and mountain chickadee diets may not overlap, thus maintaining ecological segregation between these species (Hill & Lein 1988). While there was occasional competition for nest sites among overlapping populations, Hill & Lein (1988) found little overlap in cavity use between black-capped and mountain chickadees. This reduced interspecific competition for nest sites may result from black-capped chickadees being predominately primary cavity excavators and mountain chickadees being secondary cavity users. Conversely, Carolina chickadees (*P. carolinensis*) and black-capped chickadees show extensive overlap in territories, foraging behaviour and nest sites (Brewer 1963) in eastern North America. Hybridization between black-capped chickadees and Carolina chickadees is frequent, whereas hybrids between black-capped chickadees and boreal chickadees or chestnut-backed chickadees are extremely rare (Curry 2005). I demonstrated that hybridization between black-capped chickadees and mountain chickadees occurs in my study population (chapter 2). Based on previous research on other chickadee overlap zones, the extensive hybridization might be associated with interspecific competition over resources (e.g. nest site) during the breeding season. By observing both species behaviour at the nest, measuring their reproductive success and habitat characteristics, I attempt to determine whether Hill & Lein (1988) findings were also applicable in my overlapping zone and whether they could explain some aspects of the observed hybridization pattern.

5.2. Methods

5.2.1. Study site and species

In the fall and early winter of 2008-2010, I banded birds with a unique combination of one Canadian Wildlife Service numbered aluminum band and three plastic coloured bands. Black-capped chickadees and mountain chickadees are easily distinguished based on plumage patterns, the main differences being the presence of a white supercilliary line in the mountain chickadee, which is absent in the black-capped chickadee. I determined sex of the birds by using combination of body measurements (weight and tarsus, tail and wing length), with males being larger than females in both species (Foote et al. 2010, McCallum et al. 1999), and confirmed these assessment during the breeding season with sex-specific behaviour.

5.2.2. Breeding behaviour monitoring

I followed birds from both species after the flock broke up in early spring 2009 and 2010 to determine identities of pairs and nest locations. Once a cavity was identified, I monitored it every 2 to 3 days to determine activity and stage of breeding at each nest (e.g. excavation, incubation, hatch, fledge). Dates were recorded with April 1st being day 1. I noted date of start of incubation, hatching and fledging. I observed each nest on three specific occasions while the parents were feeding the nestlings: at 4-6 days; 8-10 days and 13-15 days post-hatching. I counted the number of visits to the nest, how long they stay in the cavity and when possible I noted which parent was attending (for birds that were colour-banded) and what type of food they were bringing (e.g. caterpillar). I started the observation period when one of the two parents arrived at the cavity and recorded feeding activity and identity of the provisioning bird for 30 minutes.

Between 6 to 10 days post-hatching, I banded the chicks in all nests that were accessible. Most of the nests were in natural cavities and ranged from 2m to over 20m above ground in elevation. I used extension ladders (up to 10m) or tree-climbing equipment to access cavities. Cutting a portal a few centimetres above the nest cup, I removed the nestlings in two stages to minimise risk of nest abandonment (no nests were abandoned as a result of my activities). I counted the number of nestlings and examined the nest cup for unhatched eggs. Each nestling was banded with a CWS aluminum band and was then returned to the nest. I re-inserted the portal and secured it with tape to close the cavity. Nests that were not accessible (e.g. too high, or in trees that were too decayed to safely climb) were monitored from the ground to determine whether a successful fledge occurred.

5.2.3. Habitat and nest tree monitoring

I measured habitat variables in 11.3 m radius circular plots (0.04ha) centered on nest trees within two weeks after hatching in both 2009 and 2010. I recorded variables related to both the habitat surrounding the nest tree and the nest tree itself (table 5.1). Variables associated with the cavity-bearing tree were: species; Diameter at Breast Height (DBH); nest height (using a clinometer); and cavity tree condition. Conditions was classified into five distinct categories: 1- alive, 2- newly dead, 3- dead with internal decay evident, 4- dead with advanced internal decay and loss of upper branches, 5- stump. The variables associated with the surrounding habitat were: number of trees (higher than 10m) within each plot categorized by species, size class (1- DBH<10cm, 2- 11<DBH<20cm, 3- 21<DBH<30, 4- DBH>30cm), and condition (5 decay classes mention above). Canopy cover was estimated using a densiometer at the four cardinal edges of the plot. Species, height (using a clinometer) and DBH of a tree representative of the canopy were recorded. I counted the number of snags within each plot and recorded species, DBH size class and decay class. Shrub density was estimated by assessing the overall percentage of cover at three vertical classes (0-1, 1-3, 3-10m).

To assess the relative abundance of caterpillar prey during the breeding season, I used caterpillars' frass collected in conjunction with another project from early May to mid June in 2010. This project was focusing only on black-capped chickadees and thus samples were taken only around black-capped chickadee's nests. However, I am confident they still represent food availability for both species as mountain and black-capped chickadee territories were interspersed across the study area. I set up four buckets 5 meters from each black-capped chickadee cavity, one in each cardinal direction. The open end of each bucket was covered with a mesh in a slightly inverted funnel shape, upon which I placed paper napkin filters. Every 3 days, I collected the napkins and installed new ones. Napkins were folded in upon themselves to ensure that no frass was lost during removal, and these were immediately placed into paper bags for transport and storage. Samples were then desiccated at 40°C in a drying oven for 48 hours. Using a magnifying lens, I sorted frass from vegetal debris, and weighed the frass with an analytical scale to the 0.01mg. The four samples for each nest were then added and I averaged the data across nests (N=7) for the total amount of frass in the habitat in milligram/cm^2/hour.

Table 5.1: Habitat and nest tree variables used in the analysis.

Habitat	
% C	percentage of coniferous trees
%D	percentage of deciduous trees
T1	trees with diameter at breast height under 10 cm
T2	trees with diameter at breast height between 11 and 20 cm
T3	trees with diameter at breast height between 21 and 30 cm
T4	trees with diameter at breast height above 30cm
#trees	number of trees
#species	number of species
Ca height	Canopy height
Ca cover	Canopy cover
Cadbh	diameter at breast height of a tree representative of the canopy
Sn 2	newly dead trees
Sn 3	dead trees with evident internal decay
Sn 4	dead trees with advanced internal decay, only half of its original
Sn 5	stump
# C dead	number of dead coniferous trees
# D dead	number of dead deciduous trees
>3m	Shrub cover between 3 and 10 meters high
1 a 3 m	Shrub cover between 1 and 3 meters high
<1m	Shrub cover below 1 meter high
Nest tree	
NTsp	coniferous tree=1 and deciduous tree=0
NT dbh	nest tree diameter at breast height
Cah	cavity height
NT decay	nest tree decay, classes 1 to 5: 1 tree is alive; 2 to 5 same stages of decay as used for the snag

5.2.4. Statistical analysis

All analyses were performed with STATISTICA (version 8.0, StatSoft, Inc.). I used a Fisher exact test to compare proportion of nests that successfully fledged young between years and between species. I compared breeding data and nest tree characteristics between years and species using general linear models. Provisioning data was compared with general linear models in which within-subject effects were compared across the three sampling periods for each nest, and between subjects comparisons were made between mountain and black-capped parental behaviour. I conducted a principal component analysis on the variables associated with habitat characteristics. I then compared the factor scores from PCAs between black-capped and mountain chickadees using general linear models as outlined above.

5.3. Results

5.3.1. Reproductive success and breeding data

There was no difference in the probability of successfully fledging young from the nest between black-capped and mountain chickadees (Fisher exact test, p=0.67, n=30): 13 of 17 black-capped chickadee nests and 11 of 13 mountain chickadee nests successfully fledged offspring. There was no significant differences in brood size between species (general linear model, F(1,14)=0.35, p=0.56): black-capped chickadees had an average of 6.7 (SD ± 1.9) eggs and mountain chickadees had an average of 6.2 (SD ± 1.5) eggs per clutch.

