

UC Berkeley

UC Berkeley Electronic Theses and Dissertations

Title

Song Diversification and Speciation in the *Empidonax-difficilis-occidentalis-flavescens* Complex

Permalink

<https://escholarship.org/uc/item/3m8784nf>

Author

Rush, Andrew Christopher

Publication Date

2014

Peer reviewed|Thesis/dissertation

Song Diversification and Speciation in the *Empidonax difficilis*–*occidentalis*–*flavescens* Complex

By

Andrew Christopher Rush

A dissertation submitted in partial satisfaction of the
requirements for the degree of

Doctor of Philosophy

in

Integrative Biology

in the

Graduate Division

of the

University of California, Berkeley

Committee in Charge:

Professor Rauri Bowie, Chair

Professor Craig Moritz

Professor Rosemary Gillespie

Fall 2014

Abstract

Song Diversification and Speciation in the *Empidonax difficilis–occidentalis–flavescens*
Complex

by

Andrew Christopher Rush

Doctor of Philosophy in Integrative Biology

University of California, Berkeley

Professor Rauri Bowie, Chair

Speciation can occur when barriers to gene flow form between populations. Phenotypic differences resulting from divergent selection can act as strong pre-zygotic barriers to gene flow. Birds provide excellent opportunities to examine the effectiveness of pre-zygotic barriers, because phenotypic traits, such as song and plumage, which affect species recognition and mate choice, are often conspicuous and relatively easy to observe and quantify. In the research outlined in this dissertation, I analyze the effects of divergence in song on lineage diversification in the six principal taxa that comprise the *Empidonax–difficilis–occidentalis–flavescens* complex, a clade of suboscine passerines. This dissertation is one of the broadest examinations of the interaction between genotype and innate song type yet conducted, and provides insights relevant to the role of divergent signals in speciation in a wide range of organisms.

In Chapter 1, I examine an area of geographic contact in southwestern Canada between two of these taxa, the Pacific-slope Flycatcher (*E. d. difficilis*) and the Cordilleran Flycatcher (*E. o. hellmayri*). Contact zones between recently diverged taxa offer unique opportunities to test whether the forms are reproductively isolated and therefore distinct species. I present the first analysis of genetic variation across this region, in order to determine whether hybridization and gene flow occurs between these taxa. I determine that parental populations of Pacific-slope and Cordilleran Flycatchers have distinct mitochondrial haplotypes, and that all of the individuals sampled in interior southwestern Canada have the Pacific-slope haplotype. In contrast, analysis of nuclear DNA (AFLPs) indicates a high level of population admixture between Pacific-slope and Cordilleran Flycatchers in this region, although I find no evidence of nuclear gene flow into core parental populations. I suggest that the discordance between the mitochondrial and nuclear markers most likely results from stochastic loss of Cordilleran mitochondrial haplotype lineages facilitated by asymmetries in mating due to earlier arrival and greater abundance of Pacific-slope Flycatchers in the contact zone. Although the discovery of hybridization between Pacific-slope and Cordilleran Flycatchers in southwestern Canada calls into question their status as distinct species, the lack of evidence of gene flow into parental populations indicated a need for genetic analyses of populations representing a broader sampling of the geographic range of each species.

In the remaining two chapters of the dissertation, I address two major questions:

1. Does innate song function as a particularly strong isolating mechanism between incipient subspecies?
2. How do patterns of divergence in innate song differ from those of learned song, and how has song divergence affected lineage diversification across the *E. difficilis-occidentalis-flavescens* clade?

In Chapter 2, I perform an extensive study of song variation in Pacific-slope and Cordilleran Flycatchers to determine its effects on hybridization and gene flow. Suboscines offer interesting opportunities to investigate the effects of song divergence on lineage diversification, because songs develop without learning. When songs diverge between populations, they can create behavioral barriers to gene flow. Divergence in innate song in suboscine passerines could result in particularly strong behavioral barriers to gene flow because song type is more closely correlated with genotype, and thus a more direct marker of lineage affiliation than in passerine species with learned song. In this chapter, I demonstrate high levels of introgression in both mitochondrial and nuclear genetic markers, although the pattern of introgression is asymmetrical, with introgression limited into core Pacific-slope populations. Moreover, I highlight extensive geographic discordance between the frequencies of mitochondrial and nuclear markers. I demonstrate that the songs of the two taxa are distinct, and highly correlated with nuclear genotype, and that the songs of admixed individuals exhibit spectral characteristics intermediate to the parental species. Song playback experiments demonstrate that both species show some level of discrimination based on song, and highlight lineage-specific behavioral differences that have likely affected the outcome of secondary contact. Pacific-slope Flycatchers seem to rely more on song in territorial interactions, and may discriminate more among song types. Cordilleran Flycatchers exhibit higher levels of aggressiveness in response to playback. Based on the pattern of geographic variation in genetic and song characters, and on the results of the playback experiments, I propose a historical scenario of secondary contact in which asymmetrical introgression of nuclear alleles was facilitated by the social dominance of more aggressive Cordilleran Flycatchers. Finally, I predict that Cordilleran populations will become increasingly introgressed, while introgression into Pacific-slope populations may be limited by a combination of ecological and behavioral factors.

In Chapter 3, I extend the examination of genetic and song variation to include the six principal taxa that comprise the *Empidonax difficilis-occidentalis-flavescens* clade, to examine whether song divergence has affected lineage divergence by acting as a species discrimination trait. Numerous studies have shown that the level of complexity can affect the efficacy of song in birds, but these studies have focused mainly on birds with learned song. In this study, I offer a novel approach for examining patterns of vocal repertoire evolution, by comparing within repertoire complexity (syllable diversity) across homologous vocalization types present in all six taxa. I find varying rates of song divergence across taxa and across latitude. Songs are distinct between some taxa, but not others. Song divergence is not correlated with mtDNA distance, but it is correlated with latitudinal distance between taxa. Song complexity seems to be higher in higher latitude migratory taxa, but this is due the extremely divergent song of one taxon (*E. d. difficilis*). Moreover, a high level of divergence in one particular vocalization (Song 2) is most responsible for the overall divergence of *E. difficilis* song. Song playback experiments show varying levels of discrimination among song types, and there is at least preliminary evidence that

lower latitude species are able to use more subtle vocal cues in taxon recognition than higher latitude migratory taxa. This study provides a unique view into how vocal repertoires can evolve in birds, and how this relates to lineage diversification. Moreover, this study is consistent with other studies that have found elevated rates of signal diversification and song complexity in higher latitude migratory species.

Thus, innate song does seem to be able to function as a strong isolating mechanism, but this depends on ecological and behavioral contexts. That is, the abbreviated breeding seasons at higher latitudes might drive more extreme reproductive behaviors such as singing and territorial defense compared to lower latitude taxa. This can affect the effectiveness as song as a taxon discrimination trait, by affecting both the rate of evolution of song and the behavioral context in which the song is performed. Moreover, I found that patterns of song divergence correlate closely to patterns of genetic divergence in the comparison of song divergence and admixture between Pacific-slope and Cordilleran Flycatchers, but that varying rates of song divergence relative to genetic divergence in the broader phylogenetic comparison make this correlation weaker. Song seems capable of a high level of consistency across relatively large genetic distances or a high level of divergence over relatively small genetic distances, indicating that in at least some of these taxa, song divergence has been driven by selection. Claiming that I have evidence that song differences drove lineage diversification would be premature, but song differences do seem to have important roles in maintaining taxon boundaries. Despite the high level of admixture between Pacific-slope and Cordilleran Flycatchers at interior sites, gene flow does not occur at any significant level into core Pacific-slope populations. Multiple lines of evidence suggest that Pacific-slope song has become highly derived, relative to the songs of other taxa in this clade and that it has a greater, or at least more varied, role in reproduction. Thus, attributing an important role to song differences in decreasing gene flow from Cordilleran populations into core Pacific-slope populations seems reasonable. Moreover, the high level of discrimination in lower latitude taxa among very similar song types suggests that song could be an effective cue for assortative mating in these taxa. This comes with the caveat that the context that often exists in secondary contact zones, such as low population density, can promote hybridization in taxa that would likely mate assortatively if the cost of mate searching were lower.

I would like to dedicate this work to my mother, Martha Lee Rush, who showed me that this world, in all of its detail, deserves our curious attention.

TABLE OF CONTENTS

Introduction	iii
Chapter 1	1
Chapter 2	19
Chapter 3	58
Conclusion	89
Literature Cited	94
Appendices	106

INTRODUCTION

Speciation can occur when barriers to gene flow form between populations (Coyne & Orr 2004). Theoretical models (e.g., Kirkpatrick & Ravigne 2002) and empirical studies (e.g., Lowry et al. 2008, Schemske 2010) indicate that phenotypic differences resulting from divergent selection can act as strong pre-zygotic barriers to gene flow. Pre-zygotic barriers such as habitat isolation, temporal isolation, and behavioral isolation can all contribute to speciation in a wide range of organisms, and determining the relative importance of different types of barriers remains a major goal of speciation research (Coyne & Orr 2004, Sobel et al. 2010, Safran et al. 2013, Seehausen et al. 2014).

Birds provide excellent opportunities to examine the effectiveness of pre-zygotic barriers, because phenotypic traits, such as song and plumage, which affect species recognition and mate choice, are often conspicuous and relatively easy to observe and quantify. Recent investigations (Swenson & Howard 2004, Weir and Schluter 2004, Jetz et al. 2012) have identified western North America as a hot spot of avian diversification. Determining the roles of particular isolating mechanisms is critical to understanding the origins of this diversity. In many cases, resident tropical congeners have remained genetically isolated, while their sister taxa have evolved migratory pathways to northern breeding grounds. In many cases, northern taxa have differentiated, apparently through vicariance during periods of glaciation, into Pacific and Rocky Mountain subpopulations (Johnson and Cicero 2004, Weir and Schluter 2004). Most of these differentiated migratory populations are at an incipient stage of the speciation process, and many meet in secondary contact. Because traits continue to diverge after reproductive isolation is complete, it can be difficult in older taxa to determine whether divergent traits are the causes or the consequences of reproductive isolation. Secondary contact between incipient species, in which reproductive isolation is incomplete, provides the best opportunities to examine the strength of pre-zygotic barriers in species formation (Harrison 1993, Safran et al. 2013).

Passerines (songbirds), in which song is the most highly developed, are the most diverse group of birds, and it is thought that divergence in song has played a major role in passerine diversification (Catchpole and Slater 1995). Vocal differences have often been assumed to play a central role in creating and maintaining species boundaries between diverging passerine populations by acting as cues for species recognition and assortative mating (Slabbekoorn and Smith 2002, Price 2008). While this is intuitively satisfying, it is not clear that song differences have been effective in preventing hybridization and introgression in the majority of the cases in which its effects have been examined. Numerous examinations of secondary contact have been performed between incipient passerine species in western North America and have provided opportunities to examine the strength of song differences as pre-zygotic barriers. While differences in vocalizations have been found in nearly all of these cases, some level of hybridization is known to occur in many, if not most of these cases (e.g., Emlen et al 1975, Cicero and Johnson 1998, Pearson and Rohwer 2000, Cicero 2004, Ruegg 2007, Price 2008, Brelsford et al 2009, Irwin et al 2009, Kenyon et al 2011, Toews et al 2011; although, see Stein 1963, Toews & Irwin 2008). While this does not show that song has no effect on mate choice and hybridization, it does call into the question the effectiveness of song as a pre-mating isolating mechanism, and suggests that further investigation is warranted.

Most of the bird species that have been examined in this respect have been oscine songbirds – birds that have a strong learned component to their songs (e.g., Slabbekoorn and Smith 2002, Podos and Warren 2007). Learned song can undergo rapid cultural change because

it is not dependent on genetic change, and song learning has led to remarkable geographic diversity in oscine song. Far less is known about the role of song in maintaining species boundaries in suboscines, a diverse branch of passerines in which song is thought to be largely unaffected by learning. Innate song is far less labile, and thus may be more effective in maintaining species boundaries either by functioning as a strong *pre*-mating barrier to hybridization, or as a strong *post*-mating barrier if hybrids with intermediate songs are ineffective in attracting mates or defending territories (Stein 1963, Lanyon 1978, Sedgwick 2001, Seddon 2005, den Hartog *et al.* 2007). Because it likely diverges at a much slower rate than learned song, song differences that result in assortative mating may take longer to evolve in suboscines (*cf.* Lachlan and Servedio 2004). However, once evolved, divergent innate song types might function as stronger barriers to hybridization.

The *Empidonax difficilis-occidentalis-flavescens* complex (Pacific-slope Flycatcher, Cordilleran Flycatcher, and Yellowish Flycatcher, respectively) is a small clade of suboscine passerines that is comprised of both resident and migratory taxa, with a combined range that spans over 50° in latitude (Johnson 1980). Resident populations of Yellowish Flycatcher and Cordilleran Flycatcher reside in the highlands of Middle America, whereas migratory populations of the Cordilleran and Pacific-slope Flycatcher breed in forested habitats of western North America. These taxa have clearly homologous, but divergent, songs (Johnson 1980). By contrast, they show only slight differences in plumage coloration, and are difficult (*E. flavescens*) or nearly impossible (*E. difficilis* vs. *E. occidentalis*) to differentiate based on appearance alone. Songs are innate in this complex (Kroodsma 1984, 1985, Kroodsma and Konishi 1991), and given the very low level of plumage differentiation, likely play a disproportionate role in species recognition and mate choice.

One of the relatively few studies of the effects of divergence in innate song on species recognition focused on Willow Flycatchers (*Empidonax trailli*) and Alder Flycatchers (*E. alnorum*), congeners of the *E. difficilis-occidentalis-flavescens* clade. Stein (1963) used bioacoustic analysis and song playback experiments to show that, although morphologically they were virtually indistinguishable, sympatric Willow Flycatchers and Alder Flycatchers were divergent in song and mated assortatively based on song type. This study helped to elevate these taxa to species status, and bolstered the expectation that diagnostic song differences between suboscine taxa should act as strong behavioral barriers to hybridization. Because Willow and Alder Flycatchers are reproductively isolated (Winker 1994), however, we cannot be certain whether divergent song types reflect species differences that evolved for other reasons (such as habitat differences; Stein 1963), or whether they contributed to the evolution of reproductive isolation.

Here, I examine patterns in genetic and song variation and use experimental tests of species recognition to examine the role of song divergence in lineage diversification in the *E. difficilis-occidentalis-flavescens* complex. I address two major questions:

Does innate song function as a particularly strong isolating mechanism between incipient suboscine species?

How do patterns of divergence in innate song differ from those of learned song, and how has song divergence affected lineage diversification across the *E. difficilis-occidentalis-flavescens* clade?

In Chapter 1, I perform a multilocus genetic study of Pacific-slope and Cordilleran Flycatchers. I focus on a relatively restricted area in southwestern Canada, where individuals with songs intermediate between the two taxa were reported to occur. I demonstrate that genetic admixture and gene flow occurs between the two taxa in that area, although reference samples from parental populations outside of the contact zone showed no signs of genetic introgression. This runs counter to the expectation that innate song differences should form a strong barrier to hybridization and gene flow. The relatively restricted geographical focus, and the lack of analysis of song, however, made it difficult to assess the full scope of interactions between these taxa.

In Chapter 2, I perform a much broader multilocus analysis of genetic variation in Pacific-slope and Cordilleran Flycatchers that includes samples from throughout the 40-degree latitudinal range of these taxa. I combine this with an extensive examination of geographic variation in song to better understand the relationship between innate song type and genotype in these taxa, and to determine how song differences affect hybridization and gene flow. In addition, I performed a series of song playback experiments in Pacific-slope, Cordilleran, and contact zone populations using parental and intermediate songs to test the effectiveness of different innate song types as behavioral markers of taxon identity, and to examine whether responses to different song types can help explain the observed patterns of gene flow.

The first two chapters focus on the outcome of geographic contact between Pacific-slope and Cordilleran Flycatchers. This is an effective context within which to test the strength of divergent song types as behavioral barriers to hybridization and gene flow, but a two-taxon comparison is limited in its ability to illuminate patterns of song evolution and diversification. For this, a broader comparison is needed that takes place within a phylogenetic context.

In Chapter 3, I compare lineage diversification and vocal diversification across the entire *E. difficilis-occidentalis-flavescens* clade. I use mitochondrial DNA to examine phylogenetic relationships among the six principal taxa that comprise this clade, and compare patterns of song divergence to molecular divergence. The combined range of this clade spans over 50° in latitude and includes both migratory and non-migratory taxa. I examine how song repertoire divergence correlates with genetic divergence and whether rates of song and song repertoire divergence vary among taxa, especially between lower latitude non-migratory taxa and higher latitude migratory taxa. As in Chapter 2, I used song playback experiments to test whether varying rates of song divergence affect the efficacy of song types as taxon discrimination traits.

The study of the innate song of suboscines provides some notable advantages in understanding the relationship between divergence in acoustic signals and lineage diversification. The lack of a learned component in suboscine song makes it more comparable to the vocal signals of non-avian animals that have been studied in this context (e.g., *Laupala* crickets, Mendelson & Shaw 2002; Tungara frogs, Ryan & Rand 1993). Due to the more complex vocal apparatus of birds, however, suboscine song is often more complex than the acoustic signals of other non-avian animals, and thus has the capacity for greater phenotypic diversification. Thus, this study not only provides a better understanding of the effects of divergent acoustic signals in birds, but also may provide insight into the effects of divergent acoustic signals on lineage diversification in a wide range of organisms.

ACKNOWLEDGEMENTS

I would first like to thank the Museum of Vertebrate Zoology for support and inspiration. It was in the atmosphere of the MVZ that I could aspire without apology to become a 21st century naturalist.

I would like to thank my dissertation committee members for their advice, guidance, encouragement, and support throughout this process. Craig Moritz was especially helpful in helping me develop my thinking about hybrids, hybrid zones, and speciation. Craig always demonstrated the need to think broadly when addressing evolutionary questions. Craig also gave me an early start in field research at the Museum of Vertebrate Zoology when I was an undergraduate student. Rosemary Gillespie was always encouraging and also encouraged me to think broadly and consider examples outside of the world of birds. Finally, my graduate advisor, Rauri Bowie, provided encouragement and support and allowed me the latitude to develop my own questions and my own direction in this research. Rauri provided critical support and advice at key moments during this process, especially with respect to genetic analysis. Rauri also encouraged me to expand the scope of this project and saw the strengths of it at times when I did not. In addition to my formal committee, I received important guidance from Anthony Barnosky.

Many colleagues and fellow students helped me in the process of developing and performing this dissertation research, and in making the transformation to an evolutionary biologist.

I worked especially closely with Dr. D. Archibald McCallum. Arch, more than any other, worked through the details of this study with me, both in terms of the overarching research design and in the bioacoustic analysis. Arch was invaluable in the analysis of the vocalizations, and contributed many of his own recordings. His expertise was absolutely critical in this research. Perhaps most importantly, no one else was ever as willing to delve into the arcana of *Empidonax* vocalizations, distribution, life history, or taxonomy as Arch.

I owe much of my success in this project to my fellow students in the Museum of Vertebrate Zoology. As a graduate student, I have learned more from my fellow students than from any other source. I would like to thank the Bowie Lab Group for support and discussion. I would especially like to acknowledge Jay McEntee, Ricardo Pereira, Ângela Ribeiro, and Sonal Singhal. Without their help, this project would never have truly taken flight. Outside of the MVZ, David Toews was not only a source of intellectual input and discussion, but always an encouraging friend. Dave also taught me important molecular lab skills early in this process.

Matthew MacManes provided critical assistance with SNP discovery and bioinformatics.

J. Patrick Kelley provided critical help with the modeling of playback responses.

Richard Cannings originally suggested working on this system, and provided invaluable knowledge early in the project. Darren Irwin played a key role in the development of this project as well.

Several undergraduates helped with key parts of this project. Hillary Park, Nadjé Najar, Ji Won Yoo, Jillian Capedeville, Rachel Gulbraa, Tiffany Lue, and Angela DiRocco all helped with various aspects of this project. Their willingness to share their time helped make such a large undertaking possible.

Joshua Penalba provided invaluable assistance with this project, both in the field and in the lab. Josh was a source of expertise in the lab that I turned to with questions again and again, as well as performing some of the key lab work for the SNP genotyping. Anna Sellas, Lydia Smith, and Hanneline Smit also provided help with lab work.

The following people generously provided locality information for flycatchers, that was critical in performing the broad geographic sampling necessary in this project. Alan Brelsford, Mark Phinney, Mike Denny, Charles Gates, Charles Helm, Charles Swift, Chuck Trost, Craig Corder, Dan Casey, Jack Bowling, Jeff Marks, Jim Greaves, Kristi DuBois, Lisa Hardy, Michael Woodruff, Shirley Sturts, Tom Heindel, Jo Heindel, and Andy Stepniewski were all willing to share their knowledge of flycatcher occurrence and distribution. Cathy Koot and Jay and Amy Bailey deserve special mention for also providing accommodation.

For work in Mexico and Guatemala, I must make special mention of Dr. John Klicka. John shared his deep knowledge of the distribution of flycatchers in Mexico as well as sharing DNA sequences and tissue samples critical to developing and testing the hypotheses in this dissertation. Paul van Els was instrumental in helping to organize and perform the fieldwork in Mexico. Amy McAndrews, Francesca Albini, Robert Straub, and Javier Gomez all provided invaluable help with field knowledge and field access in Mexico. Juan Rivera generously provided access to his Finca El Pilar in Antigua, Guatemala for fieldwork. I would also like to thank the people of Omiltemí, Guerrero for their hospitality. Rosa Jiménez, Pavel Garcia, and Mafer Asturias Ramírez facilitated the fieldwork in Mexico and Guatemala in invaluable ways. Without their help, this project would have been much more difficult.

The following institutions generously provided recordings used in this dissertation: the Museum of Vertebrate Zoology, the Macaulay Library of Natural Sounds, and the Borror Laboratory of Bioacoustics. The following individuals also provided recordings: D. Archibald McCallum, Steve N. G. Howell, Kelly Bryan, Nathan Pieplow, Daniel Lane (*via* xeno-cano.org), Darren E. Irwin, and Hector and Monica Gómez de Silva.

The following institutions generously provided tissue samples used in this research: the Museum of Vertebrate Zoology, the University of Washington Burke Museum (Dr. John Klicka), and the Royal Alberta Museum.

Funding for this research was provided by the following agencies, institutions and societies: National Science Foundation (Doctoral Dissertation Improvement Grant), Museum of Vertebrate Zoology, UC Berkeley Dept. of Integrative Biology, American Ornithologists Union, Animal Behavior Society, and Wilson Ornithological Society.

The following agencies granted permission to collect specimens: U.S. Fish & Wildlife Service; Arizona Game & Fish Dept.; New Mexico Dept. of Game and Fish; California Natural

Resources Agency; Oregon Dept. of Fish & Wildlife; Utah Division of Wildlife Resources; State of Colorado Dept. of Natural Resources; Idaho Fish & Game; Washington Dept. of Fish & Wildlife; Montana Dept. of Fish, Wildlife, and Parks.

Finally, Lauren Benson helped me in the field, and more importantly, in life.

CHAPTER 1

Analysis of multilocus DNA reveals hybridization in a contact zone between *Empidonax* flycatchers

This chapter was previously published in 2009 in the *Journal of Avian Biology* with co-authors Darren E. Irwin and Richard J. Cannings. Permission was obtained from the co-authors for inclusion in this dissertation.

Full citation: Rush, A.C., Cannings, R.J. and Irwin, D.E. 2009. Analysis of multilocus DNA reveals introgressive hybridization in a contact zone between *Empidonax* flycatchers. *Journal of Avian Biology*. 40: 614-624.

ABSTRACT

Contact zones between recently diverged taxa offer unique opportunities to test whether the forms are reproductively isolated and therefore distinct species. The Pacific-slope flycatcher *Empidonax difficilis* and Cordilleran flycatcher *Empidonax occidentalis* are closely related taxa that were officially separated into two species in 1989, a treatment that has been controversial due to reports of phenotypically intermediate birds across the southern interior of British Columbia and Alberta. We present the first analysis of molecular variation across this region, in order to determine whether there is genetic introgression between the taxa. Allopatric populations of Pacific-slope and Cordilleran flycatchers belong to distinct mitochondrial clades, and all of the individuals sampled in interior southwestern Canada have the Pacific-slope haplotype. In contrast, variation in nuclear DNA (AFLPs) indicates hybridization between Pacific-slope and Cordilleran flycatchers in this region. We suggest that the discordance between the mitochondrial and nuclear markers most likely results from stochastic loss of Cordilleran mitochondrial haplotype lineages facilitated by asymmetries in mating due to earlier arrival and greater abundance of Pacific-slope flycatchers in the contact zone. The discovery of hybridization between Pacific-slope and Cordilleran flycatchers in southwestern Canada may call into question the decision to split them into two species. On the other hand, allopatric populations are genetically distinct in both mitochondrial and nuclear DNA, and the hybridization might not affect populations outside of the contact zone. This study highlights the importance of employing multiple genetic markers in studies of contact zones between closely related species.

INTRODUCTION

Zones of secondary contact have been of great interest to evolutionary biologists because they provide unique opportunities to observe the evolutionary interactions between divergent but related taxa (Barton and Hewitt 1985, Harrison 1993). Speciation occurs due to the evolution of barriers to gene flow between diverging populations. In birds, this is thought to occur most often due to divergence in allopatry (Mayr 1942, Miller 1956, Newton 2003, Coyne and Orr 2004, Price 2008). The boundary between intraspecific population differentiation and speciation is often blurry, however, and genetically distinct cryptic species pairs can be difficult to differentiate phenotypically (e.g., Irwin et al. 2001a, b, Bowie et al. 2004, Toews and Irwin 2008). Even when divergent groups are identified, it is only when they are in geographic contact that their species status can be rigorously tested (Irwin et al. 2001a,b, Cicero 2004, Price 2008).