I did not find any significant differences between species in their provisioning rates to nestlings (general linear model, F(1,34)=3.31, p= 0.08). However, I found a significant increase in provisioning visits to the nest during the nestling stage that was associated with both species (general linear model, F(2,33)=3.93, p=0.03). Post hoc analysis revealed that this increase in feeding rate occurred between day 4-6 and day 8-10 (Tukey HSD test, df=33, p=0.049) and thereafter the number of visit to the nest stayed consistent until fledging (Tukey HSD test, df=33, p=0.99). During observations on provisioning parents, I was able to identify the prey brought to the nestlings' in 53% of the visits for the black-capped and 25% of the visits for the mountains. As mountain chickadees nests were significantly higher (see below), it was more difficult to identify prey items. The identified prey was caterpillar species in 81% of instances for black-capped chickadees and in 71% of instances for mountain chickadees.

I found significant differences between years in the timing of reproduction within each species: birds bred eight days later on average in 2009 compared to 2010 (general linear model, F(1,25)=95.19, p<0.001). When controlling for this annual variation in laying date, mountain chickadees bred significantly later than black-capped chickadees in both years, with approximately a six day delay between the onset of mountain chickadee breeding relative to black-capped chickadee breeding (general linear model, F(1,25)=59.92, p<0.001).

5.3.2. Habitat characteristics

Principal component 1 (PC1) accounted for more than 25% of the total variation in habitat characteristics among plots, and principal component 2 (PC2) accounted for an additional 16%, principal component 3 (PC3) for 11%, and principal component 4 (PC4) for 10%. As I included 20 variables in the analysis, PC1, PC2, PC3 and PC4 exceeded the value of explained variation expected by chance, using the broken-stick method of factor significance (Jackson 1993, Legendre and Legendre 1998).

As a rule of thumb, Ho (2006) suggests the variable contribution to the PC value should be 0.33 or higher to be represented by the PC. Fourteen of the twenty variables exceeded this contribution to PC1 (table 5.2). Out of these 14 variables, 9 had a negative contribution to PC1 (%Deciduous, Canopy height, Canopy dbh, Snag2, Snag4, #Deciduous dead, >3m, 1 to 3m, <3m), and 5 had a strong positive contribution to PC1 (% Coniferous, Trees2, #tree, #species, #Coniferous dead). Thus, higher values of PC1 are associated with habitat that has more coniferous trees, more trees overall and a higher diversity of vegetation. As PC1 was significantly higher for mountain chickadees than for black-capped chickadees (general linear model, F(1,25)=14.75, p<0.001), these habitat characteristics are more associated with mountain than black-capped chickadees in our contact zone. Further, these results suggest the canopy was higher in black-capped territories and trees representative of the canopy were bigger. It also indicates that there were significantly more dead coniferous trees in mountain chickadee habitat. Finally, shrub density was significantly higher around black-capped chickadee nests at the three levels sampled.

PC2, PC 3 and PC 4 were not statistically different between species (general linear model, p>0.1) and none of the principal component differ significantly between 2009 and 2010 (general linear model, p>0.05).

Table 5.2: Variable contributions to PC1.

	Factor 1	Factor 2	Factor 3	Factor 4
% C	**0.78**	-0.12	0.48	0.00083
%D	**-0.78**	0.12	-0.48	-0.00083
T1	0.16	-0.13	-0.69	-0.039
T2	**0.80**	-0.23	-0.081	0.20
T3	-0.0094	0.70	-0.026	-0.18
T4	-0.22	0.69	0.446	-0.11
#trees	**0.68**	0.21	-0.386	0.029
#species	**0.60**	0.17	0.35	0.28
Ca height	**-0.46**	0.71	0.17	0.058
Ca cover	0.16	0.12	-0.096	0.13
Cadbh	**-0.54**	0.51	0. 40	-0.24
Sn 2	**-0.44**	-0.44	0.38	0.41
Sn 3	-0.32	0.41	-0.38	0.51
Sn 4	**-0.54**	-0.35	0.33	0.47
Sn 5	0.0075	0.22	-0.29	0.61
# C dead	**0.44**	0.094	0.031	0.51
# D dead	**-0.68**	-0.045	-0.0034	0.64
>3m	**-0.34**	-0.74	0.23	-0.10
1 a 3 m	**-0.58**	-0.35	-0.072	-0.22
<1m	**-0.46**	-0.26	-0.29	-0.34

The caterpillar frass collected in 2010 revealed a peak in caterpillar abundance on day 58 and day 67 relative to 1 April (fig 5.1). Those dates matched the hatching date for each species, in 2010 black-capped chickadee nests hatched on average on day 57 (May 27th) and mountain chickadee nests on day 68 (June 7th).

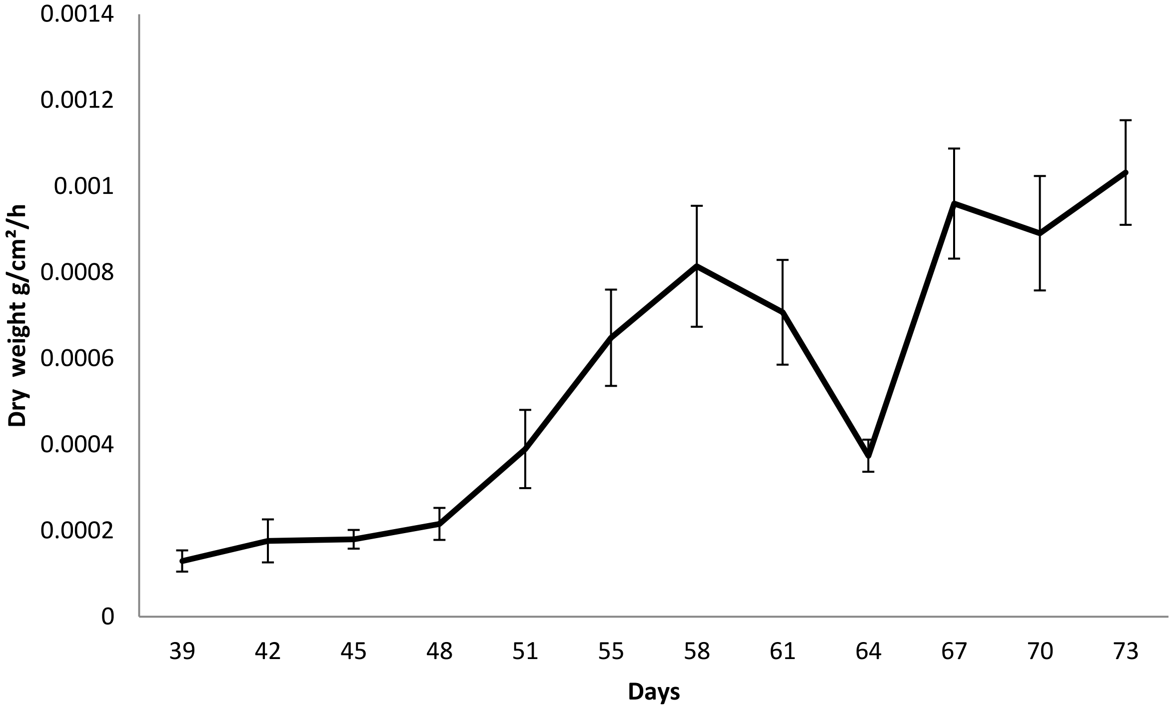

Figure 5.1: Quantity of caterpillar frass (in g/cm²/h) with corresponding standard errors from May 9th (day 39) to June 12th (day 73).

5.3.3. Cavity characteristics

I found no significant differences in nest tree species, condition and DBH or cavity height between years (general linear models, all $p>0.1$). When comparing data across species, I found that black-capped chickadee nests occurred more often in deciduous trees while mountain cavities were usually in coniferous trees (general linear model, F(1,22)=7.42, p=0.01). Also the DBH of trees in which black-capped chickadee cavities were located was significantly smaller than trees in which mountain chickadees nested (general linear model, F(1,22)=4.31, p=0.04). Both height of the cavity (general linear model, F(1,22)=1.87, p=0.19) and condition class of the nest tree (general linear model, F(1,22)=0.25 p=0.6) did not differ significantly between species, even though mountain chickadee nests were generally higher (13.8 m +/-5.4) than black-capped chickadee cavities (9.8 m +/- 6.1).

5.4. Discussion

My results are similar to those of Hill & Lein's (1988) Alberta contact zone in finding a divergence in microhabitat use by both black-capped and mountain chickadees. Although the territories of the two species overlap, mountain chickadees in our contact zone tend to center their nests in areas with more coniferous than deciduous trees, higher overall tree densities, and greater diversity of tree species than did black-capped chickadee. Within our contact zone, black-capped chickadees tend to center their nests within areas of their territories dominated by deciduous trees, whereas the mountains accepted a more mixed-species microhabitats for nest locations.

In my study site, black-capped chickadee nests were also located in areas where the canopy was higher than those of mountain chickadees. I found significantly more dead conifer trees in the areas used by mountain chickadees for breeding, suggesting they may be keying into such areas as cavity locations. Finally, shrub density was significantly higher around black-capped chickadee nests at the three shrub levels sampled.

Despite these differences in micro-habitat associated with the nests of either species, I found no differences in reproductive success between mountain and black-capped chickadees. This result may indicate that mountain chickadees, the subordinate species (chapter 2) are not necessarily forced into less preferred habitat, as has been suggested by Hill & Lein (1988). The differences in habitat may instead reflect subtle differences in foraging area and/or preferred nest tree characteristics.

Similar to Hill & Lein (1988), I found that the trees in which mountain chickadee cavities occurred were larger than those used by black-capped chickadees. Further, mountain chickadees usually nested in coniferous trees whereas no black-capped chickadees' cavities were observed in conifers within our study site. Thus, our results would support previous findings suggesting that nest site competition between black-capped and mountain chickadees is low (Hill & Lein 1988). Hill & Lein (1989a) also suggested that interspecific territoriality was limited during the breeding season and that breeding territories overlap. Within those overlapping breeding territories, they show that each species used different parts of the habitat when foraging; black-capped chickadees foraged more in deciduous trees, lower in the canopy and in smaller trees than did the mountain chickadees (Hill & Lein 1988). Whether those differences in foraging resulted from interspecific competition or ecological segregation was unclear. The differences I found in habitat characteristics immediately around the nests of either species, with black-capped chickadees occurring where there were more deciduous trees and greater shrub density, would tend to support the idea that the two species may be partitioning niches within overlap zones.

I found no differences between species in provisioning, despite both species showing significant increase in visitation rate between early- and mid-nestlings' periods. Both species are similar in size and weight and are insectivorous during the breeding season. Black-capped chickadee nestlings fledge at 16 days (Foote et al. 2010) while mountain chickadees offspring will stay in the nest for 21 days before fledging (McCallum et al. 1999). Previous research suggested that this difference in time spent in the nest before fledging might be due to a slower growth rate for mountain chickadees nestlings compared to black-capped chickadee nestlings (Grundel 1987). Because brood size between species is not significantly different in our study area, and there is no difference in overall provisioning rates between species, it would suggest that the subordinate species (mountain chickadees) are breeding in territories that provide similar availability of food items to deliver to offspring – although my data would not allow me to discern whether these items are the same in nutritional quality.

I found differences in breeding onset between years and species. Black-capped chickadees bred on average six days earlier than mountain chickadees and both species bred eight days later on average in 2009 compared to 2010. The observed difference across the two years of the study is likely due to a very early winter thaw and low snowpack in the 2010 year. Indeed the large lake that borders the research forest (Lake Tezzeron, 4km wide and 20km long) was ice-free on May 21st in 2009 and on April 25th in 2010. The asynchrony in timing of breeding between black-capped and mountain chickadees can be interpreted in various ways.

First, I found two peaks in frass abundance across the study area: the first peak coincides with hatching date for the black-capped chickadees and the second for the mountain chickadees' hatching date. As most of the foods items identified during the observations were caterpillar, both species might be timing their breeding season on caterpillar availability to feed their young and each species might be using different caterpillar species (indicated by the two different peaks). However, such intrepretation would require more detailed analysis of prey species than I was able to conduct and awaits future studies. The high proportion of caterpillars among identifiable food items might also be the result of caterpillars being more conspicuous than arthropods, which also are a significant component of chickadee diets (Foote et al. 2010, McCallum et al. 1999).

Another hypothesis would be that breeding asynchrony limits competition for food while the parents of either species are feeding their young. Indeed, I noticed an increase in provisioning rate between day 4-6 and day 8-10 in both species. As a result, when mountain chickadees are increasing their provisioning rate, black-capped chickadee's nestlings are about to fledge. Just before fledging, black-capped chickadees tend to decrease their feeding rate (Foote et al. 2010). Thus, mountain chickadees may not be in direct competition with black-capped chickadees for prey items when nestlings required the highest quantity of food.

Finally, the difference in timing of breeding between black-capped chickadees and mountain chickadees might be a mechanism to avoid interbreeding. However, I know interbreeding is occurring in our contact zone (chapter 2) with hybrids arising from extra-pair matings between female mountain chickadees and male black-capped chickadees. Because mountain chickadees breed six days later on average than black-capped chickadees, most female black-capped chickadees are incubating while mountain chickadee females are still fertile. Thus, male black-capped chickadee might be able to sire more offspring by copulating with interspecific females. However, in chickadees, females are known to be the ones driving extra-pair matings (Smith 1988, Otter et al. 1998). As a result, this difference in breeding timing might affect more nestling provisioning than extra-pair copulation pattern.

The present data confirms Hill & Lein (1988, 1989 a&b) findings in another overlapping zone between those two closely related species. Also, complementary observations allow me to clearly show that despite black-capped chickadees being dominant over mountain chickadees in winter flocks (chapter 2), interspecific competition during the breeding season is limited. Each species nests in a distinct cavity class and microhabitats around the nest are different. Mountain chickadees also breed slightly later than black-capped chickadees limiting the competition for food when feeding the nestling.

My results suggest that both species can occur in the contact zone during the breeding season without apparent fitness cost. As interspecific matings have fitness costs, one would expect that selection should act against hybridization. The data presented here tend to support isolation mechanisms to avoid interspecific breeding. As a result, the directed hybridization observed in our contact zone is likely to be induced when both species share similar territories in mix-species flocks during the non-breeding season; interspecific interactions that establish the social hierarchy of black-capped chickadees being dominant over mountain chickadees may influence future female mating behaviour. These data revealed the importance of year round observations to understand interspecific interactions between closely related species.

Hill & Lein (1989b) found few interactions between species during the breeding season; rather, they found that they seem to be niche partitioning to avoid competition. Despite the apparent low interactions and competition between species during the breeding season, however, interspecific matings do occur. This breeding pattern might also be happening in the contact zone in Alberta, but genetic analysis was not available at the time these studies were conducted. Even though behavioural observations tend to indicate that species do not interact, in this specific case genetic analysis revealed that interspecific interactions are resulting in hybridization.

6- GENERAL DISCUSSION

Black-capped and mountain chickadee ranges overlap at specific locations in western North America. These species are typically parapatric, as they are segregated by habitat, but contact zones occur where habitat associated with either species overlap. In one such contact zone, I found that black-capped and mountain chickadees form mixed-species flocks in the winter and frequently engage in interspecific interactions. However, when the flocks break up in the early breeding season, each species establishes territories within species-specific microhabitats of the matrix of mixed forest. I found that habitat around the nest differs significantly between species, with mountain chickadees favouring microhabitat with more conifers, more trees in general and higher species diversity than black-capped chickadees. However, black-capped chickadee breeding territories have a higher canopy and greater shrub density than those of mountain chickadees. Nest sites also differed within those breeding territories, black-capped chickadees using smaller deciduous trees whereas mountain chickadee cavities are found in larger coniferous trees. These differences in microhabitat may result in the reduced interspecific interactions observed during the breeding season.

Using genetic analyses, I confirmed that, despite these low levels of interspecific interactions during the breeding season, hybridization was frequent in my study area. Interspecific matings during the breeding season were higher than one would anticipate, based on observations of interspecific interactions in the field (chapter 5). I found that hybrids were only present in mountain chickadee nests (66% of the mountains chickadee nest sampled had hybrid nestlings), arising from extra-pair copulations between the attending female mountain chickadee and a male black-capped chickadee.