When divergent taxa meet in secondary contact, a number of outcomes are possible. First, if strong reproductive isolation has evolved in allopatry as an incidental byproduct of natural selection, sexual selection, or genetic drift, the two taxa can remain distinct (Coyne and Orr 2004, Price 2008). Often, however, reproductive barriers are incomplete, and taxa in secondary contact do interbreed to some extent. If reproductive isolating mechanisms are nonexistent, widespread hybridization and introgression leads to neutral diffusion of alleles between the two parental populations (Endler 1977, Barton and Gale 1993). Over time, this may overcome any divergence that has taken place in allopatry and lead to fusion of the two parental gene pools. A third potential outcome is secondary contact with partial reproductive isolation between two taxa. This can lead to the formation of a stable hybrid zone between the two taxa if some form of selection maintains the zone (Barton and Hewitt 1981). Hybridization in secondary contact zones

can make the species status of taxa debatable (e.g., Hubbard 1969, Barrowclough 1980, Rising 1996, Cicero and Johnson 1998). Despite creating taxonomic confusion, zones of secondary contact and hybridization provide unique opportunities to examine the factors that contribute to evolutionary divergence and reproductive isolation (Price 2008).

Molecular genetic data can assist in distinguishing between the possible outcomes of secondary contact. If reproductive isolation between two taxa is complete, genetically distinct individuals should coexist in sympatry in a contact zone with no genetically intermediate individuals present. If neutral diffusion of alleles is occurring, a gradual, clinal transition in genetic characters will form between the two taxa, with genetically intermediate individuals occurring over a geographic area that increases with time. If a stable hybrid zone has formed, genetically intermediate individuals will be present in a more restricted geographic area between the two parental populations. In this case, two main models describe the types of hybrid zones that may form. In a tension zone (Barton and Hewitt 1981, Barton and Gale 1993), two parental populations are separated by a narrow region in which genetically intermediate individuals occur. This region does not necessarily correspond to or track specific geographic or environmental features. In a selection gradient, the two parental populations occur in distinct environments and genetically intermediate individuals are restricted to an intervening area that is environmentally intermediate between the two parental environments (Moore 1977, Moore and Price 1993). The width and placement of this area is determined by the extent of the intermediate habitat.

Accurate inference of evolutionary dynamics in secondary contact zones generally requires examination of multiple types of genetic evidence (Edwards et al. 2005). The permeability of hybrid zones to different genes may vary due to differences in the strength of selection and demographic effects, and reliance on individual genetic markers to reconstruct species histories (e.g., mitochondrial DNA) can sometimes lead to inaccurate conclusions (Irwin 2002, Ballard and Whitlock 2004, Edwards et al. 2005; but see Zink and Barrowclough 2008). Numerous studies of hybridization between closely related species in disparate groups have shown a lack of geographical concordance between mitochondrial and nuclear markers (e.g., Patton and Smith 1994, Wake and Schneider 1998, Rohwer et al. 2001, Irwin et al. 2005, Ruegg 2007, Good et al. 2008), highlighting the importance of utilizing multiple molecular markers in molecular biogeographic studies.

A particularly intriguing group for the study of speciation is the *Empidonax* flycatchers (Passeriformes: Tyrannidae), a New World genus of perching birds that are very similar morphologically, but divergent in vocalizations and ecology (Johnson 1963, Stein 1963, Johnson 1980, Johnson and Cicero 2002). Vocalizations are innate in *Empidonax* flycatchers (Kroodsma 1984, 1985), suggesting that vocal variation should correlate more closely to genotype than it does in birds with learned song (Kroodsma et al. 1995); the presence of individuals with vocalizations intermediate between two *Empidonax* species has been taken as evidence of hybridization (Johnson 1980, Stein 1963). In general, few hybrids have been reported between *Empidonax* species (McCarthy 2006), but because such a high level of morphological similarity between species likely makes field identification of hybrids very difficult (Pyle 1997), it is likely that hybrids have been overlooked.

Pacific-slope flycatchers *Empidonax difficilis* and Cordilleran flycatchers *E. occidentalis* are sister taxa (Johnson and Cicero 2002), and are estimated to have diverged approximately 350 000 years ago (from genetic distances in Johnson and Cicero 2002), using a widely accepted molecular clock for mitochondrial DNA of 2% sequence divergence per million years (García-

Moreno 2004, Lovette 2004, Weir and Schluter 2004, Price 2008, Weir and Schluter 2008). Throughout most of their ranges, Pacific-slope and Cordilleran flycatchers occupy distinct bioclimatic regions. Pacific-slope flycatchers are distributed largely in mesic, temperate coniferous forest west of the crest of the Sierra Nevada and Cascade mountain ranges, and reach their highest densities along the Pacific Northwest coast of the United States and Canada (Johnson 1980, Lowther 2000; Fig. 1). Cordilleran flycatchers are distributed at higher elevations in cooler, more xeric coniferous forest in the interior mountain West from central Mexico to the northern Rocky Mountains, and reach their highest densities much farther south in the southern Rocky Mountains (Johnson 1980, Lowther 2000).

Pacific-slope and Cordilleran flycatchers were formerly classified as subspecies of a single species, the “Western flycatcher,” but were elevated to species status based on differences in vocal, morphological, and allozyme characters (Johnson and Marten 1988, American Ornithologists’ Union 1989, Johnson 1994). This was based on the work of Johnson (1980, 1994), and Johnson and Marten (1988), who maintained that the only confirmed area of sympatry between the two forms was in the Siskiyou Mountains in northeastern California and that the two taxa were reproductively isolated there. Largely excluded from earlier analyses were “Western flycatcher” populations from interior southwestern Canada. Subsequent observations have suggested that many flycatchers in parts of this region have vocal features that are intermediate between Pacific-slope and Cordilleran types, leading observers to suspect hybridization between the two forms in this region and to question their taxonomic status (Fig. 1; Campbell et al. 1997, Kulba and McGillivray 2000, Lowther 2000, Marshall et al. 2003, Wahl et al. 2005). There is a consensus that populations west of the Cascade and Coast mountain ranges are Pacific-slope, but sources differ on whether interior populations are comprised of non-overlapping populations of Pacific-slope and Cordilleran flycatchers, the two forms living in sympatry, or a hybrid swarm (Campbell et al. 1997, Kulba and McGillivray 2000, Lowther 2000, Marshall et al. 2003, Wahl et al. 2005). Until now, formal analyses of these populations have been lacking.

Here, we provide the first genetic analysis of Pacific-slope and Cordilleran flycatchers in southwestern Canada, comparing them with samples from allopatric areas. We conduct phylogenetic analysis of mitochondrial DNA and Bayesian admixture analysis of multilocus nuclear DNA, as surveyed using amplified fragment length polymorphisms (AFLPs; Vos et al. 1995, Campbell et al. 2003, Bensch and Åkesson 2005). AFLPs have been shown in previous studies to be effective in elucidating population structure between weakly differentiated taxa (Wang et al. 2003, Bensch and Åkesson 2005, Irwin et al. 2005, Vallender et al. 2007). We test whether populations of “Western flycatchers” in interior southwestern Canada consist of: (1) just one of the Pacific-slope or Cordilleran flycatcher species, (2) members of both species, with no evidence of hybrids, indicating reproductive isolation, or (3) a range of genetically intermediate individuals, indicating hybridization.

METHODS

Study area

Field research was conducted in southern and central British Columbia (BC) and southwestern Alberta, Canada. We chose this area because in a preliminary study, one of us (RJC, unpubl. data) had found numerous individuals in this area with vocalizations sounding intermediate between Pacific-slope and Cordilleran flycatchers, suggesting that this may be a

hybrid zone. Southwestern Canada is varied in physical geography, climate, and vegetation, and includes several biogeographically distinct regions (Parish et al. 1996). The Cascade and Coast mountain ranges (hereafter “Cascades”) run northwest to southeast and separate the more mesic coastal forests from the more xeric interior. The xeric southern interior of BC is comprised of the Okanagan and Thompson Plateaus and the Okanagan Basin and includes some of the driest areas in Canada. East of the Okanagan, the Columbia Mountains contain more mesic forests. East of the Columbia Mountains, the Rocky Mountains are again more xeric but not to the level of central interior BC (Parish et al. 1996). We sampled Pacific-slope/Cordilleran flycatcher populations at eight sites representing each of these regions, from the Pacific coast of BC to the eastern slope of the Rocky Mountains in western Alberta (Table 1).

Collections, DNA amplification, and sequencing

We captured flycatchers in mist nets and took a blood sample (5-20 μ l) from the brachial vein of each bird before releasing it. We targeted territorial males on breeding territories. Blood was stored in 500 μ l of “Queen’s lysis buffer” (0.01 M Tris, 0.01 M NaCl, 0.01 M EDTA, and 1% *n*-lauroylsarcosine, pH 7.5; Seutin et al. 1991). Extracted DNA was stored in 100 μ l of 1X TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and stored at 4° C in the lab. Tissue samples of allopatric populations of Pacific-slope and Cordilleran flycatchers were provided by the Mus. of Vert. Zool. at the Univ. of California, Berkeley and used as reference samples of the two species. Reference samples from California were collected outside of any area of suspected sympatry between the two species. Reference individuals were determined to be in breeding condition based on the date of collection and gonad size. Whole genomic DNA was extracted from all samples using a standard phenol-chloroform method. We used two methods of DNA analysis, but due to problems with amplification for some individuals, the set of individuals for each analysis differs slightly (Table 1).

We amplified the entire 1041-base pair mitochondrial gene ND2 in 10 allopatric Pacific-slope flycatchers, 12 allopatric Cordilleran flycatchers, and 24 Canadian individuals (Table 1) using the primers H1064 (Drovetski et al., 2004) and L5215 (Hackett 1996), and the following thermocycler protocol: 3 min at 95° C, 35 cycles of 95° C (30 s), 55° C (30 s), and 72° C (30 s), followed by a final extension of 72° C for 10 min. ND2 sequences were edited using the program Sequencher (Genecodes Corporation) and aligned using the programs CLC Free Workbench 4 (<http://www.clcbio.com>) and MacClade 4.06 OS X (Maddison and Maddison 2003). After uneven ends were trimmed from the sequences, we analyzed a 932-base pair segment of the gene. Sequences can be downloaded from Genbank (Accession numbers to be determined after acceptance).

To examine variation in the nuclear genome, we used the amplified fragment length polymorphism (AFLP) method according to the protocol of LI-COR Biosciences (2003), based on the method developed by Vos et al. (1995). Whole genomic DNA was digested with the endonucleases *Eco*RI and *Mse*I and then ligated to E- and M-adaptors (100 nM). We preamplified DNA fragments using complimentary E- and M-primers and then selectively amplified a subset of DNA fragments using 5 primer combinations (Table 2). We used a LI-COR 4300 to separate DNA bands in a 6.5% polyacrylamide gel and scored bands as present or absent using the program SAGA version 2.0. We generated 127 polymorphic bands that we were able to score unambiguously in 48 individuals (Table 2).

Data analysis

To test for evidence of directional selection on the ND2 gene, we used *DnaSP* to calculate Tajima's D and to perform a McDonald and Kreitman test on the Cordilleran and Pacific-slope reference samples (Rozas et al. 2003). Significant results for the Tajima's D test can indicate either selection or population expansion; the McDonald and Kreitman test is less sensitive to the effects of population expansion (McDonald and Kreitman 1991). To further test for a population expansion, we used *DnaSP* to perform Fu's F_s tests, which are more sensitive to population expansion than Tajima's D (Fu 1997). To look for evidence of population structure in mitochondrial DNA, we created a statistical parsimony haplotype network from the ND2 sequences using the program TCS v1.21 (Clement et al. 2000).

We summarized the variation in the AFLP profiles of individuals with principal components analysis (PCA), using the software package R (R Development Core Team 2006, Toews and Irwin 2008).

To quantify population differentiation among sites, we used the program *Arlequin* 3.1 (Excoffier et al. 2005) to calculate population pairwise F_{ST} values from AFLP data. Some geographically proximate sites were grouped *a priori* to increase group size (Vancouver and Hope were combined and all California samples were combined). AFLP profiles were coded as binary (0 or 1) haplotypic RFLP data and significance was calculated from 1 023 permutations. Although F_{ST} values calculated from these data provide useful measures of genetic differentiation between populations, they are not calculated from allele frequencies and cannot be compared directly to F_{ST} values calculated from codominant markers.

We used the program STRUCTURE V2.2 (Pritchard et al. 2000, Falush et al. 2007) to group samples into populations and to assess levels of gene flow between populations based on AFLP profiles. STRUCTURE uses a Bayesian model-based clustering method to infer population structure and to assign individuals probabilistically to a set of populations (k) based on allele frequencies across loci. We used STRUCTURE to estimate k given no *a priori* population information. Based on the estimated posterior probability for each run for each value of k , STRUCTURE estimates the likelihood of the data. The correct number of populations was taken as the one for which the value of k had the greatest estimated likelihood. STRUCTURE calculates a membership coefficient (q), defined as the proportion of the genotype of an individual that originated from a given population, and assigns individuals to one or more of the k populations based on the highest proportion of membership. We performed 5 independent runs of 1 000 000 MCMC repetitions with a burn-in period of 50 000 for each of $k = 1$ through $k = 5$ assuming correlated allele frequencies and a population admixture model. We also used STRUCTURE to calculate the proportion of membership in each of the 2 clusters for 10 pre-defined populations (the eight Canadian sample sites plus the Pacific-slope and Cordilleran reference samples) to assess the level of population admixture among sample sites.

To provide an independent assessment of the genetic ancestry of individuals, we performed an admixture analysis of the AFLP data using the program BAPS 5.2 (Corander and Marttinen 2006, Corander et al. 2008). Similarly to STRUCTURE, BAPS infers population structure among samples using Bayesian clustering methods, although BAPS uses a somewhat different computational approach. Both have the potential to classify individuals as genetically admixed.

RESULTS

Mitochondrial DNA analysis

ND2 analysis revealed two main haplotype clusters, one containing allopatric Cordilleran flycatchers and the other containing allopatric Pacific-slope flycatchers as well as all individuals sampled in Canada (Fig. 2). The most common haplotypes of these two clusters differed by six changes, all synonymous (0.64 % sequence divergence). We detected several rare haplotypes that differed from the main haplotype groups by a small number of changes. There was a greater number of these rare haplotypes in the Pacific-slope cluster, but this could reflect the larger number of Pacific-slope haplotypes sampled. A single divergent haplotype (separated by five mutational changes) is present in the Pacific-slope cluster. This individual was one of seven individuals sampled in Monterey County in central California (Table 1), indicating that it represents a rare haplotype rather than an under-sampled divergent population. The Tajima's D test was not significant for the Cordilleran reference samples (-1.10317; $P > 0.10$), but was significant for the Pacific-slope reference samples (-1.76515; $P < 0.05$). The McDonald and Kreitman test was not significant (two-tailed Fisher's exact test: $P = 0.56$), but this test examined only whether there is support for directional selection on sites within the ND2 gene; it would not detect selection operating elsewhere in the mtDNA. Because the entire mitochondrial genome is inherited as a single unit, selection on any part of it will affect phylogeographic patterns observed on all other parts. The Fu's F_s test was not significant for either the Pacific-slope (-0.823, $P = 0.200$) or Cordilleran (-1.410, $P = 0.136$) reference samples.

AFLP analysis

Each individual had a unique AFLP profile. Principal components analysis separated allopatric Pacific-slope and Cordilleran flycatchers into two widely separated clusters (Fig. 3), although no markers showed fixed differences between Pacific-slope and Cordilleran flycatcher reference samples. The first two principal components explained relatively little of the variation among individuals (PC1: 6.7%; PC2: 5.2%), indicating that the clear separation arose from a relatively weak signal in the genome. Thirty eigenvalues each explained more than 1%, and cumulatively explained 86% of the genetic variation. The individuals from interior southwestern Canada formed a more diffuse cluster centered between the allopatric Pacific-slope and allopatric Cordilleran clusters. Some of these interior Canadian samples occurred within the Pacific-slope cluster, but most occurred between the Pacific-slope and Cordilleran clusters, indicating the genetic intermediacy of these individuals. A small number of the Canadian samples bordered the Cordilleran cluster, but none fell within it.

Despite relatively small sample sizes in some cases, more than 50% of the pairwise F_{ST} comparisons of individual sites were significant at the $P \leq 0.05$ level (Table 3). Most sites showed little differentiation in F_{ST} (F_{ST} ca. 0.07) from their nearest neighbors. Canadian coastal samples (e.g. west of the Cascade Mountains, "Vancouver-Hope" in Table 3) and the Pacific-slope reference samples were nearly equally differentiated in F_{ST} from the Cordilleran reference samples ($F_{ST} = 0.24$, $F_{ST} = 0.27$ respectively, $P < 0.001$ for both). Cordilleran populations from Colorado and Arizona showed little differentiation from each other ($F_{ST} = 0.07$; $P < 0.001$). Princeton, on the eastern slope of the Cascades, showed low differentiation ($F_{ST} = 0.06$; $P < 0.001$) from the Pacific-slope reference samples, but moderate differentiation from the Cordilleran reference samples (avg. $F_{ST} = 0.15$, $P < 0.001$). Other sites east of the Cascades in interior BC showed intermediate levels of differentiation from both the Pacific-slope ($F_{ST} = 0.09$

– 0.17; $P < 0.001$) and Cordilleran ($F_{ST} = 0.12 - 0.19$; $P < 0.001$) reference samples. Kananaskis, on the east slope of the Rocky Mountains, showed very little differentiation ($F_{ST} = 0.07$; $P < 0.001$) from the Cordilleran reference populations (the comparison between Kananaskis and the Pacific-slope reference populations was not significant).

Based on likelihood values, STRUCTURE indicated that the samples were drawn from two populations (highest log likelihood = - 3706.68). Pacific-slope and Cordilleran reference samples were assigned to different clusters with a high posterior probability ($q \geq 0.90$) (Fig. 4). We used this $q \geq 0.90$ threshold to classify Canadian individuals of unknown taxonomic status as pure types of either species. The majority of Canadian individuals (18 of 29, 62%) showed evidence of admixture in their nuclear DNA and were not assigned with ≥ 0.90 probability to the Pacific-slope or Cordilleran cluster (Fig. 4). Of the remaining 11 (38%), 10 Canadian individuals were classified as pure Pacific-slope while only one was classified as pure Cordilleran. All five individuals sampled west of the Cascades were classified as pure Pacific-slope. East of the Cascades in interior BC and Alberta, only five of the 24 (21%) individuals sampled could be classified as pure types of either species (4 Pacific-slope, 1 Cordilleran). No individuals showed the roughly even 0.50/0.50 membership in both clusters expected for F1 hybrids, although several were close (e.g. a 0.60/0.40 probability; Fig. 1).

Based on the proportion of ancestry per sample site calculated by STRUCTURE, all sample sites from the Pacific coast to the eastern slope of the Cascades were classified as pure Pacific-slope (Table 4). In southernmost BC, the proportion of ancestry per site changed clinally from the Pacific coast to Kootenay Lake, with the highest level of Cordilleran ancestry evident in populations in south-central BC. Two neighboring sites in south-central BC (Christina Lake and Kootenay Lake) were majority Cordilleran, with no pure parental types of either species present (all other sample sites were majority Pacific-slope). The largest difference in ancestry between two neighboring sites was between Okanagan and Princeton, where proportion of Cordilleran ancestry dropped from 0.44 to 0.09 over approximately 60 km. Williams Lake and Kananaskis are east of the Cascades but north of the southernmost sites, and had proportionately less Cordilleran ancestry than the southernmost sites (Williams Lake = 0.33 Cordilleran, Kananaskis = 0.37 Cordilleran).

Results from BAPS confirmed the results from STRUCTURE. As expected, slight differences existed between the two analyses in the assignment of particular individuals to populations, but the key results remain unchanged. BAPS attributed many of the admixed individuals more evenly to the two parental populations than Structure, making them appear more like F1 hybrids than backcrosses. There were five minor discrepancies between the two analyses in terms of assignment to the parental or admixed categories. One individual that was classified as admixed by Structure was classified as pure Pacific-slope by BAPS. The one individual that was classified as pure Cordilleran by Structure was classified as admixed by BAPS. Three individuals that were classified as pure (i.e., > 0.90) Pacific-slope by Structure were classified as admixed by BAPS. One of these three was from west of the Cascades (from Vancouver). Of the two analyses, the STRUCTURE results are more similar to the PCA results, and the Vancouver individual falls well within the Pacific-slope cluster in the PCA analysis. Because sites west of the Cascades also show a clear affinity with the Pacific-slope reference samples in the F_{ST} tests, we conclude that they can be treated as Pacific-slope. Because the results from STRUCTURE and BAPS were otherwise so similar, we report only the STRUCTURE results in detail (Table 4, Figure 4).

DISCUSSION

Analysis of AFLPs and ND2 sequences shows that allopatric populations of Pacific-slope and Cordilleran flycatchers can be differentiated unambiguously using either nuclear or mitochondrial DNA. The 0.64 % divergence in ND2 sequences between Pacific-slope and Cordilleran flycatchers is consistent with the 0.7% sequence divergence reported by Johnson and Cicero (2002) based on four mitochondrial genes, and our analysis of nuclear DNA provides the first genome-wide measure of genetic differentiation between these two taxa. West of the Cascades, flycatcher populations are genetically similar to allopatric Pacific-slope reference samples, but the species identity of populations in interior BC and in Alberta is much more ambiguous. The level of genetic intermediacy in these populations strongly suggests that Pacific-slope and Cordilleran flycatchers interbreed in sympatry and that there is a broad area of hybridization between them in this region.

Analysis of AFLPs revealed a clinal transition in the nuclear DNA of these two species in our population admixture analysis, and genetic intermediacy in our F_{ST} analysis, thus offering a different view of their evolutionary history and species status. Birds of mixed ancestry are present in an area at least 400 km in width from the eastern slope of the Cascades in BC to the eastern slope of the Rocky Mountains in Alberta (Fig. 4). The Okanagan site, with roughly even proportions of Pacific-slope and Cordilleran ancestry, may form the center of a genetic transition between these two species. Christina Lake and Kootenay Lake seem to be hybrid swarms, with only admixed individuals present. It is likely that the cline of steadily increasing Cordilleran nuclear DNA continues to the southeast of Christina Lake where densities of Cordilleran flycatchers are likely higher (Lowther 2000). There is a higher level of Cordilleran ancestry at Williams Lake than at some of the sites further south (e.g., Princeton, Table 4). Because the Cascades run diagonally from northwest to southeast, Williams Lake, although west of Princeton, falls farther to the east of the Cascades, in an area somewhat intermediate in vegetation between the mesic coastal and xeric interior forests (Campbell et al. 1997), so an additional possibility is that the higher level of Cordilleran ancestry at this site may reflect greater suitability of the habitat to that species.

The pattern of genetic intermediacy displayed in nuclear DNA contrasts with the pattern in mtDNA, in which all Canadian samples grouped closely with allopatric Pacific-slope samples. Discordance between nuclear and mitochondrial DNA highlights the utility of employing multiple genetic markers in phylogeographic studies of closely related taxa. The discordance could arise for a number of reasons. First, if the Pacific-slope haplotype were under positive selection, a selective sweep on the Pacific-slope mitochondrial haplotype could have fixed that haplotype in the Canadian population. However, there is little evidence in support of selection. Although the Tajima's D test was significant for the Pacific-slope reference samples, indicating the possibility of directional selection on the gene or a population expansion, these conclusions were not supported by the McDonald and Kreitman or Fu's F_s tests respectively.

We find it more likely that the discordance between nuclear and mitochondrial DNA is due to asymmetries in mating coupled with demographic effects. Coastal British Columbian populations of Pacific-slope flycatchers are at high densities (Johnson 1980, Campbell et al. 1997), and arrive from their wintering grounds almost a month earlier than interior populations (Campbell et al. 1997). Cordilleran flycatchers are at the northern limit of their range in this region and are likely at lower densities than Pacific-slope flycatchers. If some coastal (i.e., Pacific-slope) birds disperse to the interior earlier, either by crossing through passes in the

Cascades or by moving northward to the interior via river valleys such as the Okanagan Valley, they could be in the region already when the later migrating Cordilleran flycatchers arrive. There is evidence for earlier arrival of males in populations of Pacific-slope flycatchers on the Queen Charlotte Islands, off the coast of British Columbia (Ainsley 1992). If Cordilleran males arrive earlier than females, they would encounter only Pacific-slope females upon arrival on the breeding grounds in the interior, and as a result, most hybrid offspring would have Pacific-slope mitochondrial haplotypes, whereas half of their nuclear DNA would be Cordilleran. Asymmetries in migration timing between coastal and interior groups and the earlier arrival of males on the breeding ground of the coastal form have been hypothesized to account for similar patterns of cytonuclear discordance in hybrid zones between hermit warblers *Dendroica occidentalis* and Townsend's warblers *Dendroica townsendi* (Rohwer et al. 2001), and between two subspecies of Swainson's thrush *Catharus ustulatus* (Ruegg 2007), both also occurring in the Pacific Northwest.

Asymmetries in mating could be augmented by demographic effects related to the higher densities of Pacific-slope flycatchers. Because the effective population sizes of mitochondrial genes are $\frac{1}{4}$ those of nuclear genes (Avise 2004), a mitochondrial haplotype lineage can be lost by chance in a population comparatively easily, especially if initially rare (Ballard and Whitlock 2004). If the frequency of Cordilleran females were low, the much higher density of Pacific-slope females in the region coupled with genetic drift could help fix the Pacific-slope haplotype in the Canadian population, especially in the presence of species- and sex-biased differences in arrival times to the breeding grounds.