Despite the low interspecific interactions observed during the breeding season, my genetic analysis revealed that both species do interact. This result was also confirmed by observations and experiments in the non-breeding season. First, interspecific interactions and competition are clear during the winter. Based on both field observations and experimentation in the aviary, I demonstrated that black-capped chickadees show absolute dominance over mountain chickadees – even subordinate black-capped chickadees outrank dominant mountain chickadees during interactions. This social relationship seems to differentially affect how the two species respond during interspecific communication; black-capped chickadees tend to ignore their subordinate counterparts' *chick-a-dee* calls whereas mountain chickadees respond to both conspecific and heterospecific calls. As this aviary experiment used stimuli of food-finding calls, my result might indicate that mountain chickadees perceive both conspecifics and heterospecifics as potential competitors for food. The dominant black-capped chickadees, being able to easily displace mountain chickadee competitors, tended to ignore mountain chickadee calls. This pattern of communication during the non-breeding season paralleled the observed directional pattern of intermating during the breeding season, with only mountain chickadee females having extra-pair young with heterospecific males. Black-capped chickadee females also elicit extra-pair copulations but only with conspecific males (extra-pair nestlings were found in 62.5% of the black-capped chickadees nests sampled, but no hybrid nestlings were detected).

I also found that this directional pattern of effects on species occurred during the early breeding season. In chickadees, mating displays include 10 to 15 days during the females' fertility period where males sing continuous bouts of song and/or calls at dawn. This dawn chorus is known to attract females (most conspicuously the male's social mate), and copulation typically occurs at the end of the chorus. Females also use information encoded in this chorus to assess male quality and eventually engage in extra-pair copulation (Otter et al. 1998). I found that dawn chorus of the black-capped chickadees in my contact zone was similar to other comparison populations where mountain chickadees were absent. However, mountain chickadees from the contact zone alter their dawn chorus compared to recordings from a pure population of this species. I found that mountain chickadees in my contact zone use more calls than songs and that their songs are more dissimilar to black-capped chickadee songs in the contact zone than in an area without black-capped chickadees. This difference may indicate a character displacement from the subordinate species, possibly to limit competition with the dominant heterospecific. This might also indicate a species isolation mechanism that is evolving to limit interspecific mating.

Another mechanism might reinforce reproductive isolation in the contact zone: breeding onset. Indeed, in the study area mountain chickadees are breeding on average 6 days later than black-capped chickadees. This difference in timing may serve to avoid competition for food when the nestlings are the most demanding, as black-capped chickadee offspring will be fledging when young mountain chickadees' feeding requirements are the highest.

All these behavioural observations are in concordance with the directional nature of the observed hybridization patterns. High-ranking males are known to be preferred by female black-capped chickadees as extra-pair partners (e.g. Smith 1988, Otter et al. 1998, Mennill et al. 2004), and as the black-capped chickadee is the dominant species in this relationship, similar mate choice patterns of female mountain chickadees may extend across species and account for directional hybridization. Being the subordinate species, mountain chickadees may benefit from considering black-capped chickadees as a risk to both contested resources and mates. For this reason, mountain chickadees may have been more likely to respond to calls of both hetero and conspecifics as potential threats. Further, the dawn chorus of chickadees is partially directed towards females (Otter & Ratcliffe 1993) and is known to play a role in mate assessment (Mennill et al. 2003), so there might be greater pressure on male mountain chickadees to diverge their chorusing behaviour from black-capped chickadees than vice versa.

Despite the observed bias with mountain chickadee being more affected than black-capped chickadee by overlapping population, I did not find any difference in either brood size or in reproductive success between the species. As a result, mountain chickadee populations do not seem to suffer from this directed interspecific competition. However, follow-up studies are needed to determine the impacts of hybrid matings. If hybrid viability is reduced and/or hybrids are sterile (I do not have hybrids reproductive data at this stage), mountain chickadee populations in the study area might decline and/or disappear from this contact zone. In order to prevent such a loss in local biodiversity more studies are needed to fully understand how each species is affected by the local situation.

The results I found during my thesis provide information on interspecific interactions between black-capped and mountain chickadees. To date, only one other contact zone between those two closely related species has been studied. In the late 1980s, Hill & Lein observed black-capped and mountain chickadee interactions during the breeding season. They found little interaction (Hill & Lein 1989b) with each species using different microhabitats (Hill & Lein 1988, 1989a). My results during the breeding season parallel those of Hill & Lein, but observations and experimentation outside the breeding season and genetic analysis reveals a different scenario than what Hill & Lein concluded. Indeed, despite few apparent interactions during the breeding season, mountain chickadee behaviour seems to be greatly affected by the presence of black-capped chickadee in their immediate surroundings. My thesis points out the importance of year round observations but also the relevance of experimentation and molecular techniques to fully understand population dynamics.

In my study site, hybridization between black-capped and mountain chickadee seems frequent whereas it has only been anecdotally reported before (McCallum et al. 1999). One reason that hybrids between black-capped and mountain chickadee may have not been more frequently reported might be due to the fact that hybrids I detected genetically are not phenotypically intermediate between the species – hybrids were classified phenotypically as being either black-capped or mountain chickadee, rather than as integrades of the species-specific markings. As a result, genetic analyses are needed to detect hybridization between these two closely-related species.

Interbreeding between sibling species among North American Parids occur also in Texas between Tufted and black-crested titmice and in western United States between oak and juniper titmice (Curry et al. 2007). Few researchers have explored the potential for hybridization within the clade of brown-capped chickadees, despite large areas of sympatry. Within sympatric areas of titmice and chickadees, interbreeding is extremely rare, probably due to large differences in size (Curry et al 2007). One area of hybridization has been extensively studied along an east-west line in eastern United States, where black-capped chickadee and Carolina chickadee ranges overlap. Bilingual birds seem to be frequent in the area of sympatry; however, no studies have yet addressed dominance relationships, despite

Bronson et al.'s (2003b) finding of a tendency in captivity for Carolina males to dominate black-capped chickadee males and for females to associate with the dominant males regardless of the species. As a result, information is missing to fully understand the relationship between vocal patterns, social hierarchy and mating system in this hybrid zone. My study results are also distinct from the literature on chickadees in the hybrid zone between Carolina chickadee and black-capped chickadees; these species exhibit a more symmetrical pattern of interbreeding rather than a directed pattern bias toward one species.

Among the old world Paridae species, hybridization has been reported anecdotally between at least seven species (reviewed by Harrap & Quinn 1995, McCarthy 2006, Curry et al. 2007). Interbreeding has been observed more frequently between Siberian and willow tits (Jarvinen 1997) and between coal tits and black-crested tits in Nepal (Lohrl 1994). However, hybridization between Eurasian Parids seem to be less frequent than amongst North American species (Dhondt 2007), suggesting reproductive isolation might be more developed in the Old World sympatric populations than in the New World parapatric populations.

Whether hybridization is a "reproductive mistake" (e.g. Mallet 2005, Hartman et al. 2011) or is part of evolutionary processes is under ongoing debate. Most zoologists in the past considered hybridization to result from habitat disturbances due to human practices that bring typically-allopatric species into secondary contact. Hybridization has been reported since at least the time of Linneaus, though, factors other than industrialisation and anthropogenic disturbances are likely to also influence interspecific breeding. One of the main natural occurrences of hybrids is where closely related species' ranges come into contact, at the ecotone between each species' habitats. Ecotones, first described by Clement in 1905, are ecological transitions between biogeographic regions; usually narrow and unstable ecological zones possessing a mixture of two different ecological community types (van der Maarel 1990). Hybridization between North American wolves and coyotes was probably happening at the ecotone between the forest and prairies long before deforestation started (Mallet 2005). Similarly, hybrid specimens of *Heliconius* butterflies in South America were collected long before fragmentation of the neotropical forest started. Contact in the past between *Heliconius* species probably occurred at savannah-forest boundaries or along river edges allowing parapatry of open and close canopy-species (Mallet 2005).

Hybridization has been reported in ecotones for different taxa. One of the most studied hybrid zones in mammals is situated in Denmark where house mice have been interbreeding for 5000 years (Moore 1977): M*us musculus musculus* and *M. m. domesticus* overlap in a region where climatic factors, especially precipitation, create environments where both sub-species are well adapted (Hunt & Selander 1973). In Australia, an anuran hybrid zone was established during the last Pleistocene glaciations (about 12 000 years ago) between *Litoria ewingi* and *L. paraewingi* (Watson 1972, Littlejohn 1976). This hybrid zone is situated at the ecotone between forest and grassland (Watson 1972). Among birds, a common example occurs in the Great Plains of North America where various species of woodland birds are interbreeding in intergraded habitat: flickers (*Colaptes* spp.), orioles (*Iceterus* spp.) and towhees (*Pipilo* spp.) (reviewed by Moore 1977). In Texas, the eastern tufted titmice (*Baeolophus bicolour*) and the western black-crested titmouse (*B. atricristatus*) hybrid zone is situated at the ecotone between an eastern deciduous forest assemblage and a more xeric woody assemblage. This abrupt transition in flora and fauna is also correlated with a humidity gradient and changes in soil characteristics (Dixon 1955).