The genetic similarity of populations west of the Cascades (Vancouver and Hope) and Pacific-slope populations on the coast of California, as well as their almost equal level of divergence from the Cordilleran reference samples, indicate that Canadian populations west of the Cascades are Pacific-slope. The decrease in the level of Cordilleran ancestry evident between the Okanagan and Princeton to some extent may reflect the small sample sizes at these two sites and the presence of two individuals at the Okanagan site with a high proportion of Cordilleran ancestry, but it is notable that the magnitude of change in the proportion of Cordilleran ancestry between Princeton and Okanagan is not evident in any other comparison between pairs of neighboring sites, despite comparable sample sizes in some cases. Princeton shows only a very low level of population admixture (one of four individuals sampled was 0.81 Pacific-slope; the remaining three were ≥ 0.90 Pacific-slope). This relatively sharp transition in the level of Cordilleran ancestry suggests that the Cascades may form a western boundary to this hybrid zone.

Our results indicate that there is not a strong reproductive barrier between Pacific-slope and Cordilleran flycatchers in southwestern Canada. The presence of genetically intermediate individuals in an area extending over 400 km might be suggestive of neutral diffusion resulting from a lack of reproductive isolation rather than a tension zone. On the other hand, the decrease in Cordilleran ancestry at the eastern edge of the Cascades and the occurrence of the highest proportions of Cordilleran nuclear ancestry in the relatively xeric southern interior of BC suggests that the ecological differences between Pacific-slope and Cordilleran flycatchers may play an important role in limiting areas of sympatry between the two taxa and by extension limit opportunities for hybridization. If so, the hybrid zone between these two species may be best described by a selection gradient model. However, models suggest that tension zones will tend to move to areas of low population density (Barton and Hewitt 1989) such as occur for these taxa at the Cascades (Campbell et al. 1997). Thus, without further study we cannot conclude that the

apparent break in gene flow at the Cascades is a result of ecological selection acting in an area of environmental transition rather than an effect of low population density.

CONCLUSION

The decision to split the “Western flycatcher” into the Pacific-slope flycatcher and Cordilleran flycatcher (American Ornithologists’ Union 1989) was made primarily based on studies of the contact zone in California (Johnson 1980, Johnson and Marten 1988), without data from interior southwestern Canada. We now wonder whether, given the present evidence, the decision to formally split the taxa into distinct species would have been made. On one hand, our data indicate that the two taxa hybridize within a broad region of contact, indicating that perhaps they are best treated as a single species. On the other hand, allopatric populations are genetically distinct and differentiated behaviorally, morphologically, and ecologically (Johnson 1980, Johnson and Martens 1988, Johnson and Cicero 2002); hence, they presently remain evolutionary divergent despite the hybridization. We have no evidence that the genetic introgression seen in interior southwestern Canada has affected populations outside of this region. Thus the allopatric populations might continue to remain distinct despite the presence of the hybrid zone. This situation illustrates the challenges involved in species-level taxonomy, as different species definitions contain competing ideas regarding the importance of reproductive isolation versus evolutionary distinctiveness regardless of the potential to hybridize. An accurate assessment of the species status of the “Western flycatcher” complex will require detailed studies of the amount and form of any reproductive isolation between the taxa in the contact zone, as well as an analysis of whether the allopatric populations are likely to remain differentiated despite the apparent introgression between them.

TABLES & FIGURES

Table 1. Sampling locations including total numbers of individuals used from each site as well as the numbers used in each type of analysis. Taxonomic status of individuals is assigned as Pacific-slope or Cordilleran if from allopatric populations of these species, but as ‘contact zone’ (i.e., unknown) if from the study area in Canada. ‘MVZ’ indicates the Museum of Vertebrate Zoology; ‘RCI’ indicates Rush, Cannings, and Irwin (this study).

Table 2. AFLP primer combinations and number of polymorphic fragments generated by each primer combination.

Table 3. Pairwise F_{ST} values between all sample sites calculated from AFLP data by *Arlequin*. Values in bold italics are significantly different from zero ($P \leq 0.05$).

Table 4. Proportion of Pacific-slope or Cordilleran ancestry calculated as the combined q values of individuals per sampling site by the program STRUCTURE.

Figure 1. Geographic ranges of Pacific-slope and Cordilleran flycatchers in western North America, based on the map by Lowther (2000). The lighter gray shading shows the area of the interior Pacific Northwest where intermediate vocalizations have been reported. Small circles show the locations of samples used in this study. White circles are the Pacific-slope reference samples, black circles are the Cordilleran reference samples, and light gray circles are the contact zone samples.

Figure 2. ND2 haplotype network. Black represents Pacific-slope flycatcher reference samples, white represents Cordilleran flycatcher reference samples, and gray represents Canadian samples. Circle areas are proportional to numbers of individuals. Small black circles on connecting lines indicate additional base substitutions (beyond one).

Figure 3. PCA of individuals based on AFLP profiles. Black diamonds represent Pacific-slope flycatchers from California and coastal BC (west of the Cascades), white squares represent Cordilleran flycatchers, and grey triangles represent Canadian samples from interior British Columbia and Alberta.

Figure 4. Map of sampling sites in British Columbia and Alberta, Canada and population assignments performed by the program STRUCTURE. In lower graph, each bar represents the proportion of ancestry of a given individual in a population with black corresponding to Pacific-slope and white to Cordilleran. Bars are grouped by sample site and arranged roughly west to east. Individuals labeled “Pacific-slope” (PSFL) are from California and individuals labeled Cordilleran (COFL) are from Colorado, Arizona, and South Dakota. Pie graphs in the top figure show the proportion of individuals per site that belong to the categories Pacific-slope (black), Cordilleran (white), and genetically admixed (gray). Location abbreviations: VA = Vancouver, HO = Hope, PR = Princeton, WL = Williams Lake, OK = Okanagan, CL = Christina Lake, KL = Kootenay Lake, KA = Kananaskis.

Table 1.

Taxon	Site	State/Province	Lat. N	Long. W	Sample source	Sample size	ND2	AFLP
Pacific-slope	Lake Co.	California	39.38	122.87	MVZ	1	1	1
Pacific-slope	Monterey Co.	California	36.33	121.53	MVZ	7	7	6
Pacific-slope	San Benito Co.	California	36.38	120.64	MVZ	2	2	2
Contact zone	Vancouver	British Columbia	49.27	123.23	RCI	2	-	2
Contact zone	Hope	British Columbia	49.16	121.33	RCI	3	3	3
Contact zone	Williams Lake	British Columbia	52.11	122.04	RCI	4	3	4
Contact zone	Princeton	British Columbia	49.57	120.50	RCI	4	3	4
Contact zone	Okanagan	British Columbia	49.34	119.74	RCI	4	2	4
Contact zone	Christina Lake	British Columbia	49.07	118.19	RCI	5	5	5
Contact zone	Kootenay Lake	British Columbia	49.51	116.79	RCI	3	2	3
Contact zone	Kananaskis	Alberta	51.03	115.01	RCI	6	6	4
Cordilleran	Apache Co.	Arizona	33.85	109.31	MVZ	5	4	4
Cordilleran	Graham Co.	Arizona	32.63	109.82	MVZ	2	2	1
Cordilleran	Custer Co.	Colorado	38.05	105.05	MVZ	5	5	4
Cordilleran	Lawrence Co.	South Dakota	44.16	103.88	MVZ	1	1	1
Total						54	46	48

Table 2.

Primer combination	EcoRI-primer (NNN-3')	TruI-primer (NNN-3')	No. of polymorphic fragments
1	ACA	CAC	26
2	ACT	CAC	44
3	AGC	CAT	9
4	AGG	CAC	22
5	ACC	CAC	26

Table 3.

	1)	2)	3)	4)	5)	6)	7)	8)	9)	10)
1) Vancouver-Hope	0.00									
2) Princeton	0.04	0.00								
3) Williams Lake	0.10	0.00	0.00							
4) Okanagan	0.03	0.00	0.01	0.00						
5) Christina Lake	0.07	0.07	0.10	-0.02	0.00					
6) Kootenay Lake	0.11	0.02	0.07	0.02	0.07	0.00				
7) Kananaskis	0.02	-0.04	-0.04	-0.05	-0.01	-0.06	0.00			
8) California (Pacific-slope)	0.08	0.06	0.17	0.09	0.13	0.15	0.03	0.00		
9) Colorado (Cordilleran)	0.24	0.13	0.13	0.12	0.19	0.15	0.06	0.27	0.00	
10) Arizona (Cordilleran)	0.23	0.16	0.15	0.12	0.11	0.14	0.08	0.27	0.07	0.00

Table 4.

Site	Proportion Pacific-slope	Proportion Cordilleran
Pacific-slope reference samples	0.95	0.05
Vancouver	0.97	0.03
Hope	0.96	0.05
Princeton	0.91	0.09
Williams Lake	0.67	0.33
Okanagan	0.56	0.44
Christina Lake	0.34	0.66
Kootenay Lake	0.43	0.57
Kananaskis	0.63	0.37
Cordilleran reference samples	0.04	0.96

Figure 1.

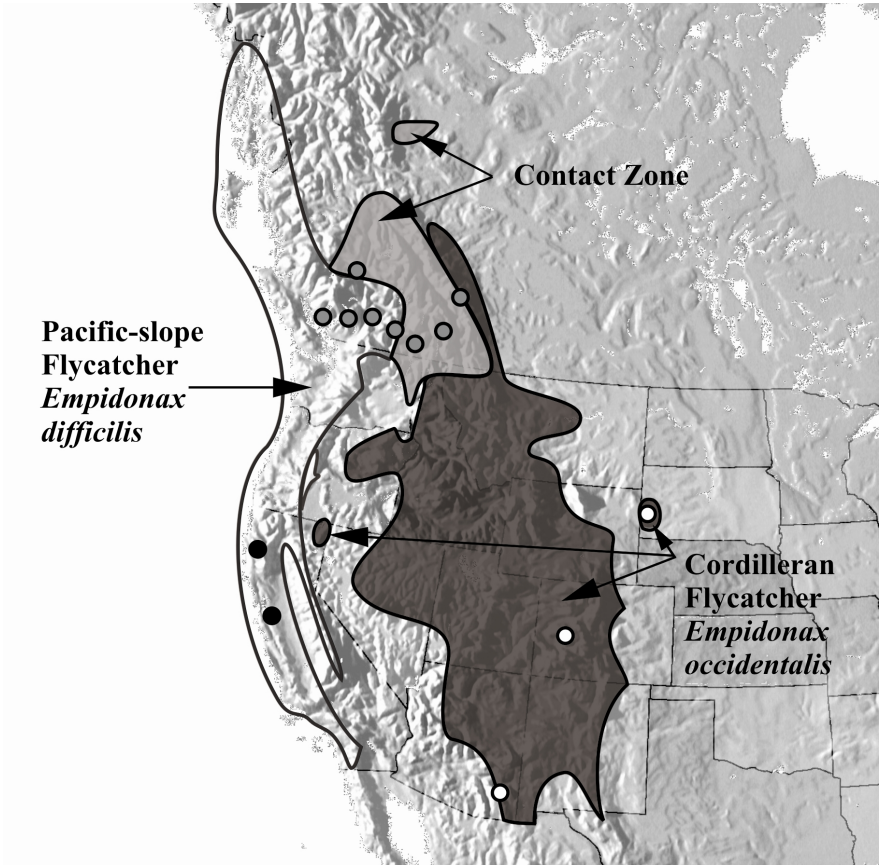


Figure 2.

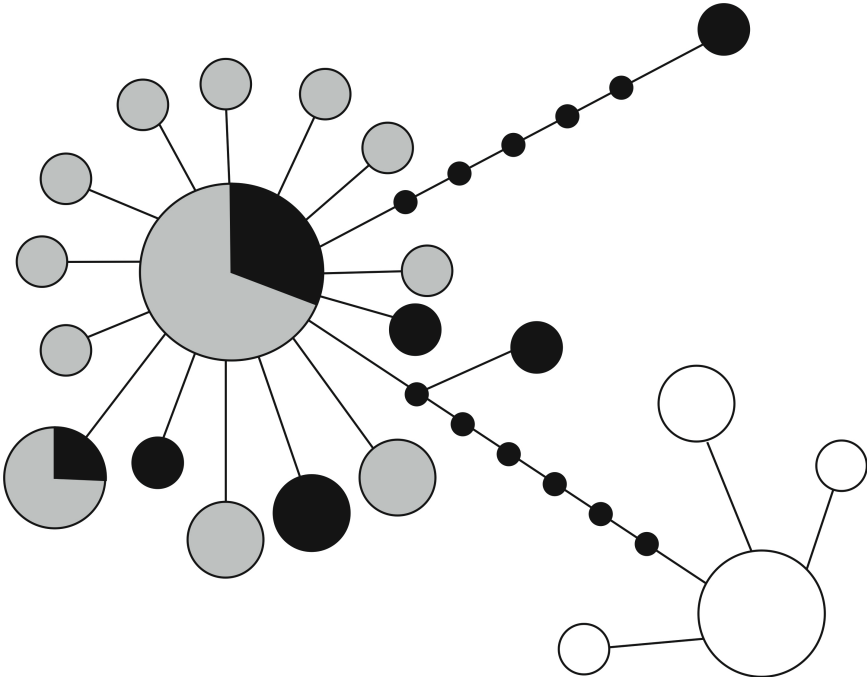


Figure 3.

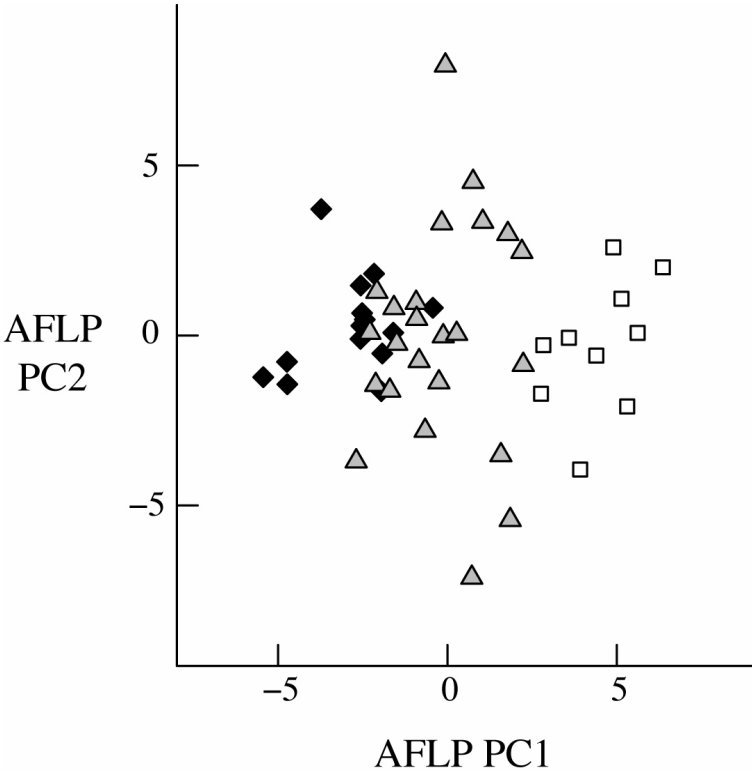
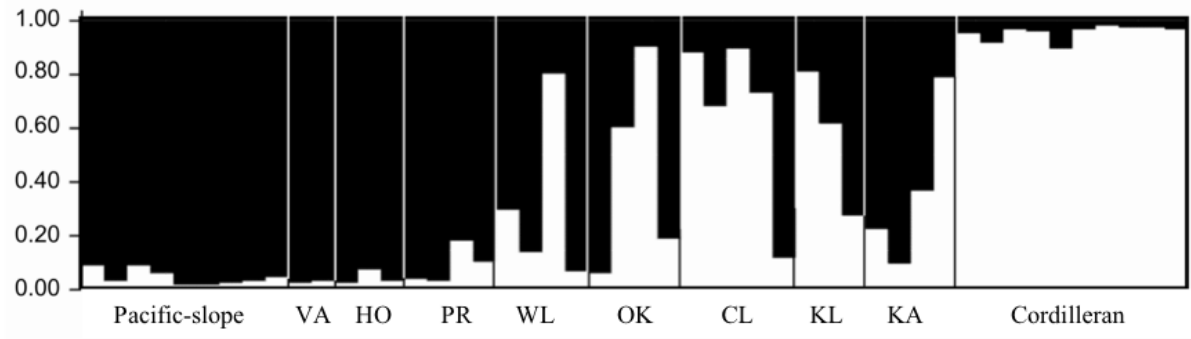
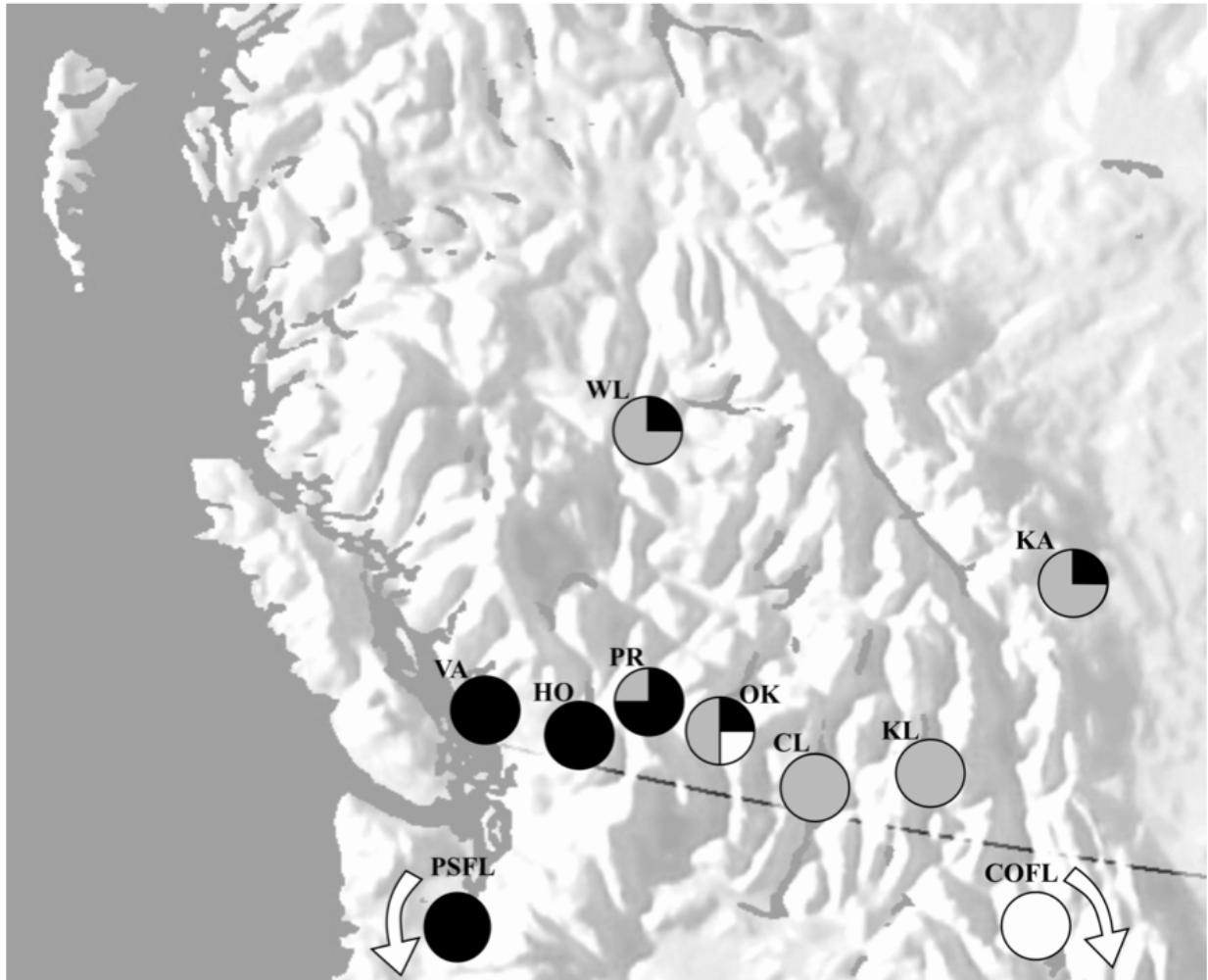


Figure 4.



CHAPTER 2

An examination of song divergence, hybridization, and introgression in two species of *Empidonax* flycatchers: Pacific-slope Flycatcher (*Empidonax difficilis difficilis*) and Cordilleran Flycatcher (*E. occidentalis hellmayri*).

ABSTRACT

Divergent song types can act as behavioral barriers to gene flow in birds because they can have direct effects on mate choice. Because songs can continue to diverge through drift or selection after speciation is complete, the effects of song divergence on the development of assortative mating and reproductive isolation are easier to infer when reproductive isolation between taxa is incomplete. Divergence in innate song in suboscine passerines could result in particularly strong behavioral barriers to gene flow because song type is more closely correlated with genotype, and thus a more direct marker of lineage affiliation than in passerine species with learned song. I perform an extensive examination of genetic variation and song variation to investigate how divergence in innate song has affected gene flow and genetic introgression in secondary contact between two suboscine species – Pacific-slope Flycatcher and Cordilleran Flycatcher. I demonstrate high levels of introgression in both mitochondrial and nuclear genetic markers, although the pattern of introgression is asymmetrical, with introgression limited into core Pacific-slope populations. Moreover, I highlight extensive geographic discordance between the frequencies of mitochondrial and nuclear markers. I demonstrate that the songs of the two taxa are distinct, and highly correlated with nuclear genotype, and that the songs of admixed individuals exhibit spectral characteristics intermediate to the parental species. Song playback experiments demonstrate that both species show some level of discrimination based on song and highlight lineage-specific behavioral differences that have likely affected the outcome of secondary contact. Pacific-slope Flycatchers seem to rely more song in territorial defense and may discriminate more among song types. Cordilleran Flycatchers exhibit higher levels of aggressiveness in response to playback. Based on the pattern of geographic variation in genetic and song characters, and on the results of the playback experiments, I propose a historical scenario of secondary contact in which asymmetrical introgression of nuclear alleles was facilitated by the social dominance of more aggressive Cordilleran Flycatchers. I predict that Cordilleran populations will become increasingly introgressed, while introgression into Pacific-slope populations may be limited by a combination of ecological and behavioral factors. This is one of the broadest examinations of the interaction between genotype and innate song type yet conducted, and provides insights relevant to the role of divergent signals in speciation in a wide range of organisms.

INTRODUCTION

Speciation occurs due to the formation of barriers to gene flow between populations (Coyne and Orr 2004). Gene flow between diverging animal populations is most often restricted due to divergence in phenotypic traits that create premating barriers, such as habitat isolation or behavioral isolation (Panhuis et al 2001, Ritchie 2007). Because they often play important roles in mate choice and reproduction, divergent signals can act as behavioral barriers to gene flow between populations by functioning as cues for assortative mating (West-Eberhard 1983, Ryan and Rand 1993, Coyne and Orr 2004). Many studies of the importance of signal divergence in species formation have focused on birds (e.g., Searcy and Andersson 1986, Price 1998). Signals that affect assortative mating, such as song and plumage, are often conspicuous and comparatively easy to observe and quantify in birds. This is especially true of passerines (songbirds). Passerine song serves as an important advertising signal that can affect the ability of males to attract mates and to defend territories from other males, and allows females to assess the

suitability of individual males as mates (Payne 1983, Read and Weary 1992, Catchpole and Slater 1995). As populations diverge, differences that develop in advertising songs can lead to assortative mating and reproductive isolation if females prefer males with song types more similar to their own population (West-Eberhard 1983, Payne 1986, Catchpole 1987, Ritchie 2007, Price 2008).

The ontogeny of passerine song, i.e., whether song develops through learning (as in oscine passerines; Slater 1989), or is innate and unaffected by learning (as in most suboscine passerines; Kroodsma 1984, 1985), has the potential to affect the strength and efficacy of song as a behavioral barrier between diverging populations. Research on the role of song divergence in speciation has focused largely on the learned songs of oscine passerines (e.g., Payne 1983, 1986, Slabbekoorn and Smith 2002, Podos and Warren 2007, Price 2008). Normal oscine song develops by naïve birds copying adult male tutors, and thus can change via non-genetic cultural evolution (e.g., due to copying errors; reviewed in Slater 1989). Because change in oscine song does not depend on a corresponding genetic change, it usually outstrips genetic evolution (Lynch 1996) and can lead to the rapid evolution of diverse, population-specific song types (dialects) that in some cases, may have important effects on mate choice (empirical studies: Baker et al 1981, Baker et al 1987, Clayton 1990a, 1990b, 1990c, Nelson 1998, Derryberry 2007, Luther and Derryberry 2012; model: Lachlan and Servedio 2004). Song must be reflective of genotype to some extent for females to gain information from song on the genetic quality of potential mates (Payne 1983), but because of the inherent plasticity of the learning process, song learning can mask the genotype of the singer. This can affect intraspecific mate choice, when lower quality males copy the songs of higher quality neighbors (Payne 1983), but can also facilitate hybridization in some cases when naïve birds learn heterospecific song and as a result attract heterospecific mates (e.g., Emlen *et al.* 1975, Grant and Grant 1998, Baker and Boylan 1999, Qvarnstrom *et al.* 2006, Table 14.1 in Price 2008). This can occur despite the negative fitness consequences of hybridization in some cases (Alatalo et al 1990, Baker and Boylan 1999, Veen et al 2001, Carling and Brumfield 2008).