Even though hybrids are sometimes fitter than parental populations (Moore 1977), narrow hybrid zone are usually maintained at ecotones due to selective pressure against hybrids. Small hybrid zones at the interface between two species' ranges are often stable through time: only a small portion of each parental population is in contact. Hybrid sterility and/or reduced fitness coupled with recruitment of pure individuals from each species limits the introgression of one species' gene into the other species' gene pool. Even though behavioural mechanisms to favour reproductive isolation are not developed, species integrity is maintained.

Ecotones, though, are also created through human disturbances. Indeed, the fragmentation of habitat increases the presence of ecotones. At the JPRF, small-scale clear cutting has resulted in a mosaic of habitat with abrupt transitions between deciduous patches and coniferous forest spread across the area. As a result, contact between mountain chickadees and black-capped chickadees in my study site might be greater than it used to be. Because hybridization is directed with solely mountain females seeking extra pair copulation with black-capped chickadee males, introgression of black-capped chickadee DNA in mountain chickadee gene pool is occurring. If backcrossing is possible between F1 hybrids and parental populations then the mountain chickadee gene pool might be at risk at a local scale over the long term. A similar scenario is occurring within the eastern United Stated, where blue-winged and golden-winged warbler populations are increasing due to farm field abandonment in 1850-1900 which induces successional habitat on fields and pastures required by both warbler species (Kinglsey 1974). However, many reports have shown that increasing populations of blue-winged warbler is coupled with decreasing population of golden-winged warblers (reviewed by Gill 1980). Blue-winged warblers might simply outcompete golden-winged warblers in overlapping area (Gill 1980). Or, as suggested by Gill (1997), because blue-winged mtDNA introgresses asymmetrically into golden-winged phenotype through hybridization, golden-winged populations are simply being lost to interbreeding at the local scale.

My work reveals the importance of adopting a holistic approach to understand biological systems. By using a combination of techniques and experimentation coupled with observations over the course of different seasons, I was able to detect cryptic patterns of interspecific interactions that escaped others studying this system. The next step in understanding this contact zone is to test fitness and reproductive success of hybrid individuals. I sampled hybrids amongst the adults during the course of this study but I was unable to follow any of these hybrids through their own breeding attempts. As a result, I was unable to ascertain whether hybrids are fertile or not. Such observation would allow us to detect whether back-crossings are possible and whether post zygotic species isolation mechanisms do exist. I also do not have data on life expectancy of hybrids versus non hybrids, thus I cannot assess hybrid viability. More studies over the long term are needed to determine the effect on the mountain chickadee population in areas of parapatry with black-capped chickadees. However, such future work in the area may require experimentation with artificial nestboxes to increase sample sizes – something that I undertook during my studies with several box designs utilized in other chickadee populations (e.g. Reudink et al 2007) but I had no occupation by mountain chickadees. Other box designs may be necessary, but acquiring larger numbers of readily-accessible nests would be a key component of this research. I can conclude only that on a year-to-year basis mountain chickadee populations do not seem to suffer from interspecific competition, but may suffer from species introgression.

Also, complementary experiments and observations are needed to understand the communication network during both the breeding season and outside of the breeding season. Playback experiments may give some insight on both species' responsiveness to heterospecific and conspecific songs and calls during the breeding season. Second, research on vocal learning for black-capped chickadees, mountain chickadees and hybrids nestlings might be undertaken, as imprinting on the wrong species' call and/or songs might enhance interbreeding. Similar experiments have already been conducted in Europe between cross-fostered tits (e.g. Hansen & Slagsvold 2004). Hansen & Slagsvold (2004) found that cross-fostered blue tit and great tit responded to both conspecific and heterospecific stimuli whereas control tits responded only to conspecific stimuli. Such heterospecific recognition from cross-fostered birds might promote hybridization between closely-related species. Finally comparisons with other overlapping areas between black-capped chickadee and mountain chickadee will determine whether my results can be expanded to the species ranges where they come into contact.

REFERENCES

Alatalo R.V. 1982. Evidence for interspecific competition among European tits Parus spp.: a review. *Annales Zoologici Fennici*. 19:309-317.

Alatalo R.V., Eriksson D., Gustafsson L. & Larsson K. 1987. Exploitation competition influences the use of foraging sites by tits: experimental evidence. *Ecology*. 68:284-290.

Alatalo R.V., Gustafsson L., Linden M. & Lundberg A. 1985. Interspecific competition and niche shifts in tits and goldcrest: an experiment. *Journal of Animal Ecology*. 54: 977-984.

Alatalo R.V., Gustafsson L. & Lundberg D.A. 1986. Interspecific competition and niche changes in tits (Parus spp.): evaluation of non experimental data. *The American Naturalist.* 127:819-834.

Banks R.C., Cicero C., Dunn J.L., Kratter A.W., Rasmussen P.C., Remsen J.V. Jr, Rising J.D., & Stotz D.F. 2002. Forty-third supplement to the American Ornithologists' Union Check-list of North American Birds. *The Auk.* 119:897–906.

Bernsohn K. 1981. Cutting up the north: the history of the forest industry in the northern interior. Vancouver: Hanock House.

Bloomfield L.L. & Sturdy C.B. 2008. All "chick-a-dee" calls are not created equally Part I. Open-ended categorization of chick-a-dee calls by sympatric and allopatric chickadees. *Behavioural Processes.* 77:73–86.

Bloomfield L.L., Charrier I. & Sturdy C.B. 2004. Note-types and coding in Parid vocalizations. II. The chick-a-dee call of the mountain chickadee (*Poecile gambeli*). *Canadian Journal of Zoology*. 82:780–793.

Braun M.J. & Robbins M.B. 1986. Extensive protein similarity of the hybridizing chickadees *Parus atricapillus* and *P. carolinensis*. *The Auk.* 103:667–675.

Brewer R. 1963. Ecological and reproductive relationships of Black-capped and Carolina chickadees. *The Auk.* 80:9-47

Bronson C.L., Grubb T.C. & Braun M.J. 2003a. A test of the endogenous and exogenous selection hypothesis for the maintenance of a narrow hybrid zone. *The society for the study of evolution*. 57: 630-637.

Bronson C.L., Grubb T.C. Jr, Sattler G.D., and Braun MJ. 2003b. Mate preference: a possible causal mechanism for a moving hybrid zone. *Animal Behaviour*. 65:489-500.

Brown J. R. & Wilson E.O. 1956. Character displacement. *Systematic Zoology*. 5:49–64.

Burg T.M., Gaston A.J., Winker K. & Friesen V.L. 2006. Effects of Pleistocene glaciations on population structure of North American chestnut-backed chickadees. *Molecular Ecology*. 15:2409-2419.

Cain A.J. 1954. Animal Species and their Evolution. London: Hutchinson House.

Charrier I., Bloomfield L.L. & Sturdy C.B. 2004. Note-types and coding in Parid vocalizations. I. The chick-a-dee call of the black-capped chickadee (*Poecile atricapillus*). *Canadian Journal of Zoology*. 82:769–779.

Charrier I. & Sturdy C. 2005. Call-based species recognition in black-capped chickadees. *Behavioural Processes*. 70:271-281.

Christie P.J., Mennill D.J. & Ratcliffe L.M. 2004. Chickadee song structure is individually distinctive over long broadcast distances. *Behaviour.* 141:101-124.

Cimprich D.A. & Grubb T.C. 1994. Consequences for Carolina chickadees of foraging with tufted titmice in winter. *Ecology*. 75:1615-1625.

Clements F.E. 1905. Research methods in ecology. Lincoln: University of Nevada.

Colquhoun M.K. & Morley A. 1943. Vertical zonation in woodland bird communities. *Journal of Animal Ecology*. 12:75-81.

Confer J.L. & Larkin J.L. 1998. Behavioral Interactions Between Golden-winged and Blue-winged warblers. *The Auk.* 115:209-214.

CourterJ. R. & Ritchison G. 2010. Alarm calls of tufted titmice convey information about predator size and threat. *Behavioral Ecology.* 21, 936-942.