Although they have been studied much less, suboscine passerines provide an interesting contrast to oscines in how song differences could function as behavioral barriers (Tobias et al. 2012). Because it is “hard-wired”, innate suboscine song is far less labile than learned song (evident, e.g., in the lack of population song dialects in suboscines), and likely to be more closely correlated with the genotype of the singer (Kroodsma 1984, 1985, 1995, Kroodsma and Konishi 1991). In the case of suboscines, females assessing males using song are assessing the underlying genotype of the singer more directly. Assuming that, in general, inbreeding is avoided through natal dispersal behavior (Greenwood 1980, Greenwood 1987, Ribeiro et al. 2012), females should benefit by selecting mates that are genetically similar to themselves, at least to the extent that it allows them to avoid mating with heterospecific males with incompatible genomes. Females could use song type as a cue to select (high quality) males by matching songs to an internal template that develops by imprinting on, or by having an innate preference for songs similar to that of their father or their natal population (Price 1998, Grant and Grant 1997, Irwin and Price 1999, ten Cate and Voss 1999, Price 2008). Divergent song types, that do not match the template of the female, would have decreased salience, and signal poor quality mates. Due to the close correlation with genotype, female preference for more similar innate song would be, by extension, a preference for more similar genomes. Thus, innate songs could function as *de facto* species recognition signals and strong barriers to hybridization, even if differences did not evolve under selective pressure to avoid hybridization (i.e., through reinforcement). By extension, males

would inherit the “correct” song for their population, and would perceive singers with song types similar to their own as potential rivals for mates. Male response strength to rival singers should decrease with decreasing song similarity and decreasing song salience. If hybridization does occur, innate songs should show the effects of hybridization and genetic admixture directly. Whether this leads to a form of extrinsic post-zygotic selection against hybrids and decreased introgression will depend on the reactions of parental populations to males with “hybrid” song types.

Responses to different song types can be affected by differences in other behaviors that have evolved between lineages. For example, asymmetries in aggression between taxa have been shown to lead to increased introgression from the aggressive, socially dominant taxon to the socially subordinate taxon in passerine hybrid zones (e.g., Kallioninen et al. 1995, Pearson and Rohwer 2000, McDonald et al. 2001). This can lead to discordance in the patterns of introgression of particular genetic markers or phenotypic traits (McDonald et al. 2001, Rohwer et al. 2001, Krosby and Rohwer 2009). These behavioral differences could evolve in allopatry through sexual selection favoring more aggressive individuals in intraspecific male-male competition for mates (e.g., McDonald et al. 2001), and subsequently confer an advantage in encounters between sister taxa resulting from secondary contact. Interactions between discriminant responses based on song similarity and differences in aggressive behavior could have important implications for gene flow and speciation, by affecting the outcomes of competitive interactions between males from divergent populations with songs similar enough that they perceive one another as rivals.

In Figure 1, I illustrate three scenarios for how responses based on song similarity could interact with non-vocal behavioral differences between taxa to affect hybridization and gene flow. For ease of communication, I refer to Species 1, Species 2, and hybrids as “taxa”. In all scenarios, each taxon responds most to its own song type. In the first scenario (Figure 1A), responses of all taxa are simple functions of song similarity. Responses of Species 1 and Species 2 are symmetrical, and show equal, but opposite rates and magnitudes of response (i.e., equal response slopes and equal ranges of response magnitude) as song types change from Species 1 to Species 2 on the x-axis. The second and third scenarios (Figures 1B and 1C) illustrate how two different types of behavioral asymmetry could affect responses based on song similarity. In the second scenario (Figure 1B), Species 2 exhibits an inherently higher level of discrimination among song types. Thus, the range of the magnitude of responses is equal between the parentals, but the change in magnitude occurs over a smaller range of song scores in Species 2 (i.e., an equal range of response values but a steeper slope). Greater discrimination among song types in one taxon could cause asymmetrical assortative mating and a reduction in hybridization. In the third scenario (Figure 1C), Species 2 shows rates of change in response equal to those of Species 1 (i.e., equal slopes), but a lower maximum magnitude of response (arbitrarily chosen to be 0.6). As a result, as in Scenario 2, responses in Species 2 decline over a shorter range of song distances than in Species 1, but in this case, although it could appear as if Species 2 were more discriminating than Species 1, this would be a result of a lower inherent level of behavioral response. If Species 2 were more discriminating as a result, this could result in some level of assortative mating, but depending on the type of response represented on the y-axis, the behavioral differences that underlie these differing magnitudes of response could result in competitive asymmetries in interspecific encounters between competing males that could modify or eclipse responses based on song type.

Hybrids behave somewhat differently in these scenarios because they cannot encounter the same level of song dissimilarity as parentals. As a result, they never reach magnitudes as low as the lowest parental responses. In the first scenario, hybrids show the same maximum magnitude of response as the parentals, and maintain a relatively high level of response to the entire range of song types. In the second scenario, hybrids have a level of discrimination intermediate between the parentals (intermediate slopes), so that they show steeper rates of decline in response per unit of song distance. As a result, hybrids reach lower minimum response values than in Scenario 1. In the third scenario, the maximum magnitude of response in hybrids is intermediate between the parentals. As a result the lowest magnitude of response is shifted downward, but the rate of decline is identical to Scenario 1. Preference for intermediate song types by hybrids would imply assortative mating due to the superior fitness of hybrids in the contact zone (Moore and Price 1993), a situation that has not been confirmed in most empirical studies of avian hybrid zones. More often, studies show a lack of preference among song types in admixed individuals in contact zones (Price 2008). This could result from a lack of assortative mating or the historical need of individuals in a contact zone to defend territories against other admixed individuals, and members of both parental species (Price 2008). In this case, hybrids would show a flat trend in each of these scenarios (not shown in Figure 1), with the magnitude of response shifted to a position intermediate between the parentals in Figure 1C. In either scenario, due to the relatively high response levels maintained across song types, hybrids could function as conduits for gene flow, but introgression is likely to depend more on the responses of parental populations outside of the area of sympatry to hybrid or “admixed” song types, than on discrimination among song types by admixed birds.

Two North American suboscine sister species, Pacific-slope Flycatcher (*Empidonax difficilis difficilis*) and Cordilleran Flycatcher (*E. occidentalis hellmayri*), provide an interesting test case for the role of song divergence in species formation. Based on mitochondrial distance, Pacific-slope Flycatcher and Cordilleran Flycatcher diverged approximately 350,000 years ago (Johnson and Cicero 2002). Together, these species are distributed across much of western North America (Johnson 1980, Lowther 2000). Geographic contact and at least some level of gene flow occurs in the interior Pacific Northwest (Johnson 1994, Rush et al. 2009). Pacific-slope and Cordilleran Flycatchers have clearly homologous but distinct songs (Johnson 1980). By contrast, they show only slight differences in morphology and plumage coloration, and cannot be assigned to taxon based on appearance alone. Songs are innate in these species (Kroodsma 1984, 1985, Kroodsma and Konishi 1991), and are used in mate attraction (Davis et al. 1963, Ainsley 1989). Johnson (1980, 1994) acknowledged a low level of hybridization between these species, but maintained that the songs of the two species remained distinct in sympatry, and functioned as cues for assortative mating. Despite Johnson’s claim, other sources describe the existence of populations with intermediate song types (Campbell et al. 1997, Marshall et al. 2003, Wahl et al. 2005). The most recent genetic analysis focused on a relatively restricted area of contact in the interior Pacific Northwest, and showed that while allopatric parental populations remained distinct, the majority of birds in the area of contact were admixed in their nuclear DNA, but had Pacific-slope mtDNA (Chapter 1, Rush et al. 2009).

The presence of suboscine sister taxa with diagnostic differences in innate song, very little plumage variation, and current geographic contact and gene flow, presents a situation in which the strength of innate song as a behavioral isolating mechanism has been put to the test. Because reproductive isolation between these taxa is incomplete, it is possible to determine whether divergent behaviors are restricting or modifying *current* gene flow, and thus to infer

their importance in the speciation process. Here, I integrate extensive examinations of genetic variation and song variation with experimental tests of behavioral isolation to assess whether differences in innate song between Pacific-slope and Cordilleran Flycatchers function as behavioral barriers to gene flow. I examine (1) the pattern and extent of genetic admixture and gene flow, (2) the geographic distribution of song types, (3) the correlation between song type and genotype, (4) the effects of genetic admixture on song, and (5) the behavioral responses to a range of parental and admixed song types in song playback experiments. Performing song playback experiments in subsocial taxa in which reproductive isolation is incomplete allowed me to investigate how the continuous variation in song characters, representing different stages of song divergence and admixture, affects song recognition (and ultimately species recognition) in parental taxa and in genetically admixed populations. In addition, song playback experiments can illustrate the competitive ability of male parentals (e.g., Pearson and Rohwer 2000) and hybrids (e.g., den Hartog et al. 2007) inside and outside of the contact zone, and thus are useful for revealing competitive asymmetries. Both the genetic and bioacoustic analyses include samples from throughout the 25-degree latitudinal range of these taxa. To my knowledge, this study is the most comprehensive investigation to date, in terms of sampling and geographic scope, of the effects of divergence in innate song on species formation.

There are over 1000 subsocial species, making up approximately 1/8 of extant bird species and approximately 1/3 of Neotropical bird species (Chesser 2004). A better understanding of how divergence in innate song can affect reproductive isolation and gene flow is important to understanding the amazing diversity of Neotropical birds (Ricklefs 2001, Tobias et al. 2012). Moreover, the lack of a learned component in subsocial song makes the effects on the speciation process more comparable to the acoustic signals of non-avian animals that have been studied in this context (e.g., *Schizocosa* spiders, Elias et al. 2010; *Laupala* crickets, Mendelson & Shaw 2002; Tungara frogs, Ryan & Rand 1993, *Microcebus* mouse lemurs, Braune et al. 2008). Thus, this study not only provides a better understanding of the effects of divergent acoustic signals in birds, but also provides important insight into the effects of divergent acoustic signals as behavioral barriers to gene flow applicable to a wide range of animal species.

METHODS

Study area.

Pacific-slope Flycatchers (*Empidonax difficilis*) and Cordilleran Flycatchers (*E. occidentalis*) are each comprised of multiple allopatric subspecies (Johnson 1980). In this study, I focus on the principal subspecies of the Pacific-slope Flycatcher (*E. difficilis difficilis*) and the northern subspecies of the Cordilleran Flycatcher (*E. occidentalis hellmayri*). Together, these taxa are distributed across much of temperate western North America north of Mexico (Figure 2), with known areas of secondary contact (Johnson 1980, Rush et al. 2009). The Pacific-slope Flycatcher occurs west of the crest of the mountain ranges that comprise the Pacific Slope (i.e., the Sierra Nevada, Cascade, and Coast Ranges), extending from northern Baja California to southwestern Alaska (hereafter the “Pacific Crest”). The Cordilleran Flycatcher is usually described as occurring in the interior western mountain ranges, from the United States–Mexican border to the northern Rocky Mountains. Sources differ regarding which taxon occupies the interior Pacific Northwest (e.g., Johnson 1980, Campbell et al. 1997, Lowther 2000, Marshall et al. 2003, Wahl et al. 2005, Dunn and Alderfer 2011, Sibley 2014), and the high frequency of

genetically admixed individuals in this region (Rush et al. 2009) makes this determination more difficult. Sampling took place at 88 sites, and spanned 28° in latitude (29° to 57°) and 32° of longitude (-135° to -103°) (Table 1). This included 18 Cordilleran sites, 30 Pacific-slope sites, and 42 sites of uncertain taxonomic status.

Genetic data collection & analysis

I collected either blood or tissue samples from breeding populations at 46 sample sites. Individuals displaying traits indicative of migrants (high levels of subcutaneous fat and the absence of enlarged gonads) were excluded from the study. Blood samples were taken from 41 birds that were caught in mist nets and subsequently released. Details on the sampling and preservation of blood are given in Chapter 1. I collected and took tissue samples (liver, kidney, or muscle) from 373 voucher specimens that are now housed in either the Museum of Vertebrate Zoology (MVZ) at the University of California Berkeley. In addition, I used 175 tissue samples from the MVZ tissue collection (9 sites), 149 tissue samples from the Burke Museum at the University of Washington (19 sites), and 6 tissue samples from the Royal Alberta Museum collection (1 site). See Chapter 1 for a more detailed explanation of the genetic extraction, amplification, and mtDNA sequencing protocol.

I examined two different types of genetic marker to establish the pattern of gene flow between Pacific-slope and Cordilleran Flycatchers. I did not analyze both types of genetic data for identical sets of sites (Table 1). I used the ND2 gene to examine variation in the frequency of mtDNA haplotypes among sites. I classified 608 individuals as belonging to either a Cordilleran or Pacific-slope haplotype group based on 6 nucleotide sites that show fixed differences between these taxa (Table A1). Because I had determined previously that these nucleotide substitutions were neutral (Chapter 1), I did not test for selection here.

I generated a large SNP database to better understand patterns of nuclear genetic variation between the Pacific-slope and Cordilleran Flycatchers. To accomplish this, I generated genome sequence data using a reduced representation approach from four unrelated individuals from each population. To accomplish this, I pooled equal amounts of extracted DNA from each of the individuals into a single sample, which was then digested with restriction enzymes following the protocol established by Groenen et al (2009). The doubly digested DNA was then prepared for sequencing using the standard Illumina sequencing preparation. Sequencing was accomplished using two lanes of 101bp paired-end data generated on an Illumina GAIIx genome sequencer operated by the Vincent Coates Genome Sequencing Center at the University of California, Berkeley.

The resultant sequence data was preprocessed as follows. Contaminating adapter sequence as well as nucleotides whose quality scores fell below a threshold of Phred < 20 were removed from the dataset using Trimmomatic (Bolger et al 2014). The remaining data were assembled using the *de novo* genome assembler ABySS (Simpson et al 2009) using default settings and a kmer of 35. Contigs whose length were less than 400 nucleotides were removed from the dataset.

SNPs were identified from the sequence data using the software package PoPoolation (Kofler et al 2011) using the following command (`perl ~/popoolation/Variance-sliding.pl --input fly.mpileup --snp-output fly.snp --output fly.out --measure pi --pool-size 16 --window-size 4000 --min-coverage 10 --min-count 5 --fastq-type sanger`). This software takes as input an mpileup file, which was generated with the Bowtie2 software package using the “very-sensitive-local” setting and samtools mpileup (Li 2011) generated here (`mpileup -C50 -q10 -Q20 -M300 -I -d`

8000 -guf snp.fa /media/hd/fly.assemb/bowtie/23Apr12.fly.bam | bcftools view -bvcb - > 24Apr12.fly.raw.bcf and bcftools view 24Apr12.fly.raw.bcf | vcfutils.pl varFilter -d20 -Q20 -w300 > 24Apr12.fly.final.bcf). The raw output was used to select SNPs with minor allele frequencies estimated to be between 40%–50% (24867 SNPs).

To enhance the genome wide coverage of the SNPs, I used blastN to locate the physical position of SNP-containing contigs in the nuclear genome of the zebra finch. This resulted in the placement on chromosomes of 16397 contigs (2084 on chr3 to 8 on chr25). Approximately 10 SNPs per chromosome (250 SNPs total; Table A2) were then randomly selected for inclusion in the Sequenom SNP array. Of the 250 SNPs, 233 performed well enough in a University of Minnesota Biomedical Genomics Center (UMBGC) Sequenom iPLEX design to be included in genotyping. Because I was able to use two runs of samples for two Illumina sequencing lanes for the SNP genotyping, I was able to genotype a maximum of 752 individuals for a maximum of ~80 SNPs (depending on the success of the reactions). SNPs were chosen by the UMBGC to be located across multiple chromosomes and to include ~25% from the Z-chromosome. SNPs were scored using the Sequenom platform by the UMBGC. Sixty-seven SNPs were scored well enough across 713 individuals to be used in subsequent analyses (Table A3). Ten individuals (4 from Cordilleran, 2 from admixed, and 4 from Pacific-slope populations) were genotyped twice to act as positive controls. Population assignment scores (see below) based on the SNP data from the two sequencing runs were nearly identical for these ten individuals ($y = 0.996x + 0.001$, $R^2 = 0.999$). I excluded three individuals with >25% missing nucleotide data from further analyses, but retained 6 samples with 17%–25% missing data and 10 samples with 10%–13% missing data.

I used the program Structure V2.3.4 (Pritchard et al. 2000, Falush et al. 2007) to assign individuals to populations based on the genotypes at the 67 nuclear SNPs. See Chapter 1 for a more detailed explanation of the general Structure methodology. I performed 10 independent runs of 1,000,000 MCMC repetitions with a burn-in period of 1,000,000 for each of $k = 1$ through $k = 5$ assuming correlated allele frequencies, a population admixture model, and no loc prior. After finding that $k = 2$ had the highest likelihood value (see Results), I calculated the mean value of q (specifically, the proportion of Pacific-slope ancestry) over the 10 runs to use as the genotype for individuals in subsequent analyses (hereafter, “genotype”). Eleven individuals were excluded from the final analysis because Structure could not assign them unambiguously to a population (each individual was assigned with high probability to either Pacific-slope or Cordilleran clusters on alternating runs). Two of these samples came from Pacific-slope sites, while the remaining came from various admixed sites. 699 genotyped individuals remained for subsequent analyses. I also used Structure to calculate q for 63 pre-defined populations with > 2 individuals (hereafter, “population genotype”). I chose a cutoff of ≥ 0.80 to classify individuals and sites as “pure” Pacific-slope and a cutoff of ≤ 0.2 to classify individuals and sites as “pure” Cordilleran. Although I realize that there is no entirely objective way to choose cutoff points to classify individuals as “pure” parentals rather than as genetically admixed (especially because I did not find any SNPs with fixed differences between parental populations), I feel that these cutoffs are reasonable based on the range of individual q values in core parental populations least affected by introgression (Pacific-slope range: $q = 0.70$ – 0.96 ; Cordilleran range: $q = 0.04$ – 0.26).

I performed linear regression to compare the frequency of ND2 haplotypes and the population genotype score from nuclear SNPs. I used the relationship between parental populations as a reference value against which to compare admixed sites. This approach is

analogous to using a hybrid index in which populations from a contact zone are compared to parental reference populations.

Song data collection & analysis

I analyzed songs from 608 individuals from 68 sites (57 sites with songs from >2 individuals, mean = 8.9 individuals per site; Table 1, Table A4). I recorded the majority of songs (417 individuals), but supplemented my recordings by including songs from sound archives (MVZ, Macaulay Library of Natural Sounds, and the Borror Laboratory of Bioacoustics) and from individual recordists (especially 106 individuals from D. Archibald McCallum; see Acknowledgements). I recorded songs digitally in linear PCM format on Marantz PMD660 or PMD671 digital recorders, at 48 kHz, using Audiotechnica AT135b and Sennheiser ME-66/K6 shotgun microphones without a parabola. Archival recordings used in the study were recorded on various analogue platforms and digitized at sample rates of 96 kHz (MVZ and Macaulay Library), or 50 kHz (Borror Laboratory). Recordings from individual recordists were made on various analogue or digital devices, and provided in various digital formats.

All sounds used for measurement were extracted from their source format with *Signal* (Engineering Design, Berkeley, CA) sound analysis software. Song samples with the highest signal-to-noise ratio (SNR) and minimal overprint of ambient sound were saved for measurement as distinct *Signal* files (16-bit, 50 kHz sample rate). To standardize resolution of subsequent measurements, the beginning and end of each *Signal*™ file were chosen to yield a 500-msec segment, with the target sound centered in this interval. The 500-msec sound segments were visualized using the fast-fourier transform (FFT) implemented in *Signal* software, version 4.x, with 512 points per spectrographic slice. This FFT-length yields frequency resolution of 43.1 Hz and time resolution of 23.2 msec. All spectrograms had a frequency range of 2000 to 9000 Hz and were plotted in a 1000 x 600 pixel window for measurement. This window had much higher resolution, at 11.67 Hz and 0.5 msec, than the spectrograms themselves, so choice of measurement platform was inconsequential.

The dawn-singing performances of these taxa are combinations of three distinct elements (Figure 3). Previous researchers (e.g., Johnson 1980) have considered a typical sequence in which these three elements are delivered as comprising a single song. I acknowledge that there is a typical order in which these elements are delivered, but this order is not fixed or inflexible. As a result, I treat these as three distinct song types (Song 1, Song 2, and Song 3) that are often delivered in a repetitive 1–2–3 order. Song 2 is the most structurally complex song type, and was more variable within individuals than the other songs types. For that reason, I measured three examples of Song 2 per individual. I included individuals for which I had fewer than three examples of Song 2 in the analysis, but this affected only a small proportion of the samples. All song measurements were performed using custom scripts in *Signal*. Because the songs of these taxa are innate, I was able to utilize a landmark-based approach that relied on the identification of homologous points that existed on song spectrograms across taxa. This allowed me to measure fine scale continuous change in the spectral characteristics of songs across taxa, rather than relying on discreet or categorical differences in songs. This allowed a better estimation of changes in the spectral characteristics of songs – i.e., the type of microevolutionary changes that would be expected between closely related taxa. Landmarks were determined based on the start and end points of song spectrograms and on internal inflection points (Figure 3). I used the means of the raw measurements to create the variables included in multivariate analysis (Figure 3, Table 2). I used the following variables in the analysis: peak frequency, duration (ms), the

sharpness of the frequency peak (i.e., the absolute value of the slope from the frequency at peak frequency minus 10 ms to peak frequency + the slope from the peak frequency to the frequency at peak frequency plus 10 ms), the proportion of the entire song (ms) comprised of the second half of the song (i.e., after the lowest inflection point), the change in frequency from the peak frequency to the frequency at the lowest inflection point, and the presence or absence of an amplitude gap at the lowest inflection point. I used Principal Components Analysis (PCA) to summarize variation in song characters across taxa.

Although song type is assumed to be closely correlated with genotype in suboscines, this correlation has rarely been demonstrated empirically. I analyzed the relationship between song type and genotype by performing linear regression of combined song PC1 score as a function of genotype for (1) the 66 individuals and for (2) the 50 sample sites (using population genotype and the mean song PC1 score for the site) for which I had both data types. Making this comparison at the level of the individual was the most direct and unambiguous way of testing the correlation between song type and genotype, but restricted me to a smaller subset of the data. Making this comparison at the level of the sample site allowed me to use genetic and song data from hundreds of additional individuals and allowed me to better represent core Cordilleran sites (for which I had fewer individuals with matched song and genetic data). As with the comparison of mtDNA frequencies and SNP scores, I used the relationship between the parental populations as a reference value against which to compare the admixed sites.

Song playback experiments.

Song playback experiments were designed to test the responses of males to parental and admixed song types across a range of genetic populations, to determine if song divergence or song admixture were causing some form of behavioral isolation. It was not feasible to conduct field tests of female preferences for different song types. Females of these taxa are inconspicuous, and responses to playback are often subtle (e.g., delivering quiet call notes from a distance). Instead, I used song playback experiments to test the reactions of males to simulated territorial intrusions by males with a range of conspecific and divergent song types. Aggressive responses in field experiments have been shown to be highly correlated with aggressive behavior in actual male-male encounters (Rohwer 1982), and this approach has been used frequently to assess the level of behavioral isolation between incipient bird species (e.g., Stein 1963, Ratcliffe and Grant 1985, Irwin et al. 2001, den Hartog et al. 2007, Seddon and Tobias 2007, Uy et al. 2009). In many passerine species, the same song type can be used in both mate attraction and territory and mate defense (Baker and Baker 1990, Searcy et al. 1997, Collins 2004, Patten et al. 2004). So, even if I did not assess female choice directly, male-male competition based on song has a direct link to mate attraction and reproduction, and males are likely to be able to distinguish potential competitors from non-competitors.

I performed 184 song playback (hereafter, "PB") experiments at 21 sites: 49 in Pacific-slope (5 sites), 67 in Cordilleran (6 sites), and 68 in admixed populations (10 sites) (Figure 2, Table 1). I chose sites to represent the three taxa based in part on existing research (e.g., Johnson 1980, 1994, Johnson and Marten 1988, Rush et al 2009), but also relied on the song scores and genetic data (population genotype) that became available as the project progressed (see Results). I collected no genetic data for two Pacific-slope PB sites that are unambiguous in their taxon affiliation (both in the San Francisco Bay Area). These sites are located clearly within the core range of Pacific-slope and the song scores of these sites fall within the range of parental scores. Bordering sites (Monterey and Lake Cos., CA) had Pacific-slope population genotypes (0.86 and

0.90 respectively; see Results). Although it was not possible to genotype the focal individual of every experiment, 38 focal individuals were genotyped. Using these, I can show a strong relationship between the population genotype and the individual genotypes of the focal individuals from those sites ($y = 1.1226x - 0.0232$, $R^2 = 0.8102$). I used this strong correlation to justify using population genotype in the analyses of playback responses.

I employed a repeated measures design in which I tested each focal individual with three song stimulus types: Cordilleran, Pacific-slope, and songs from genetically admixed sites with PC1 scores intermediate between Cordilleran and Pacific-slope. All song stimuli were scored in the song analysis. I used the distribution of 90% of the song scores for each parental taxon to choose the upper bound of Cordilleran stimulus songs and the lower bound of the Pacific-slope stimulus scores (Table 3). For intermediate stimuli, I attempted to choose songs of individuals that scored close to the midpoint of the songs that scored intermediate to the parental taxa. Selecting song recordings of high enough quality to be used in experiments reduced the number of recordings from which I could choose. As a result, admixed stimuli, although clustered around the midpoint, represented much of the range of scores between the parentals (Table 3). One stimulus (from Monterey, an unambiguous Pacific-slope site) that was used as a Pacific-slope stimulus in 5 trials falls just outside of the 90% Pacific-slope range. I used 31 unique Pacific-slope stimuli, 16 unique Cordilleran stimuli, and 26 unique admixed stimuli. Song stimulus tracks were created using the highest quality portions of the recordings of a particular individual. If the portion of the recording that was suitable for playback was < 60 seconds in length, I repeated the high quality portion of the song until the stimulus track was 60 seconds in length. The amplitude (volume) of all stimulus song tracks was standardized using the program *Audacity* (Audacity Team, 1999–2012).

Experiments consisted of three trials. A trial consisted of a 1-minute control period in which baseline behavioral observations were made of the focal individual, a 1-minute song playback period, and a 1-minute post-playback period during which I continued to make observations. Songs were broadcast using an mp3 player attached to either an SME-AFS portable field speaker or an Anchor Audio MiniVox speaker mounted on a tripod at a volume level similar to natural song. In the majority of trials (83%), the speaker was placed 15–30 m from the focal individual (median = 20 m). I allowed five minutes between trials for birds to return to their baseline activity levels. This was based on a pilot study in which I observed that the overwhelming majority of individuals returned to normal activity 1–2 minutes after playback ceased. In each *trial*, the focal individual was presented with one of the three song types. The order in which the stimulus types were presented and the choice of the particular exemplar song were both randomized across experiments. I measured the following response variables: starting distance from the sound source, approach (distance traveled toward the sound source), and number of songs sung. I made audio recordings of playback experiments and response data was extracted from these recordings.

Because song responses occurred in only a subset of experiments, I analyzed approach and song responses differently. Moreover, these are distinct behaviors, so analyzing them separately provided an opportunity to detect any differences in how taxa utilized each response type. For approach responses, I calculated “relative approach”, or the proportion of the distance between the individual and the sound source before playback commenced that the individual traveled during playback (hereafter, “approach”). This allowed me to correct for differences in approach response due to individuals simply starting at different distances from the stimulus. To measure song responses, I calculated “song persistence”, or the degree to which a focal

individual maintained its original singing rate in the presence of song playback (i.e., number of songs during playback \div number of songs during control). Because singing did not occur in all experiments (especially in Cordilleran populations, where individuals typically sang only in the pre-dawn) I analyzed song responses only from the individual trials in which singing occurred. This included 38 trials in Cordilleran populations, 63 trials in intermediate populations, and 100 trials in Pacific-slope populations. I compared only the control and playback periods for these trials, because song behavior from the post-playback period can be difficult to interpret (i.e., singing behavior from the post-playback period could represent a continued response to song playback or the resumption of pre-playback singing behavior). Song persistence ratio values indicate the following responses: > 1 = increased rate of singing, 1 = no change in rate of singing, < 1 = decreased rate of singing, and 0 = cessation of singing. If song number was 0 during the control period and > 0 during playback, the song persistence ratio could not be calculated due a 0 in the denominator. I excluded 4 trials for this reason.

I first analyzed responses using a Generalized Linear Mixed Model (GLMM), to test whether taxa differed in their approach responses to different categories of song stimulus. I used approach as the response variable in order to be able to utilize the entire playback dataset. Besides testing for differences in responses to different song types, this approach allowed me to validate our experimental design by determining whether song playback had an effect on the behavior of focal individuals (i.e., whether behavior in the playback period differed from the control period), and whether stimulus order, exposure to multiple stimuli in individuals, or calendar day on which the experiment took place affected responses. I specified the individual PB sites and individual experiment as random effects. I logit-transformed the proportion data (approach). The model was specified with taxon, stimulus type, and an interaction between taxon and stimulus type as fixed effects. Prior to fitting models with these fixed effects, I also eliminated the random effects of "previous stimulus" (stimulus order effect) and "ordinal day" (calendar day on which the experiment occurred) since they accounted for none of the variation. I used Akaike's Information Criterion corrected for small sample sizes (AIC_c) values to determine which model best explained approach responses. I gauged relative support for each candidate model by first ordering models by increasing AIC_c (lowest AIC_c is the best supported model) and calculating the change in AIC_c (ΔAIC_c) relative to the best supported model. Models with $\Delta AIC_c \leq 2$ were considered to be the models with the greatest amount of support (Burnham and Anderson, 2002). I used *post hoc* tests to determine which responses were significantly different from one another. These analyses were carried out using the *lme4* package in the R statistical platform (R Core Development Team 2011).

To examine whether different genotypes differed in general response behavior, I performed linear regression of both approach and song responses across all genotypes for all trials combined (i.e., responses of all focal individuals to all song stimuli). This analysis included up to three responses (to three stimulus types) per individual (Pacific-slope: $n = 147$ trials; Cordilleran $n = 204$ trials; admixed: $n = 207$ trials). I used this broad scale approach to illustrate whether, irrespective of the level of similarity between the song of the focal individual and the song stimulus, certain responses or certain magnitudes of response were more likely to occur in individuals with particular genotypes. In addition, I performed linear regression of *song* response as a function of genotype for each stimulus type to determine if the pattern elucidated using the broad scale approach above masked response differences between taxa to particular song types (*approach* responses of taxa to particular song types were analyzed using the GLMM).

Behavioral isolation could occur if individuals in each taxon responded most to songs that

sites were located relatively close to interior admixed sites (Figure 4). By contrast, both haplotypes are present at sites across much of the western United States and Canada east of the crest of the Pacific-slope, including 5/10 Cordilleran sites (9/95 haplotypes = 9.5%), with a general pattern of decreasing frequency of Pacific-slope haplotypes from northwest to southwest. Taken as a whole, admixed sites had a much higher frequency of Pacific-slope haplotypes (274/371 individuals = 73.9%). A geographic break between sites that have predominantly Pacific-slope haplotypes and sites that have predominantly Cordilleran haplotypes occurs along the approximate northern boundary of the Great Basin, from northeastern Nevada, through southern Idaho and Montana, to the Black Hills in South Dakota. Thus, a significant part of what is currently considered the range of the Cordilleran Flycatcher has predominantly Pacific-slope or a mixture of Pacific-slope and Cordilleran mtDNA.

Nuclear DNA. Output from the Illumina GAIIX resulted in 197,286,548 101np paired end reads. These reads, which were reduced in number by approximately 10% after quality trimming were assembled into 93,987 contigs greater than 400 nt in length (400nt-44415nt, N50=1949).

None of the SNPs that I analyzed showed fixed differences between Pacific-slope and Cordilleran Flycatchers. Nevertheless, frequency differences in the occurrence of alleles allowed me to differentiate between Pacific-slope and Cordilleran Flycatchers in the *Structure* analysis (highest likelihood for $k = 2$; estimated Ln probability of data = -53726.8). As in the distribution of mtDNA haplotypes, genotype scores indicate little introgression evident from interior to coastal populations (Figure 5). The range in q values among the 165 individuals from pure Pacific-slope sites (i.e., *sites* for which $q \geq 0.80$) was 0.52–0.96 (mean = 0.87), and 19 individuals (12%) from 8 sites had genotypes that could be classified as introgressed ($q < 0.8$). The range in q values among the 125 individuals from pure Cordilleran sites (i.e., *sites* for which $q \leq 0.20$) was 0.04–0.38 (mean = 0.13), and 17 individuals (14%) from 8 sites had genotypes that could be classified as introgressed ($q > 0.2$). I was able to compare population genotypes between sampling periods ~30 years apart for seven sites (three Pacific-slope, three admixed, and one Cordilleran). The scores were very highly correlated, showing no significant difference in genetic makeup across eras ($y = 1.04x - 0.023$, $R^2 = 0.99$). Importantly, these include sites that Johnson (1980, 1994, Johnson and Marten 1988) used to establish the presence of reproductive isolation between these taxa (i.e., his northern California transect through the Siskiyou contact zone), and shows that the differences in the findings between his study and the current study are not due to population changes that have taken place in the intervening time.

Similar to the distribution of ND2 haplotypes, sites admixed in nuclear DNA span a broad geographic area from the east slope of the Pacific Crest to central Nevada, central Utah, and northern Colorado, well within what is currently considered the range of Cordilleran Flycatchers. No site east of the Pacific Crest scored >0.70 Pacific-slope. Most sites located on the immediate east slope of the Pacific Crest had similar population genotype scores (for 5/6 sites $q = 0.64$ – 0.70 ; for Deschutes in central Oregon $q = 0.48$), and 22% of individuals (15/69) from these 6 sites had Pacific-slope genotypes ($q \geq 0.8$). Among admixed sites, there is a general northwest to southwest cline from more Pacific-slope sites in the interior Pacific Northwest to more Cordilleran sites in the Great Basin and central Rocky Mountains. The range of individual genotype scores from admixed sites was 0.05–0.95. Thus, individuals that could be classified as pure parentals exist at admixed sites, although, as expected, these individuals tend to occur at the margins of the admixed zone, closest to their respective parental populations (this is especially true for Pacific-slope individuals). There is almost no evidence of current sympatry between

individuals with parental genotypes; one site (Coeur d'Alene) near the center of the admixed sites had one individual with $q = 0.16$ and another individual with $q = 0.8$.

I found that the discordance in the geographic distribution of nuclear and mitochondrial DNA reported in Chapter 1 occurs over a broader area. Based on the relationship between the parental sites, many genetically admixed sites, especially those in the interior Pacific Northwest, have a much higher frequency of Pacific-slope ND2 haplotypes than predicted by the nuclear population genotype (Figure 6).

Song analysis.

The first two PC axes of song variation had eigenvalues > 1 (3.56 and 1.03 respectively) and explained 51% and 15% of the variation respectively. PC1 separated individuals from parental populations into two, largely non-overlapping, clusters (Figure 7), and was composed mainly of peak frequency, sharpness of the frequency peak, and the proportion of the song comprised of the second half of the song (Table 2). PC1 was used in all subsequent analyses as “song score.” PC2 described only intra-group variation, and was composed mainly of the frequency change from the peak to the lowest inflection point, and the presence or absence of an amplitude gap at the lowest inflection point. Thus, the difference between the songs of the parental taxa is due to both frequency differences and spectral characteristics that affect spectrogram “shape”. Admixed birds are intermediate in all of these characters, and when included in the PC plot, overlapped with and filled the gap between the parental clusters (not shown, but see Figure 8). I did not find a stereotypical intermediate song – i.e., songs can have a variety of spectral profiles for a similar intermediate PC score. I found no differences in the composition of the song repertoire between the parental taxa or in admixed individuals; the same three song types were present in all and the typical sequence of dawn song delivery was maintained in all taxa

I found a very strong relationship between song type and genotype, both at the individual and population levels. Based on 66 individuals for which I have both song scores and genotype, song score is highly correlated with nuclear genotype ($R^2 = 0.68$, Figure 6). The correlation is even greater at the level of 50 sample sites for which I have both data types ($R^2 = 0.93$, Figure 8). Song score was also highly correlated with the frequency of Pacific-slope haplotypes per site ($R^2 = 0.78$), but not as highly correlated as with population nuclear genotype.

The geographical pattern in song variation is largely congruent with the genetic patterns. There is little evidence of introgression of Cordilleran song characters into Pacific-slope (i.e., of individuals with song PC1 scores lower than the main distribution of Pacific-slope song scores) beyond a small number of sites along the west slope of the Pacific-slope, but fairly extensive introgression of Pacific-slope song characters into interior populations (Figure 9). Intra-site variation in song PC1 scores was greatest among Cordilleran sites (SD per site range: 0.24–0.88; median SD = 0.70) and lower among admixed sites (SD per site range: 0.36–0.76; median SD = 0.56) and Pacific-slope sites (SD per site range: 0.25–0.73; median SD = 0.51). It is notable that the level of intra-site variation among admixed populations is not particularly high compared to parental sites. This reflects the relative uniformity of song types and the lack of sympatry of among individuals with divergent song types at most admixed sites. A transition to sites with more purely Cordilleran song occurs along a similar line along the northern edge of the Great Basin and the central Rocky Mountains as the genetic transitions. I found no evidence of geographic variation in song that does not seem to be related to genetic admixture. For example, despite the presence of a fairly dramatic north–south environmental gradient, I found no evidence of a north–south cline in song along the $\sim 25^\circ$ latitude Pacific-slope Flycatcher range.

Evidence for clinal change in song within Cordilleran populations is harder to detect because introgression occurs at the northern limit of the Cordilleran range.

Playback experiment analysis.

Responses by taxon or genotype

Results of the GLMM showed that playback had an effect on the behavior of focal individuals and ruled out any effects of repeated playbacks on the same individual, the calendar day on which the experiment occurred, or the order in which stimuli were presented. The model with the lowest AIC value was: *taxon * playback period * stimulus type* (Table B1). Thus, the taxon affiliation of the focal individual and the song type that it was presented with determined its approach response. *Post hoc* tests show that Cordilleran individuals approached more to conspecific song and to admixed song than to Pacific-slope song (Table 4, Figure 9). Cordilleran approach to conspecific song was generally greater than to admixed song, but the difference was not quite significant ($p = 0.06$). Cordilleran approach to Pacific-slope song was not different than Pacific-slope approach to Pacific-slope song, illustrating a higher general level of approach response in Cordilleran populations. Pacific-slope individuals approached more to conspecific song than to either of the other song types, and responded more to admixed song than to Cordilleran song. Admixed individuals did not show significant differences in response to any song type. Thus, Pacific-slope individuals seem to discriminate the most among song types, Cordilleran individuals discriminate Pacific-slope song from the other two song types, and admixed individuals do not show any significant discrimination. Pacific-slope approach to conspecific song was not significantly different from the approach of admixed individuals to any stimulus type, indicating that the greatest approach response achieved in Pacific-slope populations is comparable to the approach responses of admixed individuals.

Results of the GLMM show that approach responses by the three taxa differed not only in terms of the song types to which they reacted most, but also in the magnitude of the approach responses (Figure 10). Median Cordilleran approach to conspecific song (0.71) was more than twice that of Pacific-slope (0.31). Median Cordilleran approach to heterospecific song (0.31) was equivalent to the Pacific-slope approach to conspecific song (0.33); median approach in Pacific-slope populations to heterospecific song was 0. Median Cordilleran approach to admixed song (0.68) was nearly equivalent to the approach to conspecific song, and 4 times that of Pacific-slope (0.17). Median approach responses in admixed individuals were similar across stimuli (0.40 to Pacific-slope, 0.47 to admixed, and 0.30 to Cordilleran), and were approximately intermediate between the parentals. The broad scale analysis of approach response as a function of genotype for all trials (i.e., across all song types) combined further illustrated the greater approach response of individuals with more Cordilleran genotypes to playback (Figure 11; $p = 6.22e-12$ ***; $R^2 = 0.07991$), and an intermediate response in admixed populations.

The broad scale analysis of song responses across all trials showed that there is a greater tendency in individuals with more Pacific-slope genotypes to either maintain the same rate of singing or to increase the rate of singing (22% of trials) in response to playback (Figure 11; p -value = 0.018, R -squared: 0.023), indicating that Pacific-slope individuals have a greater tendency to respond vocally to playback. An increase in singing rate never occurred in Cordilleran individuals.

Song responses differed across genotypes in response to Cordilleran song ($p = 0.004$ **, $R^2 = 0.104$) but not in response to intermediate song ($p = 0.260$, $R^2 = 0.004$) or to Pacific-slope song ($p = 0.177$, $R^2 = 0.014$) (Figure 11). As indicated in the broader analysis, the different trends in

song response result from the tendency of individuals with more Cordilleran genotypes to decrease or cease singing in response to all song types, while individuals with more Pacific-slope genotypes tended to cease singing in response to Pacific-slope song, but to maintain or increase singing in response to admixed or Cordilleran song. Singing increased in response to playback in 11% (21/198) of the trials in which singing occurred. No instances of increased singing (in response to any song type) took place in Cordilleran populations, 30% took place in intermediate populations, and 70% took place in Pacific-slope populations. Increased singing occurred in 7% of total trials in intermediate populations and in 22% of total trials in Pacific-slope populations. Notably, the overwhelming majority of instances of increased singing (15/21, 71%) were in response to Cordilleran song, and almost none occurred in response to Pacific-slope song (1/21, 5%). In 79% (11/14) of the trials in Pacific-slope populations in which song persistence occurred, there was no approach (this was true in 3/6 of the trials in which song persistence occurred in admixed populations).

Responses by song similarity

The analyses of approach as a function of song distance are congruent with the analysis of the approaches of individuals with different genotypes as functions of song stimulus category. The best-supported GAM (lowest AICc; Table B2) was the model that included all terms: taxon + song distance + (taxon x song distance). The model results show a decrease in approach response for both Cordilleran and Pacific-slope populations as songs become more dissimilar to the song of the focal population (Figure 13). So, although the analysis of approach response by song stimulus category (GLMM) showed that Cordilleran populations did not approach differently to the conspecific or admixed song categories, using song distance I was able to show that their approach declined to admixed songs that were increasingly dissimilar to conspecific song. Individuals from admixed populations showed no significant difference in approach with song distance. Importantly, the estimates of approach response from linear regression show that the approach responses of the two parental taxa change at the same rate with song distance (Pacific-slope slope = -0.41, $p = 0.001$; Cordilleran slope = 0.41, $p < 0.001$), but that the magnitudes of the approach responses are very different (Pacific-slope y-intercept = 0.34, Cordilleran y-intercept = 0.60).

Song responses as functions of song distance (Figure 14) were very different from approach responses. Both Pacific-slope ($R^2 = 0.28$, $p \ll 0.001$) and Cordilleran ($R^2 = 0.10$, $p \ll 0.03$) individuals tended to cease singing in response to conspecific song (y-intercepts ~ 0 for both taxa), but only Pacific-slope populations increased singing in response to the most dissimilar songs (admixed populations increased singing in response to more Cordilleran song, but the trend was not significant). Pacific-slope individuals showed much higher magnitudes of song persistence and much higher rates of change in response to song distance than Cordilleran individuals (Pacific-slope slope = 1.6, Cordilleran slope = 0.35). Comparison of the Pacific-slope frames in Figure 13 and Figure 14 shows that as approach reaches its lowest values in Pacific-slope populations, song persistence reaches its highest values, indicating that in some proportion of Pacific-slope individuals, a change in response behavior from approach to increased singing occurs at higher levels of song distance. A similar change does not take place in Cordilleran populations.

DISCUSSION

I took advantage of the lack of reproductive isolation, the range of song types and genotypes, and the ability to quantify microevolutionary change in innate song in Pacific-slope and Cordilleran Flycatchers to investigate how song divergence and subsequent admixture affect social recognition and gene flow.

Geographic variation in genes and songs

The presence of largely admixed populations (hybrid swarm) across a large geographic area in the interior Pacific Northwest and northern Rocky Mountains indicates an absence of strong negative selection on individuals with admixed genotypes. A model of neutral diffusion of alleles (Endler 1977) in which genetic differences between populations erode as functions of time since the establishment of secondary contact seems to fit the observed pattern better than models of stable hybrid zones (Barton and Hewitt 1985, Barton and Gale 1993, Harrison 1993). There do not seem to be strong barriers to gene flow from Pacific-slope to Cordilleran populations. On the other hand, although there is evidence that gene flow occurs from Cordilleran to Pacific-slope populations, there is a steep decrease in the presence of Cordilleran associated alleles at the Pacific Crest. The frequency of Cordilleran associated alleles in Pacific-slope populations west of the Pacific Crest remains very low, and Cordilleran associated alleles remain restricted to the margins of the Pacific-slope range. The broad congruence between the geographic patterns of song variation and genetic variation reflects the close correlation between song type and genotype (Figure 6). To my knowledge, this correlation has never been demonstrated so extensively in birds. As in the genetic pattern, I observed extensive phenotypic admixture and apparent introgression of Pacific-slope song characters into interior populations, and a lack of introgression of Cordilleran song characters into Pacific-slope populations.

An alternative explanation to secondary contact with introgression for the observed pattern of nuclear DNA variation is simply incomplete lineage sorting. This scenario could explain the lack of fixed differences in alleles and the presence of Pacific-slope associated nuclear alleles in core Cordilleran populations. While I acknowledge that some level of incomplete lineage sorting is likely in such closely related taxa, I argue that based on genetic, phenotypic, and geological evidence, these patterns are more likely the results of secondary contact following divergence in allopatry. Incomplete lineage sorting would be expected to produce a more stochastic pattern in the distribution of alleles. The broadly concordant clinal transitions between two genetically distinct parental populations in both mitochondrial and nuclear DNA is more consistent with genetic introgression following secondary contact (Coyne and Orr 2004). Furthermore, the discordance that exists between the mitochondrial and nuclear genetic patterns is suggestive of a “mitochondrial wake” that can occur when the range of one of two hybridizing taxa in secondary contact is expanding into the range of the other (Excoffier et al 2009, Toews and Brelsford 2012). Furthermore, intraspecific geographic variation in song (evident, e.g., in the Pacific-slope, Cordilleran, and in the range of admixed song types) is not seen in other *Empidonax* species with similar broad distributions in western North America (e.g., *E. oberholseri*, Sedgwick 1993; *E. hammondi*, Sedgwick 1994), and thus seems unlikely to have evolved in these taxa through drift or selection. Finally, secondary contact zones have been described in this area for several avian and non-avian taxon pairs that were affected in similar ways by the Pleistocene glaciations (Brunsfeld et al. 2001, Johnson and Cicero 2002, Weir and Schluter 2004, Swenson and Howard 2005). Post-Pleistocene reforestation of the areas where

most Pacific-slope–Cordilleran admixed sites occur was relatively recent (estimates are as recent as 2500–1500 years BP; Mack 1978a, b, Pielou 1991). This presents a relatively narrow time range in which populations could have existed and differentiated in interior northwestern North America.

Interpretation of song playback results

The high levels of gene flow and phenotypic introgression evident in interior populations suggest that divergent song types are not functioning as strong behavioral barriers, but the discordance between nuclear and mtDNA at admixed sites and the apparent barriers to gene flow into Pacific-slope populations suggest that divergent behavior related to reproduction could be affecting gene flow. This situation provided an interesting opportunity to use song playback experiments to simulate the range of behavioral interactions between males with different song types and genotypes that likely would have occurred as secondary contact and admixture proceeded between these taxa. The potential to measure microevolutionary changes in the innate songs of Pacific-slope and Cordilleran Flycatchers, and the inclusion of both stimulus songs and focal population songs in the analysis enabled me to employ a unique approach in which I used the exact distance between stimulus songs and the songs of focal populations (“song distance”) to create a continuous measure of song distance over which to examine responses. Comparing responses against a continuous scale of song distance provided an effective way to evaluate evidence for decreasing song salience with increasing song distance, especially with respect to the range of admixed song stimuli.

Approach responses. I found evidence for the effects of song distance and lineage-specific differences in aggressive behavior on responses. Approach responses declined with increasing song distance in both parental taxa, but not in admixed populations. Pacific-slope and Cordilleran Flycatchers showed equivalent rates of decline in approach response to increasingly dissimilar song types (Figure 13), suggesting that the way in which song recognition is affected by song similarity may be symplesiomorphic in these taxa. This symmetrical, gradual decline in approach with decreasing song similarity matches the pattern that I predicted if song similarity allowed taxa to recognize and react to conspecifics. The much higher value for the y-intercept for the response slope in Cordilleran populations, however, indicates that although response patterns in both taxa are affected in very similar ways by song similarity, the two taxa have pronounced differences in the magnitude of approach response. Cordilleran Flycatchers seem to be a more aggressive taxon. In all analyses of approach responses, individuals with more Cordilleran genotypes were more likely than individuals with more Pacific-slope genotypes to approach in response to playback, and the magnitude of their approach responses tended to be greater. Cordilleran populations seem to have a higher baseline for aggressive response behavior as seen in the fact that the lowest approach response in Cordilleran populations is equivalent to the highest approach in Pacific-slope populations (both to Pacific-slope song).

Thus, differences in aggressive response behavior have evolved in these taxa that have had important effects on hybridization and introgression. Because Cordilleran Flycatchers are larger than Pacific-slope Flycatchers (Johnson 1980, Pyle 1997), it is possible that more aggressive behavior is a function of size, rather than an intrinsic behavioral difference *per se*. While, without a more detailed analysis, I cannot rule this out, preliminary analysis of size and approach in admixed populations (i.e., avoiding the correlation between species differences and size differences in a comparison of the parentals) shows no significant correlation between size and approach (unpublished data). Moreover, higher levels of aggressive behavior are not

correlated with larger size in other species in which similar social dominance asymmetries have been observed in contact zones (e.g., *Poecile* chickadees, Bronson et al. 2003; *Setophaga* warblers, Pearson and Rohwer 2000; *Manacus* manakins, Stein and Uy 2006).

Song responses. Song response changed with song distance in all taxa, but important differences in the patterns of change indicate that Pacific-slope populations utilize song as a response behavior in ways that Cordilleran populations do not. The slopes of song response as functions of increasing song dissimilarity were much steeper in Pacific-slope populations indicating a rate of change in song persistence > 4 times greater (slope = 1.56 in Pacific-slope; slope = -0.35 in Cordilleran), although the x- and y-intercepts were very similar (~0 for both in both taxa). This is due to a decline to 0 response in both taxa in response to conspecific song, and an increase in singing in response to heterospecific song in Pacific-slope populations. The broad scale comparison of song responses to all song types combined illustrated that individuals with Pacific-slope genotypes had a greater tendency to increase singing rate in response to more dissimilar playback (Figure 11). Comparisons of song response behavior by stimulus type (Figure 12) and by taxon (Figure 14) confirmed that increased singing is a response to playback exhibited mainly by Pacific-slope populations (it never occurred in Cordilleran populations), and that it occurred specifically as a response to more Cordilleran song types. In admixed populations, although the trend was not significant, the instances in which increased singing occurred were in response to more Cordilleran song types. Thus, there is less variation in song response to conspecific song than there is in approach response, because all taxa tend to cease singing in response to conspecific song (i.e., this is the minimum reaction to conspecific playback). In more aggressive individuals, this is followed by an approach. Those individuals comprise a greater proportion of Cordilleran populations than of Pacific-slope populations.