Curry R.L. 2005. Hybridization in chickadees: much to learn from familiar birds. *The Auk* 122:747-758.

Curry R.L., Rossano L.M. & Reudink M.W. 2007. Behavioral aspects of chickadee hybridization. In: *Ecology and behavior of chickadees and titmice: an integrated approach* (Ed. by K. A. Otter), pp 95-110. Oxford: Oxford University Press.

Dawson M.R.W., Bloomfield L.L., Charrier I. & Sturdy C.B. 2006. Statistical classification of black-capped (*Poecile atricapillus*) and mountain chickadee (*P. gambeli*) call notes. *Journal of Comparative Psychology*. 120:147–153.

Desrochers A. 1989. Sex, dominance, and microhabitat use in wintering black-capped chickadees: A field experiment. *Ecology*. 70:636–645.

Dhondt A.A. 1980. Competition and the regulation of numbers in Great and Blue tit. *Ardea* 68:121-132.

Dhondt A.A. 1989. Ecological and evolutionary effects of interspecific competition in tits. *The Wilson Bulletin.* 101:198-216.

Dhondt A.A. 2007. What drives differences between North American and Eurasian tit studies? In: *Ecology and behavior of chickadees and titmice: an integrated approach* (Ed. by K.A. Otter), pp 299-310. Oxford: Oxford University Press.

Dhondt A. A. 2011. Interspecific competition in birds. Oxford: Oxford University Press.

Dixon K.L. 1955. An ecological analysis of the interbreeding of crested titmice in Texas. *University of California Publications in Zoology*. 54: 125-206.

Dixon K.L. 1961. Habitat distribution and niche relationships in North American species of Parus. In: *Vertebrate speciation*. (Ed. by W.F. Blair), pp. 179-219. Austin: University of Texas Press.

Dobzhansky T. 1951. Genetics and the Origin of Species 3rd edn. New York: Columbia University Press.

Doutrelant C., Blondel J., Perret P. & Lambrechts M.M. 2000a Blue tit song repertoire size, male quality and interspecific competition. *Journal of Avian Biology.* 31:360-366.

Doutrelant C., Leitao A., Otter, K.A. & Lambrechts, M.M. 2000b Effect of blue tit song syntax on great tit territorial responsiveness- an experimental test of the character shift hypothesis. *Behavioral Ecology and Sociobiology.* 48:119-124.

Doutrelant C. & Lambrecht M.M. 2001 Macrogeographic variation in song- a test of competition and habitat effect in blue tits. *Ethology*. 107:533-544.

Ekman J. 1989. Ecology of non-breeding social systems of *Parus*. *The Wilson Bulletin*. 101:263-288.

Emlen S.T., Rising J.D. & Thompson W.L. 1975. A behavioral and morphological study of sympatry in the Indigo and Lazuli Buntings of the Great Plains. *The Wilson Bull*etin. 87: 145-179.

Erokene C., Rigstad E., Dale M. & Iifjeld J.T. 1998. The function of extrapair paternity in blue tits and great tits: good genes or fertility insurance? *Behavioral Ecology.* 9: 649-656.

Falush D., Stephens M. & Pritchard J.K. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics.* 164:1567-1587.

Ficken M.S., Weise C.M. & Popp J.W. 1990. Dominance and resource access in winter flock of black-capped chickadees. *The Wilson Bulletin*. 102:623–633.

Ficken M.S., Ficken R.W. & Witkin S.R. 1978. Vocal repertoire of the black-capped chickadee. *The Auk.* 95:34–48.

Foote J.R., Mennill D.J., Ratcliffe L.M. & Smith S.M. 2010. Black-capped chickadee (*Parus atricapillus*). . In: *The Birds of North America Online,* (Ed. by A. Poole). Ithaca : Cornell Lab of Ornithology.

Freedberg T.M. 2008. Complexity in the *chick-a-dee* call of carolina chickadees (*Poecile carolinensis*): associations of context and signaller behaviour to call structure. *The Auk*. 125:896-907.

Gaddis P.K. 1985. Structure and variavility in the vocal repertoire of the mountain chickadee. *The Wilson Bulletin*. 97:30-46.

Gammon D.E. 2004. Black-capped chickadee dawn chorus and subsequent sexual activity. *The Wilson Bulletin*. 116:252-256.

Gammon D.E. 2007. How postdispersal social environement may influence acoustic variation in birdsong. In: *Ecology and behavior of chickadees and titmice: an integrated approach* (Ed. by K. A. Otter), pp 183-198. Oxford: Oxford University Press.

Gil D. & Gahr M. 2002. The honesty of bird song: multiple constraints for multiple traits. *Trends in Ecology & Evolution.* 17:133-141.

Gill F.B. 1980. Historical aspects of hybridization between blue-winged and golden-winged warblers. *The Auk* 97:1-18.

Gill F.B. 1997 Local cytonuclear extinction of the golden-winged warbler. *Evolution.* 51:519-525.

Gill F.B., Mostrom A.M. & Mack A.L. 1993. Speciation in north American chickadees: I. Patterns of mtDNA genetic divergence. *Evolution.* 47:192-212.

Gill F.B., Slikas B. & Sheldon F.H. 2005. Phylogeny of titmice (Paridae): II. Species relationships based on sequences of the mitochondrial cytochrome-b gene. *The Auk.* 122:121-143.

Grainger S. 2002: Analysis of historical photography to support ecological restoration. Forest renewal British Columbia, unpublished report.

Grant P.R. & Grant B.R. 1992. Hybridization of birds species. *Sciences* 256: 193-197.

Grant P.R. & Grant B.R. 1997. Genetics and the origin of bird species. *Proceedings of the national academy of sciences.* 94:7768-7775.

Grant P.R. 1972. Convergent and divergent character displacement. *Biological Journal of the Linnean Society* 4:39-68.

Grant P.R. 1994. Ecological character displacement. *Science*. 266:746-747.

Griffith S.C., Stewart I.R.K., Dawson D.A., Owens I.P.F. & Burke T. 1999. Contrasting levels of extra-pair paternity in mainland and island populations of the house sparrow (*Passer domesticus*): is there an 'island effect'? *Biological Journal of the Linnean Society*. 68:303-316.

Grava T., Grava A. & Otter K.A. 2009. Supplemental feeding and dawn singing in black-capped chickadees. *Condor.* 111:560-564.

Grundel R. 1987. Determinants of nestling feeding rates and parental investment in the mountain chickadee'. *The condor*. 89:319-328.

Haftorn S. 1993. Is the coal tit *Parus ater* really the most subordinate of the Scandinavian tits? *Ornis Scandinavia.* 24:335–338.

Hailman J.P. & Ficken M.S. 1996. Comparative analysis of vocal repertoires, with reference to chickadees. In: *Ecology and Evolution of Acoustic Communication in Birds* (Ed. by D.E. Kroodsma & E.H. Miller), pp. 136–159. New York: Cornell University Press.

Hailman J.P. 1989. The organisation of the major vocalizations in the Paridae. *The Wilson Bulletin.* 101:305-343.

Hanotte O., Zanon C. & Puga A. 1994. Isolation and characterization of microsatellite loci in a passerine bird: the reed bunting Emberiza schoeniclus. *Molecular Ecology*. 3:529-530.

Hansen B.O. & Slagsvold T. 2004. Early learning affects social dominance:interspecifically cross-fostered tits become subdominant. *Behavioral Ecology*. 15:262-268.

Harrap S. & Quinn D. 1995. Tits, Nuthatches and Treecreepers. Princeton: Princeton University Press.

Hartley P.H.T. 1953. An ecological study of the feeding habits of the English titmice. *Journal of Animal Ecology*. 22:261-288.

Hartman P., Wetzel D., Crowley P. & Westneat D. 2011. The impact of extra-pair mating behavior on hybridization and genetic introgression. *Theoretical Ecology*. DOI: 10.1007/s12080-011-0117-1.

Hasselquist D., Bensch S. & von Schantz T. 1996. Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature*. 381:229-232.

Hill B.G. & Lein M.R. 1988. Ecological relations of sympatric black-capped and mountain chickadees in southwestern Alberta. *Condor*. 90:875-884.

Hill B.G. & Lein M.R. 1989a.Territory overlap and habitat use of sympatric chickadees. *The Auk*. 106: 259-268.

Hill B.G. & Lein M.R. 1989b. Natural and simulated encounters between sympatric black-capped and mountain chickadees. *The Auk*. 106:645-652.

Ho R. 2006. Handbook of univariate and multivariate data analysis and interpretation with SPSS. Boca Raton: Chapman & Hall/CRC.

Hogstad O. 1988. The influence of energy stress on social organization and behaviour of willow tits *Parus montanus*. *Fauna Norvegica Series C*. 11:89–94.

Hogstad O. 1992. Mate protection in alpha pairs of wintering willow tits, *Parus montanus*. *Animal Behaviour*. 43:323–328.

Howard D.J. 1993. Reinforcement: Origin, dynamics, and fate of an evolutionary hypothesis. In: *Hybrid Zones and the Evolutionary Process* (Ed. by R.G. Harrison), pp 46-49. Oxford: Oxford University Press.

Hunt W.G. & Selander R.K. 1973. Biochemical genetics of hybridisation in European house mice. *Heredity*. 31:11-33.

Irwin D.E., Bensch S. & Price T.D. 2001. Speciation in a ring. *Nature*. 409:333-337.

Jackson D.A. 1993. Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecology*. 74:2204–2214.

Jarvinen A. 1997. Interspecific hyrbidization between the Siberian tit *Parus cinctus* and the willow tit *Parus montanus* produces fertile offspring. *Ornis fennica*. 74:149-152.

Jordan D.S. 1905. The origin of species through isolation. *Science*. 22: 545–562.

Kempenaers B., Verheyen R.F. & Dhondt A.A. 1997. Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. *Behavioral Ecology*. 8:481–492.

Kingsley N.P. 1974. The timber resources of southern New England. *United States Departement of Agriculture Forest Service.* NE-36.

Kroodsma D.E., Albano D.J., Houlihan P.W. & Wells J.A. 1995. Song development by black-capped chickadee (*Parus atricapillus*) and Carolina chickadee (*P. Carolinensis*). *The Auk.* 112:29-43.

Kvist L. Martens J. Nazarenko A.A. & Orell M 2003. Paternal leakage of mitochondrial DNA in the great tit (*Parus major*). *Molecular Biology and Evolution.* 20:243-247.

Lack D. 1954. Natural regulation of animal numbers. Oxford: Oxford University Press.

Lack D. 1969. Tit niches in two worlds; or homage to Evelyn Hutchinson. *The American Naturalist*. 103:43-49.

Legendre P. & Legendre L. 1998. Numerical ecology: developments in environmental modelling. 2nd edN. Amsterdam: Elsevier Science.

Lemmon D., Withiam M.L. & Barkan C.P.L. 1997. Mate protection and winter pair-bonds in black-capped chickadees. *Condor*. 99:424–433.

Lister B.C. 1980: Ressource variation and the structure of British bird communities. *Proceedings of the Natural Academy of Sciences.* 77:4185-4187.

Littlejohn M.J. 1976. The Litoria ewingi complex (Anura: Hylidae) in south-eastern Australia IV. Variation in mating-call structure across a narrow hybrid zone between L. ewingi and L. paraewingi. *Australian Journal of Zoology*. 24:283-293.

Loftus Hills J.J. & Littlejohn M.J. 1992. Reinforcement and reproductive character displacement in *Gastrophryne carolinensis* and *Gastrophryne olivacea* (Anura: *Microhylidae*): a reexamination. *Evolution*. 46:896–906.

Lohrl H. 1994. Contribution to the still unsettled question complex of hybridization between coal tit and black-crested tit (*Parus ater*, *P.melanolophus*)(Aves: Passeriformes: Paridae). *Zoologische Abhandlungen.* 48:149-157.

Lucas J.R. & Freeburg T.M. 2007. Information and the *chick-a-dee* call: communicating with a complex vocal system. In: *Ecology and behavior of chickadees and titmice: an integrated approach* (Ed. by K.A. Otter), pp 199–215. Oxford: Oxford University Press.

Lynch A. &Baker A.J. 1990. Increased vocal discrimination by learning in sympatry in two species of chaffinches. *Behaviour*. 116:109–126.

Maarel E. van der. 1990. Ecotones and ecoclines are different. *Journal of Vegetation Science.* 1:135-138.

MacGregor M.K. 2002. Msc thesis: Developping reference conditions for ecological restoration of forest in sub-boreal British Columbia. University of Northern British Columbia, Canada.

Mahurin E. J. & Freeberg T.M. 2009. Chick-a-dee call variation in Carolina chickadees and recruiting flockmates to food. *Behavioral Ecology*. 20:111-116**.**

Mallet J. 1998. Isolating mechanisms. In: *Encyclopaedia of Ecology and Environmental Management* (Ed. by P. Calow), pp. 379-380. Blackwell Press.

Mallet J. 2005. Hybridization as an invasion of the genome. *Trends in Ecology and Evolution.* 20:229-237.

Mallet J., Jiggins C.D. & McMillan W.O. 1998. Mimicry and warning color at the boundary between races and species. In: *Endless Forms: Species and Speciation* (Ed. by D.J. Howard and S.H. Berlocher), pp. 390–403. Oxford: Oxford University Press.

Marten K. & Marler P. 1977. Sound transmission and its significance for animal vocalisation: I. Temperate habitats. *Behavioral Ecology and Sociobiology*. 2:271-290.

Martin K., Aitken K.E.H. & Wiebe K.L. 2004. Nest Sites and Nest Webs for Cavity-Nesting Communities in Interior British Columbia,Canada: Nest Characteristics and Niche Partitioning. *Condor*. 106:5-19.

Martin K. & Norris A.R. 2007. Life in the small-bodied cavity-nester guild: Demography of sympatric mountain and black-capped chickadees within nest web communities under changing habitat conditions. In: *Ecology and behavior of chickadees and titmice: an integrated approach* (Ed. by K. A. Otter), pp 111-130. Oxford: Oxford University Press.

Martinez J.G., Soler J.J., Soler M., Møller A.P. & Burke T. 1999. Comparative population structure and gene flow of a brood parasite, the great spotted cuckoo (*Clamator glandarius*), and its primary host, the magpie (*Pica pica*). *Evolution*. 53:269-278.

Mayden R.L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In *Species: The units of diversity* (Ed. by M.F. Claridge, H.A. Dawah and M.R. Wilson), pp. 381-423. London: Chapman and Hall.

Mayr E. 1942. Systematics and the Origin of Species. New York: Dover Publications.

Mayr E. 1963. Animal Species and Evolution. Cambridge : Harvard University Press.

Mayr E. 1970. Populations, species, and evolution. Cambridge: Harvard University Press.

Mayr E. & Short L.L. 1970. Species taxa of North American birds: A contribution to comparative systematics. *Nuttall Ornithological Club*. 9.

McCallum D.A., Grundel R. & Dahlsten D.L. 1999. Mountain chickadee. In: *Birds of North America,* (Ed. by A. Poole & F.B. Gill), No. 453, pp 1-28. Philadelphia: Academy of Natural Sciences.

McCarthy E. M. 2006. Handbook of avian hybrids of the world. Oxford: Oxford University Press.

Mennill D.J. & Otter K.A. 2007. Status signalling and communication networks in chickadees: Complex communication with a simple song. In: *Ecology and behavior of chickadees and titmice: an integrated approach* (Ed. by K. A. Otter), pp 215-240. Oxford: Oxford University Press.

Mennill D.J., Ramsay S.M., Boag P.T. & Ratcliffe L.M. 2004. Patterns of extrapair mating in relation to male dominance status and female nest placement in black-capped chickadees. *Behavioural Ecology*. 15:757–765.

Mennill D.J., Boag P.T. & Ratcliffe L.M. 2003. The reproductive choices of eavesdropping females black-capped chickadees, *Poecile atricapillus. Naturwissenschaften*. 90:577-582.

Miller R.S. 1968.Condition of competition between Redwings and Yellow-headed blackbirds. *Journal of Animal Ecology*. 37:43-62.

Minock M.E. 1972. Interspecific aggression between black-capped and mountain chickadees at winter feeding stations. *Condor*. 74:454-46.

Moore W.S. 1977 An evaluation of narrow hybrid zones in vertebrates. *The Quarterly Review of Biology*. 52:263-277.

Murray B.G.Jr. 1981. The origins of adaptive interspecific territorialism. *Biological Review*. 56:1-22.