The differences in song responses are evidence of an additional behavioral asymmetry between these taxa. In my experiments, maintaining a similar rate of song in the presence of playback was generally an indication that the singer was not responding (Searcy and Beecher 2009), and was not usually accompanied by an approach. Interpreting an increase in song rate, however, is not as straightforward. It is possible that in Pacific-slope populations, the presence of a singing male with a very similar song type warrants the full response of the territorial focal male – i.e., cessation of singing accompanied by close approach – but the presence of a singing male with a less similar (though homologous) song type warrants a lesser response – an increase in singing to offer some level of resource defense, but no approach. An increase in the rate of singing is observed in aggressive interactions in a number of passerine species (reviewed in Collins 2004), and may be more expected than other types of aggressive song behavior (such as song switching or song matching) in species with innate song. Moreover, increased song rate may be energetically expensive (reviewed in Read and Weary 1992 and in Collins 2004), and females have been shown to prefer males with higher song rates (Collins 2004). So, by increasing the rate of singing, a male could be both sending a signal of escalating aggression to male rivals and reminding its mate of its own quality (Collins 2004, Searcy and Beecher 2009). Moreover, these taxa may differ in their aggressive response behavior, such that there are a greater number of changes in vocals signals for Pacific-slope males as aggressive encounters escalate toward an actual approach (and attack) (Searcy and Beecher 2009). In any event, the fact that an increase in singing rate in response to heterospecific song never occurred in Cordilleran populations, but occurred in nearly ¼ of trials in Pacific-slope populations (in which singing occurred), is an indication that song is used differently in resource defense by these taxa.

Behavioral explanations for observed patterns in gene flow

The results of these behavioral analyses, in combination with the genetic analysis (especially the mito-nuclear discordance), help to explain the roles of behavioral differences as barriers to gene flow between these species. Non-zero slopes in parental populations in both approach and song responses show that heterospecific song is not perceived as the same as conspecific song in either parental taxon. A continuum of signal salience seems to exist such that males are less stimulated to react as songs become more divergent from conspecific song. Thus, some level of behavioral isolation based on song type seems to exist between these taxa. Importantly, intermediate songs as a whole elicit responses that are equivalent to conspecific song in Cordilleran populations, suggesting a tendency to react to admixed individuals as conspecifics and perhaps a greater potential for backcrossing between Cordilleran and admixed individuals. Pacific-slope individuals approach less to song types other than conspecific song, but from the results it is difficult to determine whether they discriminate more among song types than Cordilleran individuals (Figure 1B), or whether this is a result of inherently lower levels of aggression (Figure 1C). Greater discrimination among song types could help to explain lower levels of gene flow into Pacific-slope populations. Besides the steeper decline in approach response with song distance, two additional lines of evidence suggest that Pacific-slope populations may be more discriminating. First, the unique tendency of Pacific-slope individuals to respond to dissimilar songs with an *increase* in singing suggests that song may be more important in territorial interactions in Pacific-slope populations. Second, the narrower range of song PC1 scores in Pacific-slope vs. Cordilleran populations could be a result of selection due to a narrower preference function in Pacific-slope females.

Proposed scenario for history of secondary contact and introgression

While other scenarios are possible, here I outline a plausible scenario of the history of secondary contact and genetic introgression between these taxa. Lineage-specific differences in aggression level apparent in the approach responses seem to have had a greater effect on genetic introgression than differences in song behavior, at least in contact zone populations. Higher levels of aggressiveness could have facilitated a range expansion by socially dominant Cordilleran males and introgression of Cordilleran nuclear alleles into a former interior Pacific-slope population. Higher aggression levels have been shown to be correlated with a propensity for dispersal and range expansion in *Sialia* bluebirds (Duckworth and Badyaev 2007, Duckworth 2008), and may have had similar effects in Cordilleran males. The high frequency of Pacific-slope mtDNA in admixed populations in the interior Pacific Northwest suggests that these sites may have once represented an interior extension of the Pacific-slope Flycatcher range, or at least that expanding Pacific-slope populations had a larger presence in this region when secondary contact occurred. A number of other forest bird species and subspecies with similar distributions along the Pacific-slope have range extensions into the interior in this region (e.g., Vaux's Swift, Cassin's Vireo, Steller's Jay, Chestnut-backed Chickadee, Varied Thrush, Nashville Warbler; Poole 2005). This region corresponds largely to the disjunct distribution of a mesic cedar-hemlock forest type that is more widespread along the Pacific Coast, and in which several non-avian more coastally distributed organisms have disjunct distributions as well (Brunsfeld et al. 2001). Moreover, some of the avian taxa have Great Basin/Rocky Mountain sister taxa with range limits that correspond closely to the interior step evident in the clinal transition between Pacific-slope and Cordilleran mitochondrial and nuclear DNA along the northern edge of the

Great Basin (e.g., Cassin's–Plumbeous Vireo, Steller's Jay subspecies, Nashville–Virginia's Warbler), suggesting that this could have been a former range limit for Cordilleran populations.

In this scenario, colonizing Cordilleran males encountering the interior Pacific-slope population would have displaced Pacific-slope males and hybridized with Pacific-slope females. Social dominance has been shown to lead to displacement of competing heterospecific males and increased reproductive success for more aggressive males in several studies of avian hybrid zones (e.g., Olson and McDowell 1983, Brodsky et al 1988, Bronson et al. 2003). Moreover, if there is partial assortative mating based on song and greater male aggression is needed to overcome it, the most likely hybrid pairings are of males of the more aggressive taxon with females of the less aggressive taxon (Parker and Partridge 1998). Higher levels of aggression in males are correlated with higher levels of resistance in females in some taxa (although in *Drosophila*; Parker and Partridge 1998), and this also could have reduced hybridization between Pacific-slope males and Cordilleran females. Aggressive hybridization led by Cordilleran males would have led to asymmetric introgression of Cordilleran nuclear alleles into the interior Pacific-slope population and created the discordance between distribution of mtDNA and nuclear DNA now evident at the admixed sites. Hybridizing with Cordilleran males may ultimately have been beneficial to Pacific-slope females if Cordilleran males were able to defend the best territories (especially as there seems to be a lack of negative genetic consequences to hybridization). Thus, behaviors unique to each species that likely evolved in allopatry, perhaps as results of intrasexual competition within each species, do not seem to have had equal effects on the outcome of secondary contact between Pacific-slope and Cordilleran Flycatchers. Higher levels of aggression in Cordilleran populations seem to have “trumped” a greater reliance on song in Pacific-slope populations in territorial contests, at least at some point in the history of secondary contact.

What are the implications of these behavioral asymmetries for core Pacific-slope and Cordilleran populations? Cordilleran populations seem likely to become increasingly introgressed. A pattern of isolation-by-distance is likely to develop in interior populations, with populations farthest from admixed populations remaining phenotypically and genetically Cordilleran for some time. Partial pre-mating isolation based on song in Pacific-slope populations likely helps to decrease gene flow from interior sites. Although more aggressive Cordilleran responses seem to trump the more multi-faceted Pacific-slope responses in territorial interactions, and migrants with Cordilleran-associated alleles successfully interbreed to some extent in Pacific-slope populations, introgression of Cordilleran alleles into core Pacific-slope populations remains very low. This is despite the expectation that alleles associated with the higher aggression levels and social dominance of Cordilleran-type males would introgress at higher rates due to positive selection. It is possible that enough time has not yet elapsed for significant introgression into core Pacific-slope populations to have occurred; admixture at sites on the immediate east slope of the Pacific Crest is still relatively low (mean $q = 0.64$, $SD = 0.18$ for 70 individuals from immediate east slope sites, and for 20/70 individuals $q \geq 0.80$). It is also possible that an aggressive phenotype is not as favorable (and thus less frequent) when populations are no longer at the leading edge of an expanding population (Duckworth and Badyaev 2007, Duckworth 2008). On the other hand, it may be that the social dominance of Cordilleran individuals is mitigated by the demographic dominance of larger and more geographically proximate Pacific-slope populations. Contact zone sites are closer to core Pacific-slope sites, with population sizes that far outnumbered the invading Cordilleran individuals. Moreover, core Pacific-slope populations arrive to the breeding grounds up to two months earlier

than interior populations (Johnson 1973, Sullivan et al 2009), and populations on the east slope of the crest of the Pacific-slope arrive earlier than populations further east in the interior (Sullivan et al. 2009).

Because the difference in migration and breeding times between the coastal and interior populations are likely to favor dispersers from west slope Pacific-slope populations to interior sites, contact zone sites currently may experience more Pacific-slope gene flow. Moreover, the competitive asymmetries that I have hypothesized to have existed in the original encounters between Pacific-slope and Cordilleran Flycatchers may be less pronounced in current encounters between Pacific-slope males and admixed males. Populations across the area of genetic admixture exhibit aggressive responses intermediate between Cordilleran and Pacific-slope populations. Determining whether this is enough of a difference to result in the social dominance of admixed birds over more genetically Pacific-slope birds would require further study. Thus, the original introgression of Cordilleran alleles into interior Pacific-slope populations may be counterbalanced currently by introgression of Pacific-slope alleles into admixed populations. Moreover, less aggressive admixed individuals from contact zone populations that disperse to core Pacific-slope populations may be less likely to displace resident males. More detailed analysis of gene flow between these populations is necessary to understand these patterns with more certainty.

Although I do not focus on this in the present study, niche divergence has been hypothesized to have played an important role in the speciation process in these taxa (Johnson 1980, Johnson and Cicero 2002). Thus, ecological factors could have important effects on the ability of interior alleles to diffuse into Pacific-slope populations as well. First, the Pacific Crest forms an important geological and ecological barrier to individuals that would potentially disperse from interior to coastal populations. In my fieldwork, I never observed Pacific-slope/Cordilleran Flycatchers above approximately 1400 m elevation on the eastern slope of the Pacific Crest (unpublished data). Because the Pacific Crest is substantially higher than 1400 m along most of its extent, dispersal across this barrier due to ecological factors alone would be reduced. Moreover, a congener (Hammond's Flycatcher) occupies the higher elevation habitats (Johnson 1980, ACR personal observation), and because interspecific territoriality can exist between these taxa (Johnson 1966, Beaver and Baldwin 1975), this could create a competitive barrier (in addition, Hammond's Flycatchers arrive approximately two weeks earlier than Pacific-slope/Cordilleran Flycatchers to the interior Pacific Northwest; Sullivan et al. 2009). Second, due to the earlier arrival of migrants west of the crest of the Pacific Crest, dispersers from interior populations to Pacific-slope populations might experience a significant temporal barrier as well. Thus, while song differences alone might not have been sufficient to prevent hybridization between Pacific-slope and Cordilleran populations in the original secondary contact, it is likely that the combination of the ecological barrier presented by the Pacific Crest, temporal differences in migration timing, and lower inherent levels of aggression in admixed individuals act in combination with differences in song to reduce gene flow into Pacific-slope populations. Detailed analyses of migratory timing and habitat use, in combination with the results of the current study, would help to determine which factors have the greatest effects on gene flow between these taxa.

CONCLUSION

In summary, two related, but different types of behavioral traits have diverged in these taxa: (1) song behavior and (2) aggression behavior. Both types of traits have affected the speciation process in these taxa, but in countervailing ways. Song divergence apparently has contributed to *population divergence* by causing some level of assortative mating (especially in Pacific-slope populations). Asymmetries in aggressive behavior apparently have contributed to *population fusion*, by overriding the effects of song divergence and enabling more aggressive and socially dominant Cordilleran males to hybridize with Pacific-slope females. Asymmetrical gene flow due at least in part to behavioral asymmetries may result in asymmetrical species collapse, as Cordilleran populations become increasingly admixed, but Pacific-slope populations remain genetically distinct. Gene flow may reach equilibrium between these two taxa, because, although asymmetries in aggression may favor more Cordilleran males, demographic and temporal factors may favor Pacific-slope males. Thus, evolution of lineage-specific behaviors with important effects on mate choice and territorial defense, even in taxa as closely related as these, can lead to complex evolutionary outcomes that do not fit well with most conceptions of species. This highlights the utility of examining long held conceptions about the role of divergence in behavior in the speciation process by integrating laboratory-based examinations of genetic and phenotypic variation with in-depth field-based examinations of species interactions.

TABLE & FIGURES

Table 1. Locations for genetic data, song data, and song playback experiments (“PB Expts”). The “Taxon” category is based on the proportion of Pacific-slope or Cordilleran ancestry from the Structure analysis of nuclear SNP data (see text). “CO” indicates Cordilleran, “PS” indicates Pacific-slope, and “AD” indicates admixed populations. The “SNP”, “mtDNA”, “Song”, and “PB Expts” columns show the number of individuals per site sampled for each data type. “SNP Score” shows the proportion of Pacific-slope ancestry from the Structure analysis, “% PS mtDNA” shows the proportion of Pacific-slope ND2 haplotypes per site, and “Song Score” shows the mean song PC1 score per site.

Table 2. Factor loadings for PC1 and PC2 from the Principal Components Analysis of song. PC1 has an eigenvalue of 3.56 and explains 51% of the variation in song. PC2 has an eigenvalue of 1.03 and explains an additional 15% of the variation in song.

Table 3. The range of genotype (population genotype) and song (PC1) scores for all sites and for sites in which playback experiments were conducted.

Table 4. Results of post-hoc Z-tests for significance in approach responses modeled in the GLMM. Values below the diagonal show Z-scores for each pairwise comparison. Values above the diagonal show the corresponding p-values. “CO” indicates Cordilleran, “PS” indicates Pacific-slope, and “AD” indicates admixed populations. For each couplet (e.g., “PS-CO”), the first abbreviation is for the focal population and the second abbreviation is for the stimulus that it was responding to.

Figure 1. Three scenarios modeling how song dissimilarity can affect responses in a two-species comparison. Species 1 is represented by a solid black line, Species 2 is represented by a red line, and hybrids are represented by a dashed black line. The x-axis shows song score, with 0 = Species 2 song and 1 = Species 1 song. Three trends are shown in each plot representing different rates of change in (generic) response to song types with varying scores. See text for description of different plots (the plots are referred to, left to right, as 1A, 1B, and 1C).

Figure 2. Map of the geographic ranges of Pacific-slope and Cordilleran Flycatchers. Circles indicate data collection sites for this study (genetic data, song data, or both). Black circles show core Pacific-slope sites, gray circles show admixed sites, and white circles show core Cordilleran sites. Data collection sites cover virtually the entire distributions of both taxa. Circles marked with stars indicated sites where song playback experiments were conducted.

Figure 3. Spectrograms showing complete Pacific-slope Flycatcher (top; coastal Washington), Cordilleran Flycatcher (bottom; Colorado), and intermediate (middle; northern Idaho) song phrases. Part 1 and Part 2, peak frequency, and the sharpness of the frequency peak are marked for Song 2. Parameters measured on Song 2 that were used in this study: peak frequency, duration, the sharpness of the frequency peak, the proportion of the entire song comprised of Part 2 of the song, the change in frequency from the peak frequency to the lowest post-peak point in Part 1, and the presence or absence of an amplitude gap between Parts 1 & 2.

Figure 4. The frequency of Pacific-slope and Cordilleran Flycatcher mtDNA (ND2) haplotypes per sample site. Black shading indicates the proportion of Pacific-slope haplotypes and white indicates the proportion of Cordilleran haplotypes.

Figure 5. Geographic variation in the nuclear DNA of Pacific-slope and Cordilleran Flycatchers. The proportion of each circle shaded black shows the proportion of Pacific-slope ancestry per site from the Structure analysis (white shows the proportion of Cordilleran ancestry). E.g., an evenly black and white circle does not necessarily imply equal numbers of parental Pacific-slope and Cordilleran genotypes at a site, but rather a 0.50 Structure score (“population genotype”) calculated from the SNP genotypes of all individuals sampled at the site.

Figure 6. The frequency of Pacific-slope Flycatcher mtDNA (ND2) haplotypes per site as a function of population nuclear genotype score (from Structure) for 54 samples sites ($R^2 = 0.63$). The trendline shows the relationship between the parental sites only ($R^2 = 0.96$).

Figure 7. Plot of the first and second principal components from a Principal Components Analysis of Song 2 characters for core Pacific-slope and Cordilleran sites only. White circles show individuals from Cordilleran sites and dark gray circles show individuals from Pacific-slope sites. Individuals from admixed sites (not shown) overlap with and fill the space between the parental clusters.

Figure 8. Correlation between individual song PC1 and individual genotype for 66 individuals (left; $R^2 = 0.68$) and between mean song PC1 and population genotype for 50 sites (right; $R^2 = 0.93$). Black circles indicate Pacific-slope sites or individuals, gray indicates admixed, and white indicates Cordilleran. Trendlines (in red) show the relationship between the parental sites only for both comparisons (individuals: $R^2 = 0.88$; sites: $R^2 = 0.97$).

Figure 9. Geographic variation in the songs of Pacific-slope and Cordilleran Flycatchers. To represent the variation, the range of mean Song 2 PC1 scores per site were divided into 11 equal bins. The proportion of black in the pie graph represents which bin the site falls within, with a score of 0 (bin 1; most Cordilleran song) being 100% white and a score of 1 (bin 11; most Pacific-slope song) being 100% black.

Figure 10. Box plots of modeled approach responses (GLMM) of three taxa to three stimulus types. Because the raw response data are effectively pseudoreplicated (clustered), the points constituting a boxplot of the raw response data would not be independent. Instead, boxplots are used here to visualize the *model predictions* from GLMMs, created by generating $n = 1000$ predictions, using the beta of the model term as the mean, and the standard deviation (SD) for each unique taxon-stimulus type combination. I could assume that I was drawing from a normal distribution because the effect sizes (betas + SE) were on the link-scale.

Figure 11. Left: linear regression of approach as a function of genotype for 204 trials in Cordilleran populations, 147 trials in Pacific-slope populations, and 207 trials in genetically admixed populations ($p = 6.22e-12$ ***; $R^2 = 0.080$). This includes the approach responses of all individuals of all taxa to all stimuli. Right: Linear model of song response (song persistence) as a function of population genotype for 38 trials in Cordilleran populations, 100 trials in Pacific-

slope populations, and 63 trials in genetically admixed populations ($p = 0.018$, $R^2 = 0.023$). This includes the song responses of all individuals of all taxa to all stimuli. Each plot includes up to three responses (to three stimulus types) per individual.

Figure 12. Linear regression of song response (song persistence) as a function of genotype for Cordilleran (left), Pacific-slope (middle), and admixed (right) song stimuli. Values on the y-axis indicate the following song responses: >1 = increased rate of singing, 1 = no change in rate of singing, <1 = decreased rate of singing, and 0 = cessation of singing.

Figure 13. Generalized Additive Model of approach as a function of song distance for each focal taxon. Gray boxes indicate distance from Cordilleran and Pacific-slope song stimuli for each population. Distances from the two stimulus types overlap in Cordilleran and admixed populations due to the wider range of song scores in these taxa. The box representing distance from conspecific song stimuli is to the right in Cordilleran populations, and to the left in Pacific-slope populations. The box representing distance from Pacific-slope is to the left in the admixed populations (Cordilleran is to the right). The red bar at the bottom of each plot shows the range of song distances from that taxon to admixed song stimuli (see also Table 3). Dotted lines indicate lower and upper 95% confidence intervals.

Figure 14. Song response (song persistence) as a function of song distance for Cordilleran (left), Pacific-slope (middle), and admixed (right) populations. Values on the y-axis indicate the following song responses: >1 = increased rate of singing, 1 = no change in rate of singing, <1 = decreased rate of singing, and 0 = cessation of singing.

Table 1.

LOCATION							GENOTYPE				SONG & PLAYBACK		
Site name	State	County	Country	Taxon	Lat	Long	SNP	SNP score	mtDNA	% PS mtDNA	Song	Song Score	PB Expts
Rocky Mtn NP	CO	Larimer	USA	CR	40.67	-105.39	5	0.19	11	0	12	-2.91	-
W Elk Mtns	CO	Gunnison	USA	CR	38.81	-106.74	1	0.10	-	-	2	-1.52	7
Wet Mtns	CO	Custer	USA	CR	38.07	-105.11	15	0.12	9	11	20	-1.91	14
Wasatch Mtns	UT	Juab, Utah	USA	CR	39.77	-111.72	10	0.19	-	-	20	-1.81	19
Pine Vly Mtns	UT	Washington	USA	CR	37.38	-113.47	7	0.17	8	38	-	-	-
Snake Range	NV	White Pine	USA	CR	38.91	-114.16	15	0.16	10	10	2	-2.21	-
Taos Mtns	NM	Taos	USA	CR	36.73	-105.52	10	0.11	11	0	-	-	-
Zuni Mtns	NM	Cibola, McKinley	USA	CR	35.20	-108.14	-	-	-	-	17	-3.32	-
Black Range	NM	Grant, Sierra	USA	CR	32.91	107.80	9	0.10	-	-	21	-2.14	12
Sacramento Mtns	NM	Lincoln, Otero	USA	CR	32.83	-105.74	12	0.11	14	0	17	-2.17	6
San Francisco Peaks	AZ	Cocconino	USA	CR	35.31	-111.72	10	0.12	10	0	8	-2.43	-
Hualapai Mtns	AZ	Mohave	USA	CR	35.10	-113.88	4	0.12	6	33	1	-1.27	-
White Mtns	AZ	Apache	USA	CR	33.92	-109.12	10	0.15	-	-	7	-1.95	7
Pinaleno Mtns	AZ	Graham	USA	CR	32.64	-109.82	8	0.13	4	0	10	-2.96	2
Santa Catalina Mtns	AZ	Pima	USA	CR	32.44	-110.79	-	-	-	-	6	-3.28	-
Chiricahua Mtns	AZ	Cochise	USA	CR	31.93	-109.27	9	0.09	12	17	9	-2.64	-
Chisos, Davis & Guadalupe Mtns	TX	Brewster, Culberson, Jeff Davis	USA	CR	29.24	-103.30	-	-	-	-	8	-2.88	-
Sitka	AK	Sitka	USA	PS	57.05	-135.33	5	0.88	-	-	-	-	-
Haida Gwaii	BC	-	CA	PS	53.59	-132.17	-	-	-	-	5	2.56	-
Vancouver	BC	-	CA	PS	49.27	-123.23	-	-	-	-	15	1.82	-
Hope	BC	-	CA	PS	49.15	-121.30	7	0.89	8	100	5	1.87	-
Skagit	WA	Skagit, Whatcom	USA	PS	48.62	-121.41	10	0.90	11	100	11	2.02	12
Olympic Pen	WA	Clallum	USA	PS	47.93	-123.04	10	0.90	11	100	6	2.11	-
Mt. Ranier	WA	Cowlitz, Pierce	USA	PS	46.90	-121.65	-	-	-	-	3	-	-
Mount Hood	OR	Clackamas	USA	PS	45.45	-122.24	-	-	-	-	9	1.74	-
Central Oregon Cst	OR	Coos, Lincoln	USA	PS	44.48	-123.92	8	0.89	8	100	7	1.88	-
Willamette Vly	OR	Lane, Douglas	USA	PS	43.96	-123.13	-	-	-	-	15	1.83	-
Umpqua	OR	Douglas	USA	PS	42.85	-122.86	-	-	-	-	2	1.61	-
Rogue Rv	OR	Jackson	USA	PS	42.74	-122.39	13	0.82	5	100	8	1.61	-
Shasta-E	CA	Shasta	USA	PS	41.03	-121.70	11	0.85	11	100	-	-	-
Shasta-W	CA	Shasta	USA	PS	40.87	-122.12	26	0.86	24	96	18	1.93	16
N California Cst	CA	Humboldt, Del Norte	USA	PS	40.86	-123.99	9	0.90	10	100	7	2.27	-
Red Bluff	CA	Tehama	USA	PS	40.30	-122.18	3	0.88	-	-	-	-	-
Mendocino	CA	Mendocino	USA	PS	39.82	-122.99	8	0.89	10	100	-	-	-
Crockett Peak	CA	Lake	USA	PS	39.44	-122.82	14	0.90	12	100	2	1.89	-

Table 1. (cont.)