Noor M.A.F. 1999. Reinforcement and other consequences of sympatry. *Heredity*. 83:503-508.

Nowicki S. & Searcy W.A. 2005. Song and mate choice in birds: How the development of behavior helps us understand its function. *The Auk*. 122:1-14.

Nowicki S. 1983. Flock-specific recognition of chickadee calls. *Behavioral Ecology and Sociobiology*. 12:317-320.

Oort van H., Otter K.A.,Fort K. & Holschuh C.I. 2006. Habitat quality affects the reliability of a condition-dependant signal in black-capped chickadees. *Ethology*. 112:772-778.

Odum E.P. 1942. Annual cycle of the black-capped chickadee - III. *The Auk*. 59:499-531.

Otter K.A., Chruszcz B. & Ratcliffe L.M. 1997. Honest advertisement and song output during the dawn chorus of Black-capped Chickadees, *Parus atricapillus*. *Behavioral Ecology*. 8:167-173.

Otter K.A., McGregor P.K., Terry A.M., Burford F.R.L., Peake T.M. & Dabelsteen T. 1999. Do female great tits (*Parus major*) assess males by eavesdropping? A field study using interactive song playback. *Proceedings of the Royal Society London*. 266:1305-1310.

Otter K.A., Ratcliffe L.M. & Boag P.T. 1994. Extra-pair paternity in the black-capped chickadee. *Condor*. 96:218-222.

Otter K.A. & Ratcliffe, L.M. 1993. Changes in the singing behaviour of male black-capped chickadees (*Parus atricapillus*) following mate removal. *Behavioral Ecology and Sociobiology*. 33:409-414.

Otter K.A. & Ratcliffe L.M. 1996. Female initiated divorce in a monogamous songbird: abandoning mates for males of higher quality. *Proceedings of the Royal Society London*. 263:351-354.

Otter K.A., Ratcliffe L., Michaud D. & Boag P.T. 1998. Do female black-capped chickadees prefer high ranking males as extra-pair partners*? Behavioral Ecology and Sociobiology*. 43:25-36.

Otter K.A., van Oort H. & Fort K.T. 2007. Habitat quality and reproductive behaviour in chickadees and tits; potential for habitat matrix use in forest generalists. In: *Ecology and behavior of chickadees and titmice: an integrated approach* (Ed. by K. A. Otter), pp 277-291. Oxford: Oxford University Press.

Peakall R. & Smouse P.E. 2001. GenAlEx v5.1: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research., (5.1 eds),. Canberra, Australia :Australian National University.

Phillimore L.S., Sturd, C.B., Turyke M.M. & Weisman R.G. 2002. Discrimination of individual vocalizations by black-capped chickadee (*Poecile atricapilus*). *Animal learning and behaviour*. 30:43-52.

Pritchard J.K., Stephens M. & Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics*. 155:945-959.

Pyle P. 1997. Identification guide of North American birds. California: Slate Creek Press.

Randler C. 2002. Avian hybridization, mixed pairing and female choice. *Animal Behaviour.* 63:103-119.

Ratcliffe L.M., Mennill D.J. & Schubert K.A. 2007. Social dominance and fitness in black-capped chickadees. In: *Ecology and behavior of chickadees and titmice: an integrated approach* (Ed. by K. A. Otter), pp 131-146. Oxford: Oxford University Press.

Reudink M.W., Mech S.G. & Curry R.L. 2006. Extrapair paternity and mate choice in a chickadee hybrid zone. *Behavioral Ecology*. 17:56-62.

Reudink M.W., Mech S.G., Mullen S.P. & Curry R.L. 2007. Structure and dynamics of the hybrid zone between black-capped chickadee (*Poecile atricapillus*) and Carolina chickadee (*P. carolinensis*) in soustheastern Pennsylvania. *The Auk.* 124:463-478.

Rohwer S., Bermingham E. & Wood C. 2001. Plumage and mitochondrial DNA haplotype variation across a moving hybrid zone. *Evolution.* 55:405–422.

Saetre G.P., Moum T., Bures S., Kral M., Adamjan M. & Moreno J. 1997. A sexually selected character displacement in Flycatchers reinforces premating isolation. *Nature.* 387:589-592.

Schluter D. 1994. Experimental evidence that competition promotes divergence in adaptive radiation. *Science.* 266:798-801.

Smith S.M. 1988. Extra-pair copulation in black-capped chickadees: the role of the female. *Behaviour.* 107:15-23.

Smith S.M. 1991. The black-capped chickadee: behavioural ecology and natural history. Ithaca, New York: Comstock Publishing.

Smith S.M. 1967. An ecological study of winter flocks of black-capped and chestnut-backed chickadees. *The Wilson Bulletin*. 79:201-207

Soard C.M. & Ritchison G. 2009. *Chick-a-dee'* calls of Carolina chickadees convey information about degree of threat posed by avian predators. *Animal Behaviour*. 78:1447-1453.

Stallcup P.L. 1968: Spatio-temporal relationships of nuthatches and woodpeckers in ponderosa pine forests of Colorado. *Ecology*. 49:831-843.

Stebbins R.C. 1949. Speciation in salamanders of the Plethodontid genus *Ensatina*. *University of California publications in zoology*. 48:377–526.

Sturdy C.B., Bloomfield L.L., Charrier I. & Lee T.T.Y. 2007. Chickadee vocal production and perception: An integrative approach to understanding acoustic communication. In: *Ecology and behavior of chickadees and titmice: an integrated approach* (Ed. by K. A. Otter), pp 153-166. Oxford: Oxford University Press.

Sturman W.A. 1968. The foraging ecology of *Parus atricapillus* and *P. Rufescens* in the breeding season, with comparisons with other species of Parus. *Condor*. 70:309-322.

Sueur J., Aubin T. & Simonis C. 2008. Seewave: a free modular tool for sound analysis and synthesis. *Bioacoustics*. 18:213-226.

Tamura T., Dudley J., Nei M. & Kumar S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*. 24:1596-1599.

Templeton C. N. & Greene E. 2007. Nuthatches eavesdrop on variations in heterospecific chickadee mobbing alarm calls. *Proceedings of the Natural Academy of Sciences*. 104:5479-5482.

Templeton C.N, Greene E. & Davis K. 2005. Allometry of alarm calls: black-capped chickadees encode information about predator size. *Science*. 308:1934–1937.

Ticehurst C.B. 1938. A systematic review of the genus *Phylloscopus*. Trustees of the British Museum, London.

Waite T.A. & Grubb T.C.Jr. 1988. Copying of foraging locations in mixed-species flocks of temperate-deciduous woodland birds: an experimental study. *Condor*. 90:132- 140.

Wake D.B. & Schneider C.J. 1998. Taxonomy of the plethodontid salamander genus *Ensatina*. *Herpetologica*. 54: 279-298.

Wake D.B. & Yanev K.P. 1986. Geographic variation in allozymes in a ‘ring species,’ the plethodontid salamander *Ensatina eschscholtzii* of western North America. *Evolution* 40:702-715.

Wallin L. 1986. Divergent character displacement in the song of two allospecies: the pied flycatcher *Ficedula hypoleuca*, and the collared flycatcher *Ficedula albicollis*. *Ibis*. 128:251–259.

Walsh P.S., Metzger D.A. & Higuchi R. 1991. Chelex 100 as a medium for PCR based typing from forensic material. *Biotechniques.* 10:506-513.

Wang M.T., Hsu Y.C., Yao C.T., Li S.H. 2005. Isolation and characterization of 12 tetranucleotide repeat microsatellite loci from the green-backed tit (*Parus monticolus*). *Molecular Ecology Notes*. 5:439-442.

Watson G.F. 1972. The Litoria ewingi complex (Anura: Hylidae) in south-eastern Australia. II. Genetic incompatibility and delimitation of a narrow hybrid zone between L. ewingi and L. paraewingi. *Australian Journal of Zoology*. 20:423-433.

Wheeler Q.D. & Meier R. 2000. Species Concepts and Phylogenetic Theory: a Debate**.** New York: Columbia University Press.

Wiley R.H. & Richard D.G. 1982. Adaptations for acoustic communication in birds: sound propagation and signal detection. In: *Acoustic Communication in Birds, Vol. 1* (Ed. by D. E. Kroodsma & Miller E. H.), pp. 131-181. New York: New York Academic Press.