LOCATION							GENOTYPE				SONG & PLAYBACK		
Site name	State	County	Country	Taxon	Lat	Long	SNP	SNP score	mtDNA	% PS mtDNA	Song	Song Score	PB Expts
San Francisco Bay N	CA	Alameda, Marin, Sonoma	USA	PS	38.00	-122.76	-	-	-	-	29	1.98	5
Yosemite	CA	Tuolumne	USA	PS	37.75	-119.84	3	0.91	-	-	7	1.86	-
San Francisco Bay S	CA	San Mateo	USA	PS	37.19	-122.33	-	-	-	-	26	2.07	13
Monterey	CA	Monterey, San Benito	USA	PS	36.38	-121.57	17	0.87	13	100	7	1.83	3
Walker Pass	CA	Kern	USA	PS	35.66	-118.04	5	0.87	-	-	-	-	-
SantaBarbara	CA	SantaBarbara	USA	PS	34.53	-120.17	1	0.84	-	-	1	-	-
San Bernadino Mtns	CA	SanBernadino	USA	PS	34.16	-116.92	6	0.82	12	92	-	-	-
Santiago Oaks	CA	Orange	USA	PS	33.83	-117.77	-	-	-	-	4	2.33	-
San Jacinto Mtns	CA	Riverside	USA	PS	33.81	-116.75	4	0.88	5	100	-	-	-
San Diego	CA	SanDiego	USA	PS	32.73	-116.94	-	-	-	-	2	2.27	-
San Pedro Martir	BCN	-	MX	PS	30.82	-115.74	5	0.92	-	-	-	-	-
Peace Rv	BC	-	CA	AD	55.63	-121.91	2	0.51	2	50	5	0.76	-
William's Lk	BC	-	CA	AD	52.16	-122.20	7	0.64	7	100	5	0.90	-
Lillooet	BC	-	CA	AD	50.83	-121.69	1	0.44	1	100	-	-	-
Princeton	BC	-	CA	AD	49.57	-120.50	7	0.65	7	86	9	0.90	-
Kootenay Lk	BC	-	CA	AD	49.51	-116.79	4	0.41	5	80	4	0.03	-
Penticton	BC	-	CA	AD	49.34	-119.77	7	0.56	7	86	10	0.75	-
Christina Lk	BC	-	CA	AD	49.12	-118.24	10	0.50	11	91	16	0.40	-
Kananaskis	AB	-	CA	AD	51.05	-114.96	19	0.57	16	88	9	0.82	-
Okanogan E	WA	Okanogan	USA	AD	48.80	-119.05	19	0.59	20	85	3	0.75	-
Sullivan Lk	WA	Pend Oreille	USA	AD	48.77	-117.29	6	0.47	6	100	10	0.44	-
Okanogan W	WA	Okanogan	USA	AD	48.44	-119.98	16	0.55	18	94	16	0.30	11
Kittitas	WA	Kittitas	USA	AD	46.93	-120.83	10	0.70	10	100	4	0.78	-
Blue Mtns N	WA	Asotin, Columbia, Walla Walla	USA	AD	46.21	-117.71	3	0.34	3	67	13	-0.45	-
Lk Pend Oreille	ID	Bonner	USA	AD	48.13	-116.23	9	0.41	10	100	8	-0.22	-
Coeur d'Alene	ID	Kootenai, Shoshone	USA	AD	47.71	-116.37	26	0.47	23	88	5	-0.07	-
Clearwater Rv	ID	Idaho, Nez Perce	USA	AD	45.97	-116.36	18	0.35	19	79	8	-0.33	7
Pattee Creek	ID	Lemhi	USA	AD	44.98	-113.59	6	0.31	6	67	-	-	-
Payette Rv	ID	Boise	USA	AD	44.05	-115.90	-	-	2	100	1	-	-
Pocatello	ID	Bannock, Power	USA	AD	42.46	-112.72	12	0.23	12	25	1	-	2
Palouse	ID, WA	Latah, Whitman	USA	AD	46.82	-116.97	-	-	-	-	7	-0.21	-
Hungry Horse	MT	Flathead	USA	AD	48.38	-114.10	1	0.31	1	100	-	-	-
Thompson Rv	MT	Sanders	USA	AD	47.63	-115.17	15	0.45	15	80	8	0.13	5
Sawtooth Range	MT	Lewis & Clark, Teton	USA	AD	47.40	-112.75	5	0.46	4	50	4	-	1

Table 1. (cont.)

LOCATION							GENOTYPE				SONG & PLAYBACK		
Site name	State	County	Country	Taxon	Lat	Long	SNP	SNP score	mtDNA	% PS mtDNA	Song	Song Score	PB Expts
Bitterroot Mtns	MT	Mineral	USA	AD	47.08	-114.93	3	0.45	4	100	-	-	-
Big Belt Mtns	MT	Broadwater, Lewis & Clark	USA	AD	46.63	-111.57	23	0.29	33	61	18	-1.05	10
Lolo Vly	MT	Ravalli	USA	AD	46.40	-113.90	11	0.39	11	64	3	-0.39	1
Crazy Mtns	MT	Sweet Grass	USA	AD	46.04	-110.24	1	0.15	1	0	-	-	-
Pryor Mtns	MT	Carbon	USA	AD	45.15	-108.44	5	0.26	6	17	-	-	-
Blue Mtns S	OR	Grant, Umatilla, Union, Wallowa	USA	AD	45.34	-118.72	10	0.42	10	90	3	-0.02	-
Ochoco Mtns	OR	Crook	USA	AD	44.46	-120.73	7	0.40	6	83	-	-	-
Deschutes	OR	Deschutes	USA	AD	44.18	-121.67	10	0.48	5	60	12	0.11	-
Paulina Lk	OR	Deschutes	USA	AD	43.71	-121.30	4	0.26	4	100	-	-	-
Ft. Klamath	OR	Klamath	USA	AD	42.70	-122.08	2	0.64	1	100	16	0.87	-
Warner Mtns N	OR	Lake	USA	AD	42.35	-120.73	37	0.36	14	71	10	-0.48	-
Sinks Canyon	WY	Fremont	USA	AD	42.76	-108.80	-	-	-	-	1	-0.77	-
Black Hills	SD	Lawrence	USA	AD	44.42	-103.88	20	0.23	19	26	5	-1.11	-
Siskiyou	CA	Siskiyou	USA	AD	41.88	-122.17	33	0.67	13	62	25	1.30	13
Modoc	CA	Modoc	USA	AD	41.51	-120.23	25	0.29	26	69	11	-0.39	18
Mono	CA	Mono	USA	AD	38.12	-119.27	1	0.92	-	-	7	-0.43	-
Jarbidge Mtns	NV	Elko	USA	AD	41.78	-115.70	10	0.22	10	60	-	-	-
Ruby Mtns	NV	Elko	USA	AD	40.65	-115.40	-	-	-	-	2	-	-
Spring Mtns	NV	Clark	USA	AD	36.24	-115.73	4	0.22	5	40	-	-	-
TOTAL							212	-	210	-	152	-	26

Table 2.

Measurement variable	PC1	PC2
Peak frequency	0.468	0.144
Duration	-0.280	-0.279
Sharpness of frequency peak (abs. val. of Δ freq. \pm 10 ms of the peak freq.)	0.454	0.052
Proportion of total song made up by second half of song	0.452	-0.296
Duration from peak of song to lowest inflection point	-0.359	0.139
Δ frequency from peak of song to lowest inflection point	0.211	0.779
Presence/absence of amplitude gap	-0.346	0.431

Table 3.

Taxon	Total Expts	Range	Genotype				Song			
			Genotype: all sites range	Genotype: all sites mean	Genotype: PB sites range	Genotype: PB sites mean	Song: all sites range	Song: all sites mean	Song: PB sites means range	Song: PB sites mean
Cordilleran	67	high low	0.19 0.09	0.14	0.19 0.10	0.13	-1.52 -3.32	-2.44	-1.52 -2.96	-2.07
Admixed	68	high low	0.70 0.22	0.44	0.67 0.29	0.41	1.30 -1.11	0.12	1.30 -1.05	-0.04
Pacific-slope	49	high low	0.92 0.82	0.88	0.90 0.86	0.88	2.56 1.61	1.97	2.07 1.83	1.97

Table 4.

	CO-PS	CO-AD	CO-CO	AD-PS	AD-AD	AD-CO	PS-PS	PS-AD	PS-CO
CO-PS		0.001	4.41E-07	0.623	0.393	0.691	0.702	0.091	1.50E-05
CO-AD	3.053		0.058	0.027	0.058	0.025	0.043	4.75E-05	4.11E-10
CO-CO	4.804	1.873		3.20E-04	0.001	3.30E-04	0.001	1.73E-07	8.44E-13
AD-PS	0.238	-2.410	-3.912		0.659	0.919	0.952	0.032	2.44E-06
AD-AD	0.782	-1.907	-3.450	0.638		0.598	0.703	0.013	5.78E-07
AD-CO	0.244	-2.343	-3.807	0.013	-0.603		0.987	0.042	4.15E-06
PS-PS	0.248	-2.122	-3.452	0.033	-0.461	0.022		0.015	3.39E-07
PS-AD	-2.110	-4.424	-5.683	-2.350	-2.855	-2.324	-2.560		0.002
PS-CO	-4.427	-6.286	-7.268	-4.634	-5.051	-4.598	-4.878	-2.777	

Figure 1.

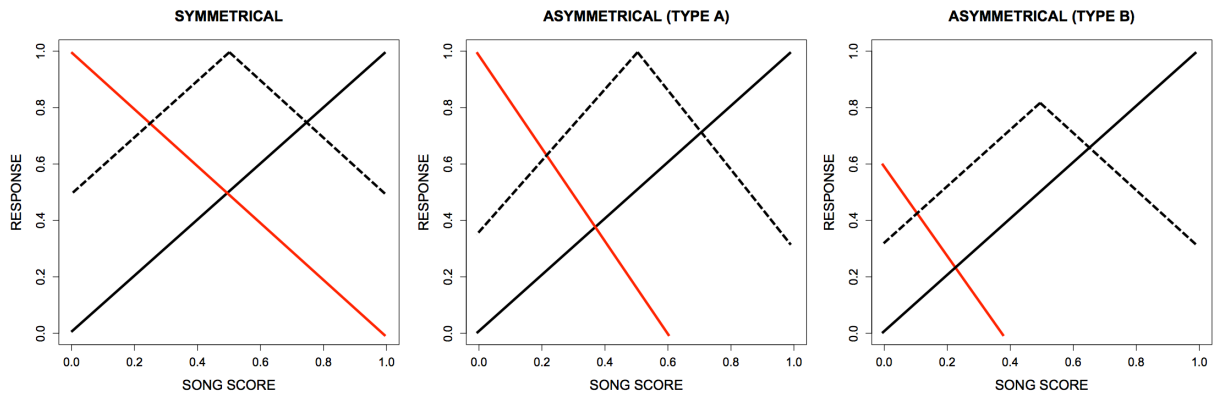


Figure 2.

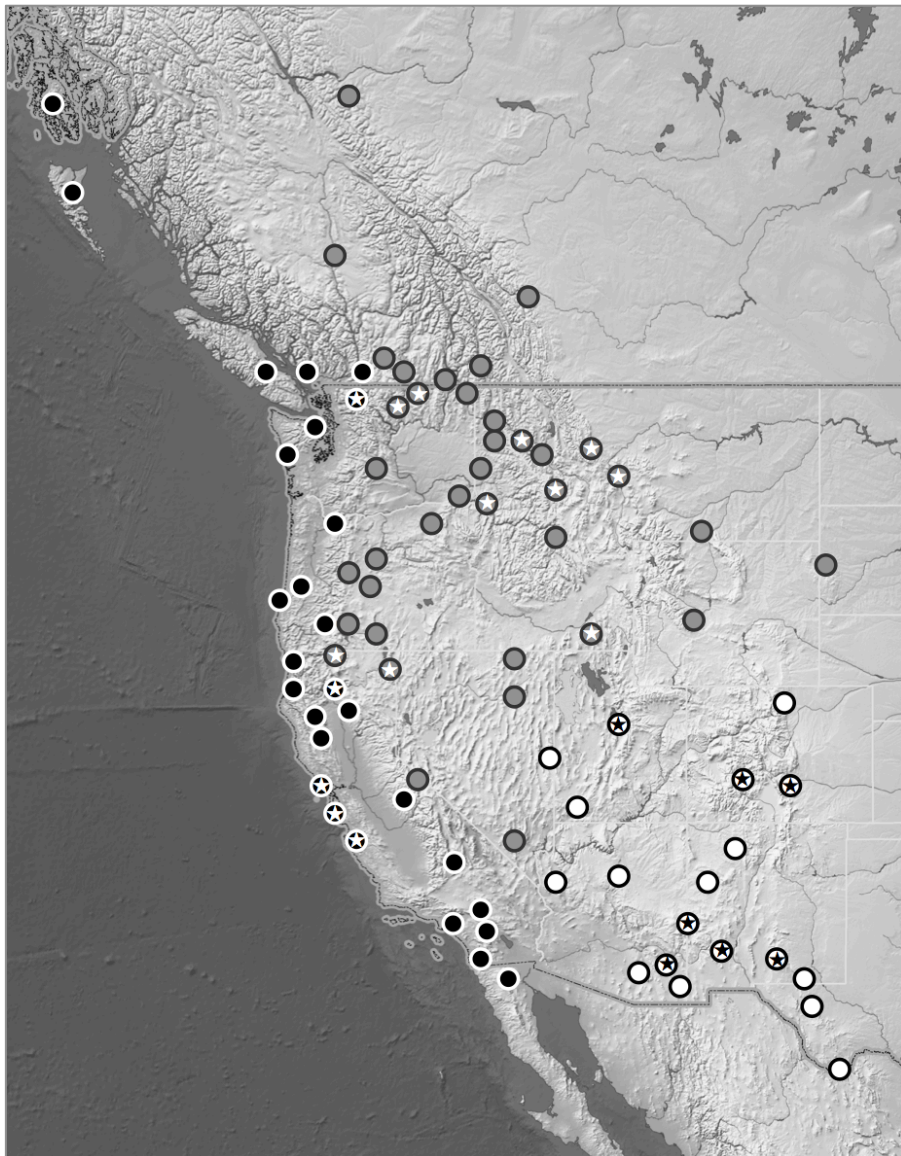


Figure 3.

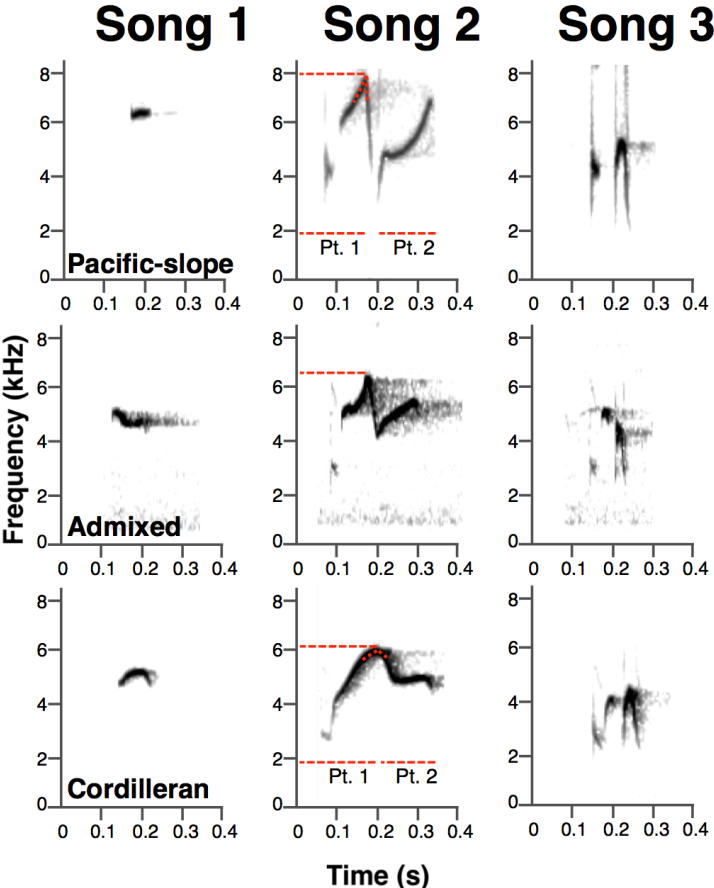


Figure 4.

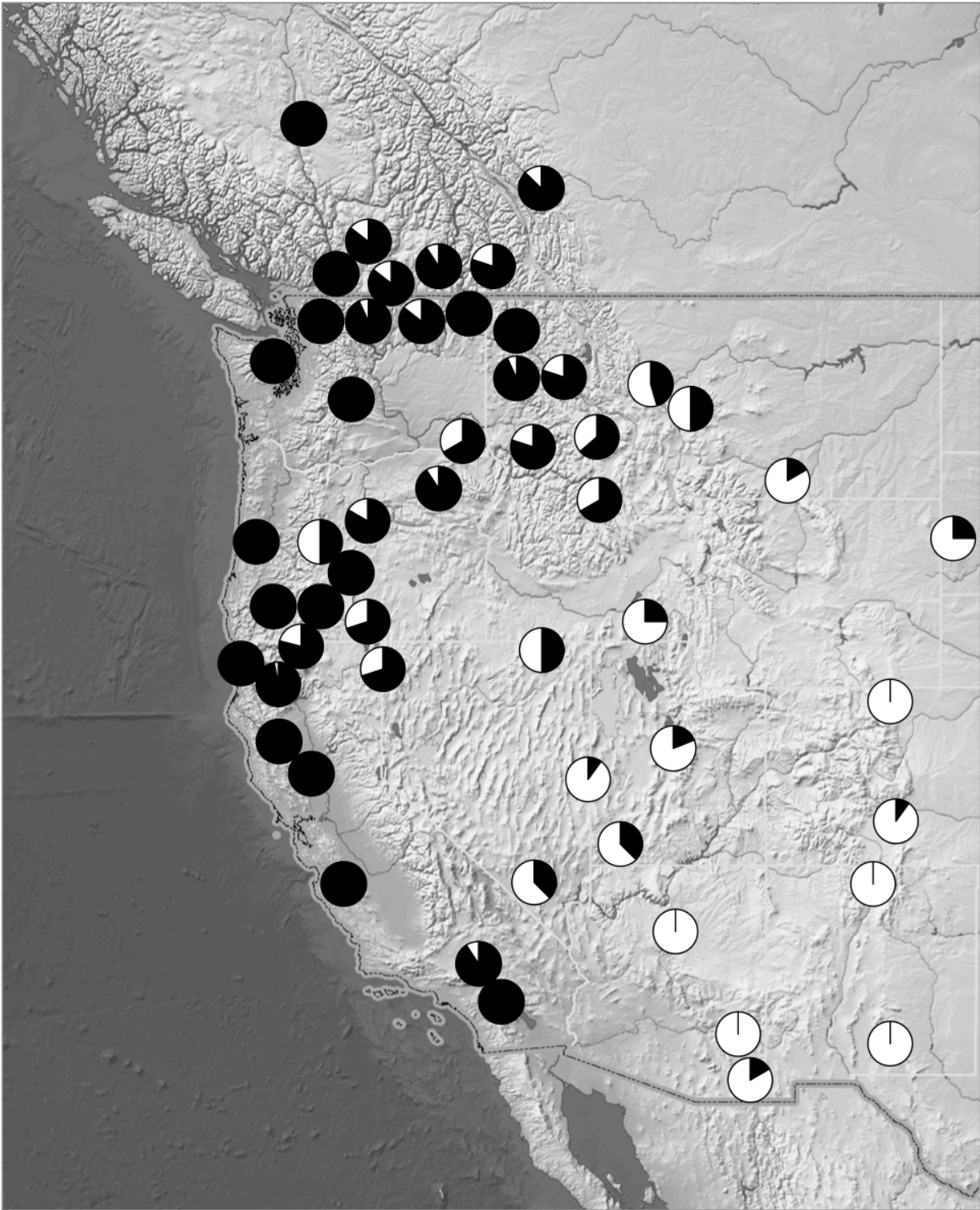


Figure 5.

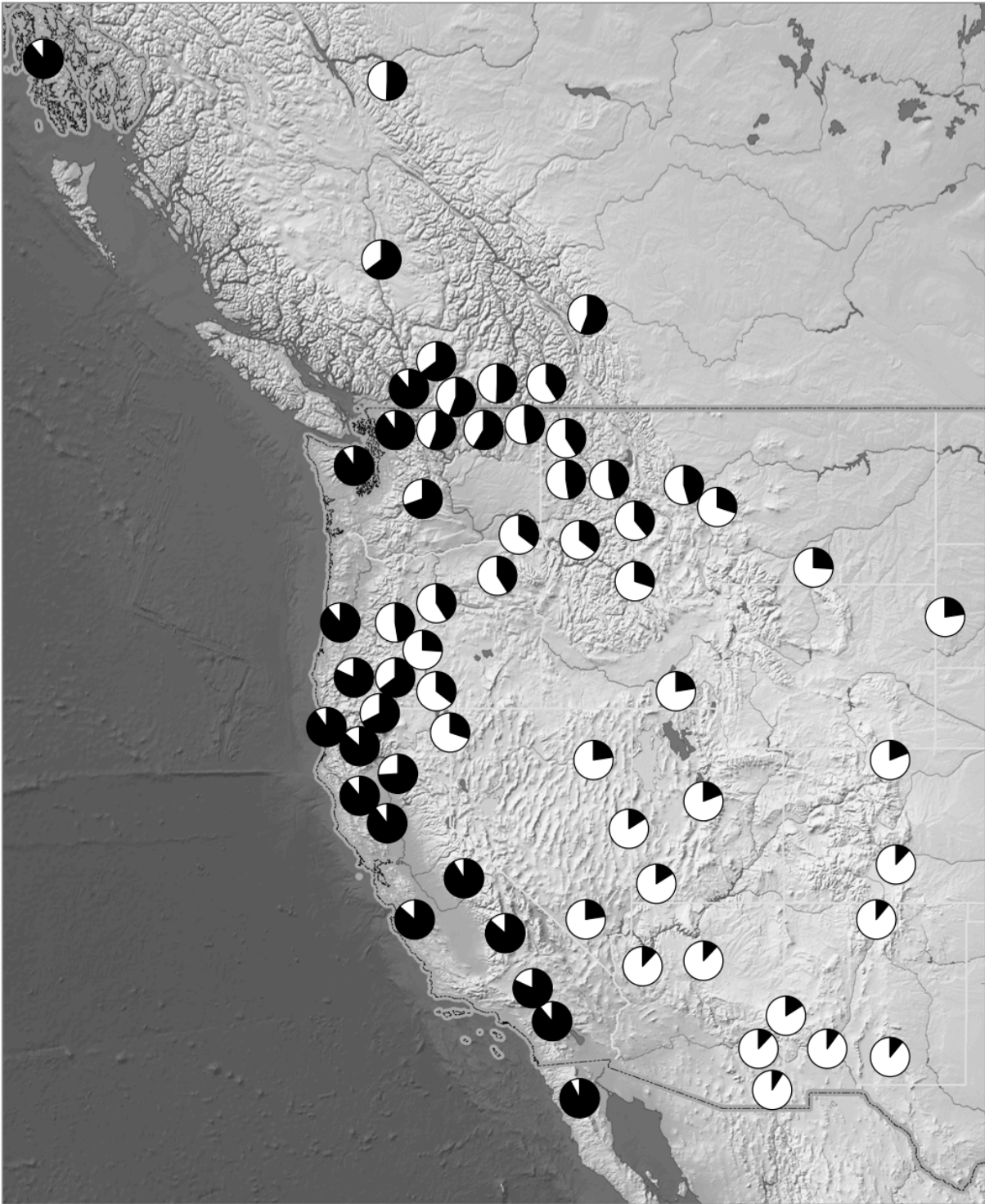


Figure 6.

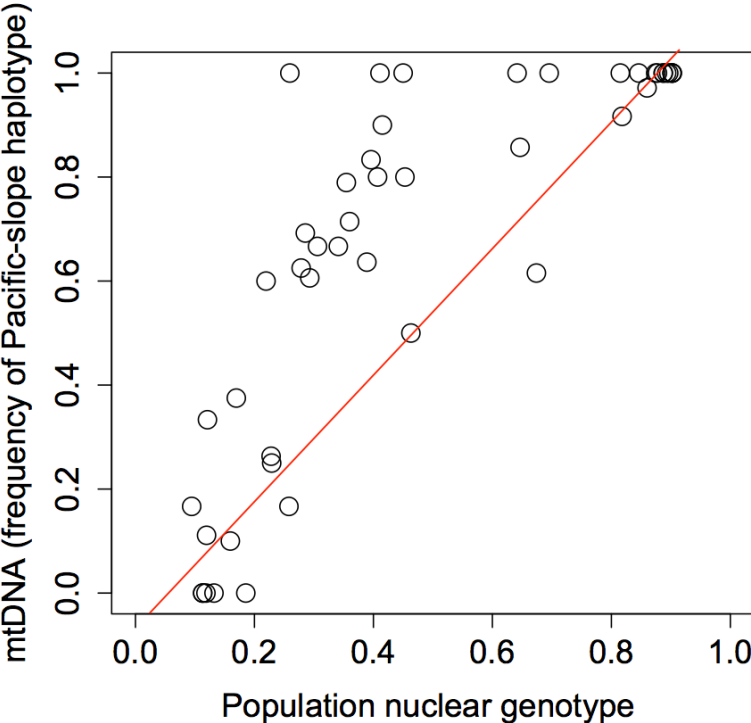


Figure 7.

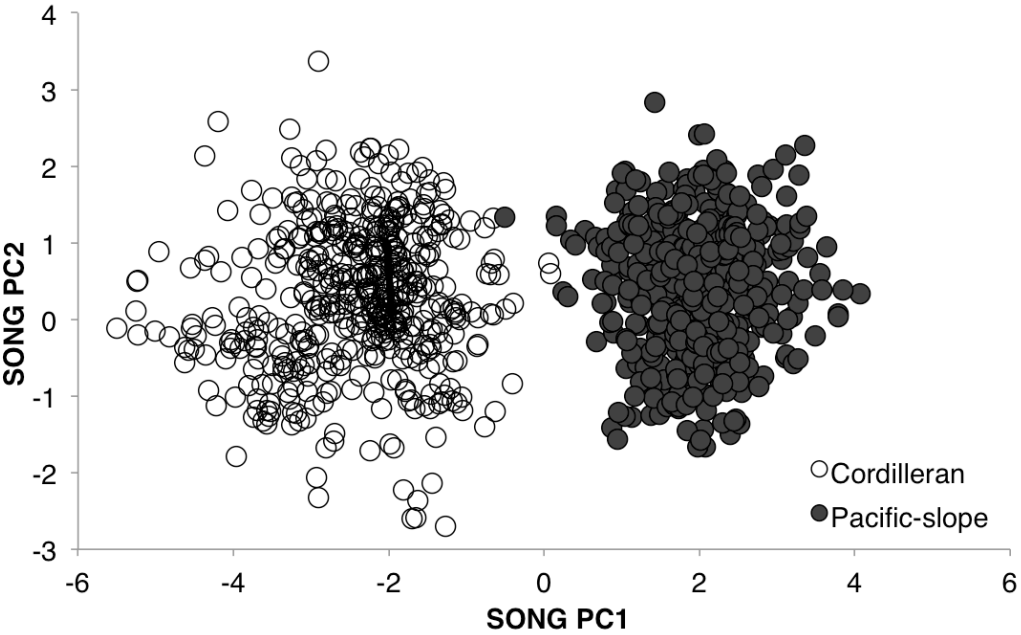


Figure 8.

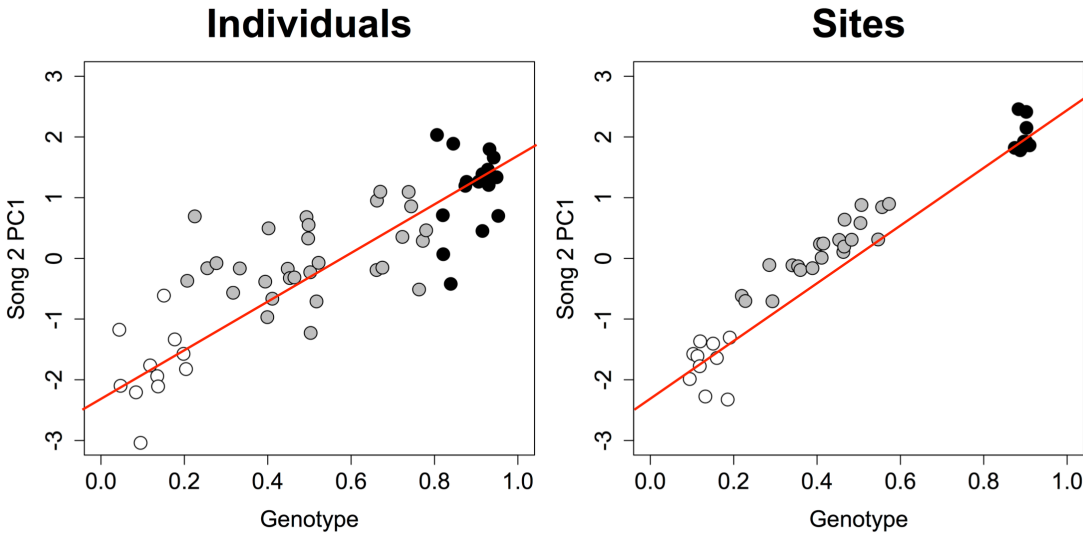


Figure 9.

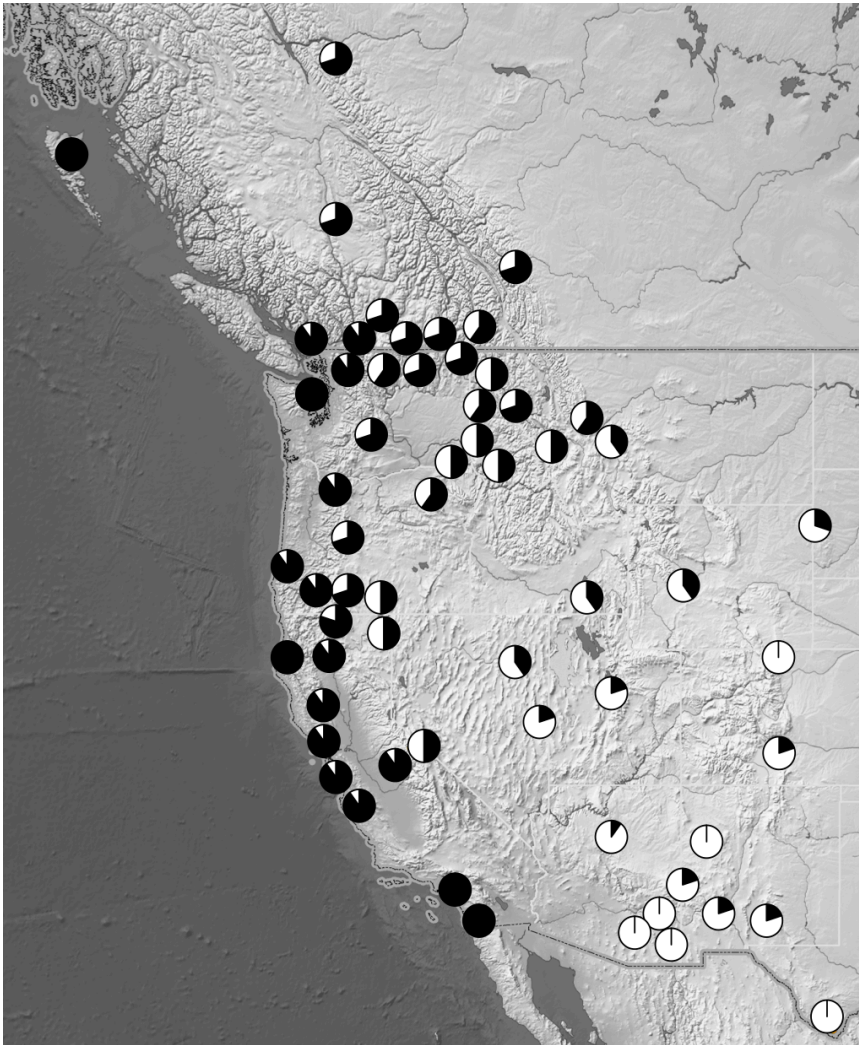


Figure 10.

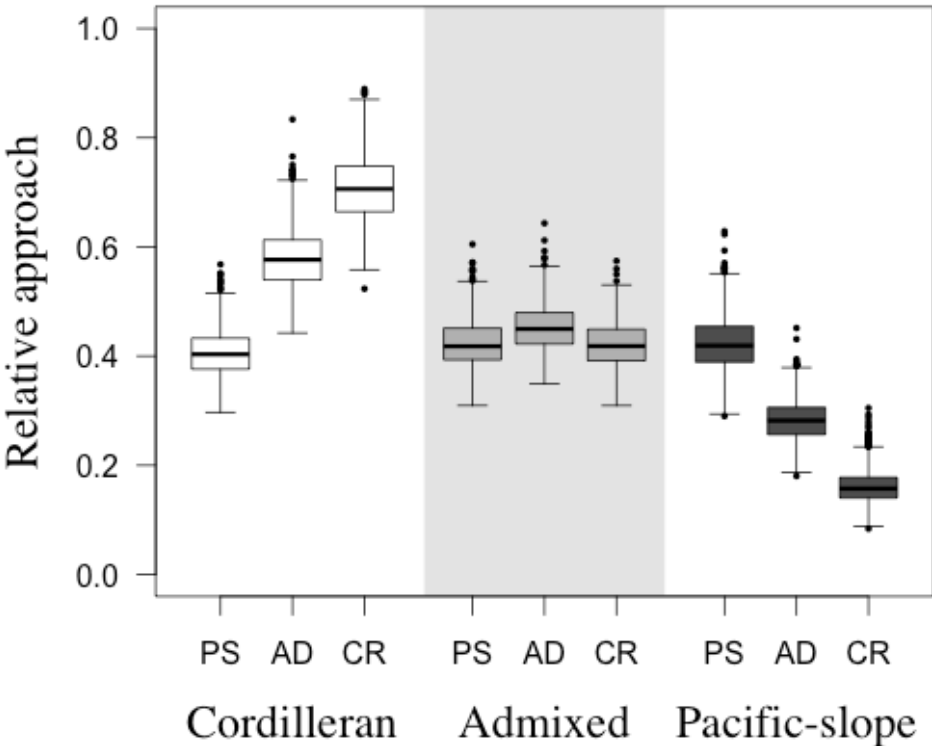


Figure 11.

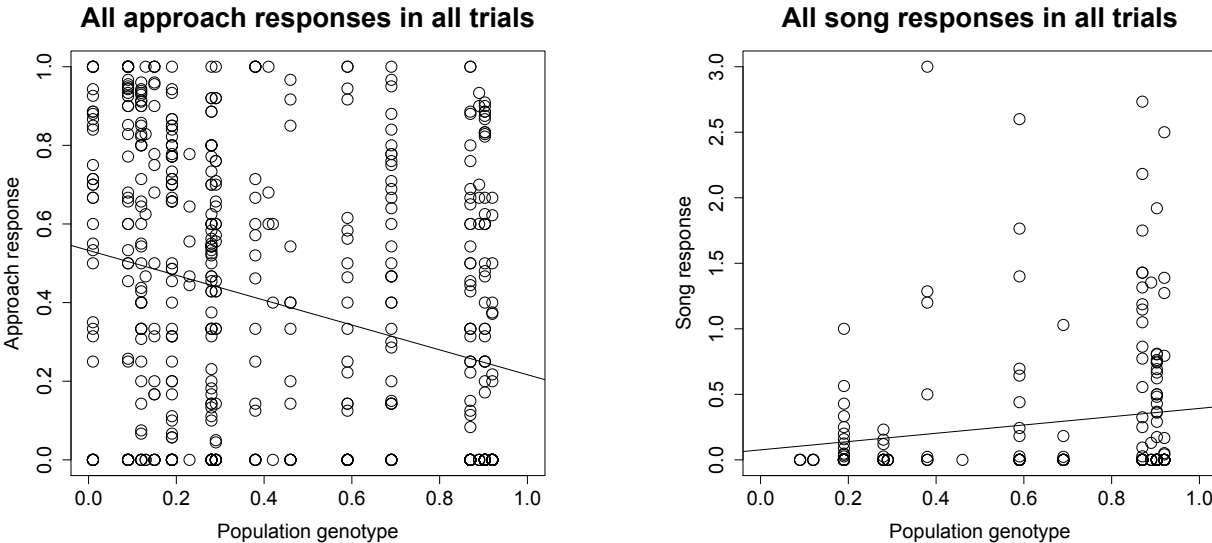


Figure 12.

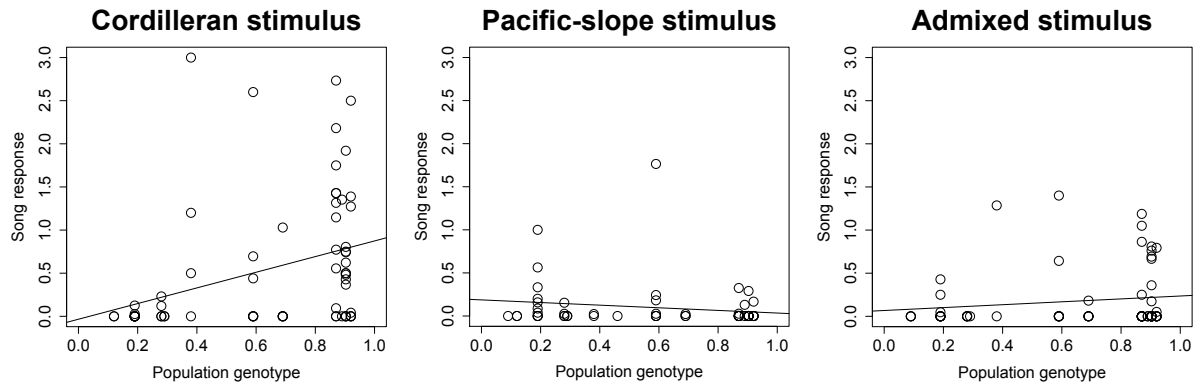


Figure 13.

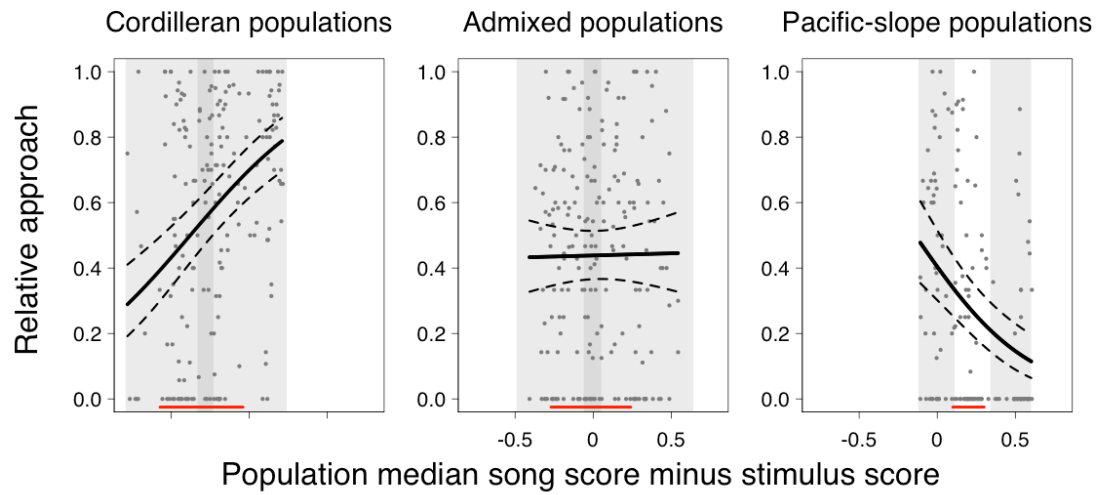
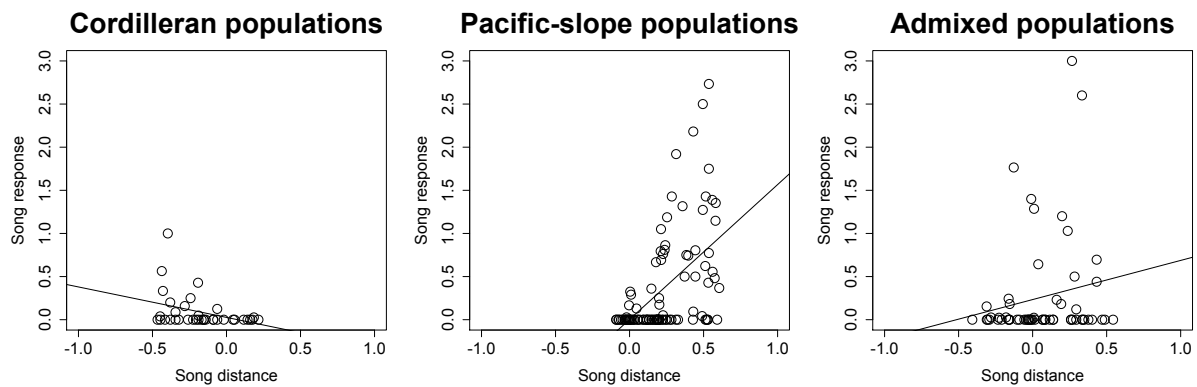


Figure 14.



CHAPTER 3

Vocal divergence and lineage divergence in a clade of tyrannid flycatchers

ABSTRACT

When signals that affect reproductive behaviors diverge between populations, they can create behavioral barriers to gene flow. Numerous studies have shown that the level of complexity can affect the efficacy of song in birds, but these studies have focused mainly on birds with learned song. Elevated rates of signal diversification and song complexity have been observed in higher latitude migratory species, ostensibly as a result of greater sexual selection on song, related to an accelerated breeding season. Suboscines offer interesting opportunities to investigate the effects of song divergence on lineage diversification, because songs develop without learning. Here, I examine genetic and song variation across the entire *Empidonax difficilis-occidentalis-flavescens* clade, and perform song playback experiments to test whether song divergence has affected lineage divergence by acting as a species discrimination trait. I offer a novel approach for examining patterns of vocal repertoire evolution, by comparing within repertoire complexity (syllable diversity) across homologous vocalization types present in all six taxa. I find varying rates of song divergence across taxa and across latitude. Songs are distinct between some taxa, but not others. Song divergence is not correlated with mtDNA distance, but it is correlated with latitudinal distance between taxa. Song complexity seems to be higher in higher latitude migratory taxa, but this is due the extremely divergent song of one taxon (*E. difficilis*). Moreover, a high level of divergence in one particular vocalization (Song 2) is most responsible for the overall divergence of *E. difficilis* song. Song playback experiments show varying levels of discrimination among song types, and there is at least preliminary evidence that lower latitude species are able to use more subtle vocal cues in taxon recognition than higher latitude migratory taxa. This study provides a unique view into how vocal repertoires can evolve in birds, and how this relates to lineage diversification.

INTRODUCTION

Acoustic signals can play an important role in the speciation process (Ryan and Rand 1993, Andersson 1994, Coyne and Orr 2004, Price 2008). When signals that affect reproductive behaviors, such as territory defense and mate choice, diverge between populations, they can create behavioral barriers to gene flow by functioning as cues for assortative mating or more generally, as species discrimination traits (Catchpole and Slater 1995, Martens 1996, Mendelson & Shaw 2002, Ryan & Rand 1993, Marler and Slabbekorn 2004, Price 2008). The effects of divergence in acoustic signals has been studied extensively in birds, because (1) acoustic signals tend to be relatively conspicuous and easy to observe, and (2) the structure of the syrinx (the avian sound production organ) allows the production of complex sounds in many species, leading to extensive acoustic diversification.

Numerous studies have shown that the level of complexity can have important effects on the efficacy of song in birds (Podos 1997, Read and Weary 1992, Irwin et al 2001 Slabbekorn and Smith 2002, Ballentine et al 2004, Dooling 2004, Searcy and Nowicki 2005, DuBois et al 2008, Price 2008, Weir and Wheatcroft 2010). Measures of song complexity that have been shown to have important effects on territorial defense and mate choice include: repertoire size, syllable diversity, song rate, and the production of non-linear sounds (Catchpole 1980, 1986, Searcy and Yasukawa 1990, 1996, Irwin 2000, ten Cate 2004). The effects of song complexity on reproductive behavior suggest that evolutionary changes in these characteristics could have important effects on lineage diversification and speciation. There is a general consensus that the

evolution of complex song traits is the result of interactions between natural selection and sexual selection (Slabbekoorn and Smith 2002). Signals must be heard to be effective and there is often an important role for environment-based selection for effective sound propagation (i.e., the acoustic adaptation hypothesis; Morton 1975, Nottebohm 1975, Slabbekoorn 2004). Because songs can convey information to both male and female receivers (Payne 1983, Collins 2004), songs and song complexity can evolve via intra- or intersexual sexual selection as well (Payne 1983, West-Eberhard 1983, Catchpole and Slater 1997).

The level of complexity favored by sexual selection may vary depending on the signaling context. For example, shorter songs (i.e., reduced complexity) are often used in male-male competition while longer, more complex songs are often used in male-female interactions (Catchpole 1982, Collins 2004, Collins et al 2009). In addition, more complex songs, that are thought to have evolved due to sexual selection, have been shown to be more common in migratory populations (Read and Weary 1992, Irwin 2000, Collins et al 2009, Price 2008).

Determining how song complexity evolves in any particular case has been hindered by the fact that song tends to be a highly derived and plastic trait (Hailman and Ficken 1996, ten Cate 2004). This is related to the fact that an overwhelming proportion of the studies that have focused on song evolution have focused on the songs of oscine passerines (e.g., Payne 1983, 1986, Slabbekoorn and Smith 2002, Podos and Warren 2007, Price 2008, Tobias et al 2012), in which there is an important learned component. Although critical, species-specific characteristics of oscine song are heritable (Dooling and Searcy 1980, Marler and Peters 1980), normal oscine song development requires copying of adult male tutors by naïve birds (reviewed in Slater 1989). As a result, song structure or syntax can change due to ‘cultural drift’ resulting from imperfect copying during the learning process (e.g., Payne 1996, Derryberry 2007), and requires no concomitant genetic change. In other words, transmission of song characteristics to naïve singers occurs both vertically (genetic component) and horizontally (learned component). The decoupling of genes and song can result in the rapid evolution of diverse, and often population-specific song types (Baptista 1975, Lynch 1996, Soha et al 2002, Ritchie 2007), but can make it difficult or impossible to differentiate homology from homoplasy in song characters between even closely related taxa (Badyeav and Leaf 1997, ten Cate 2004, Price et al 2007). As a result, comparative studies of diversification in learned song often must rely on more general (or ‘secondary’) measures of song differentiation such as repertoire size, the presence or absence of certain syllable types, or spectral characteristics of an overall song series, rather than on detailed measures of change in the structure of the individual vocal elements themselves (e.g., Read and Weary 1992, Badyeav and Leaf 1997, Price and Lanyon 2002, Weir and Wheatcroft 2011). At this level of resolution, the correlation with the underlying genotype often is less evident (especially for more deeply diverged taxa), and this has complicated attempts to examine the rate or direction of song or repertoire evolution within a phylogenetic context (ten Cate 2004).

Suboscines represent a diverse evolutionary radiation of songbirds that offer interesting opportunities to investigate the effects of song divergence on lineage diversification. Although a few exceptions are known (Kroodsma et al 2013), the songs of almost all suboscine species are thought to be innate and develop normally without learning (Kroodsma 1984, 1985, 1995, Kroodsma and Konishi 1991). Because it requires genetic change, evolutionary change in innate suboscine song is likely to be far less rapid than in oscine song in most cases. The relative simplicity of suboscine songs, and the close correlation between innate song type and genotype provide the opportunity not only to compare broader repertoire characters across taxa, but also to use homologous landmarks to quantify fine-scale evolutionary change in the spectral

characteristics of individual vocal elements that could contribute to song and repertoire complexity (see Chapter 2). Due to the constraints on evolutionary changes in song and repertoire in suboscines, divergent song types should reflect genetic divergence and lineage affiliation more closely (Kroodsma 1995; Chapter 2). That is, in the absence of cultural drift, divergence in suboscine song due to neutral processes is likely to be closely correlated with divergence in neutral genetic markers (see Chapter 2), and more likely than learned song to reflect phylogenetic relationships. Mismatches between the rates of song divergence and neutral genetic divergence, either due to slower rates (e.g., stabilizing selection) or more rapid rates of change in song (e.g., divergent selection) are more likely to indicate that song is evolving due to some form of selection. This correlation has led researchers to hypothesize that divergent innate song types should function as strong behavioral barriers to gene flow between divergent populations, because song type is likely to be a particularly effective species discrimination trait (Lanyon 1978, Johnson 1980, Sedgwick 2001, Seddon 2005).

Suboscines in the family Tyrannidae ('tyrant flycatchers') offer unique opportunities to study the patterns of effects of divergence in song and song repertoire. Tyrannidae is a diverse family of New World suboscines (> 100 genera, > 400 species; Fitzpatrick et al 2004) that exhibits its greatest species diversity in the Neotropics. Species within tyrannid genera are often very difficult to differentiate based on appearance alone, but usually have diagnostic song types (Lanyon 1978, Johnson 1980, Howell and Webb 1995). Suboscine song can be very simple (e.g., the simple songs of woodcreepers in the family Funariidae; Derryberry et al 2012). Tyrannids, however, feature some of the most elaborate song types among suboscines, and song divergence has been hypothesized to have had an important role in the diversification of this group by functioning as a species discrimination trait (Johnson 1963, 1980, Stein 1963, Lanyon 1978, Sedgwick 2001; see also Chapter 2).

The *Empidonax difficilis-occidentalis-flavescens* (Pacific-slope, Cordilleran, and Yellowish Flycatchers) species complex comprises a clade of suboscine songbirds in the family Tyrannidae (Figure 1). The geographic range of the group spans over 50 degrees of latitude, with resident (non-migratory) populations of *E. occidentalis* and *E. flavescens* in the highlands of Middle America, and migratory populations of *E. occidentalis* and *E. difficilis* breeding in forested habitats in western North America. Each species is comprised of multiple subspecies, representing varying stages of genetic divergence (Johnson and Cicero 2002) and song divergence (Johnson 1980). Members of the *E. difficilis-occidentalis-flavescens* clade are difficult to impossible to differentiate based on appearance alone (Johnson 1980, Howell and Webb 1995, Pyle 1997), although the breeding ranges of most taxa in the clade do not overlap (Johnson 1980, Howell and Webb 1995). Experimental work has shown that song is innate in this genus (Kroodsma 1984, 1985, 1995, Kroodsma and Konishi 1991), and *E. difficilis*, *E. occidentalis*, and *E. flavescens* have homologous, but distinct song types.

Here, I extend the analysis of song diversification and lineage diversification outlined in Chapters 1 and 2, to include the entire *E. difficilis-occidentalis-flavescens* clade. Placing these taxa within a broader phylogenetic context provides a better opportunity than a two-taxon comparison to analyze how the level of song divergence relates to lineage divergence. I examine genetic variation and song variation across a suboscine species complex with varying levels of evolutionary divergence and geographic contact to test whether divergence in innate song has affected lineage divergence by functioning as an important species discrimination trait. I examine the interactions between genetic divergence, song divergence, and differences in latitudinal range and migratory behaviors to determine the direction and rate of evolutionary

change in song in this complex, and the factors that have affected song diversification. The ability to identify homologous song characters across taxa makes a comparison of song divergence and genetic divergence, and how they have interacted in the process of lineage diversification possible at greater evolutionary distances and at a much finer resolution than is usually possible with learned song. Moreover, by comparing changes in multiple homologous vocalization types, within and across taxa, I address whether the rates of change in vocalizations depart from patterns of neutral genetic divergence, and whether this contributes to song complexity. Finally, I test the responses of multiple taxa to homologous, but increasingly divergent song types, to examine how the rate of change in song and song repertoire affects taxon recognition.

METHODS

Study area

Three species are currently recognized in the *Empidonax difficilis–occidentalis–flavescens* complex. Each species is comprised of multiple allopatric subspecies (Johnson 1980). In this study, I focused on the following taxa: *E. difficilis difficilis* (Pacific-slope Flycatcher), *E. occidentalis occidentalis* and *E. o. hellmayri* (both recognized as Cordilleran Flycatcher) and *E. flavescens flavescens* and *E. f. salvinii* (both recognized as Yellowish Flycatcher). In addition, I analyzed populations of *E. o. occidentalis* from south of the Rio Balsas drainage in the Sierra Madre del Sur mountain range in southern Mexico (Guerrero and southwestern Oaxaca). This population has not heretofore been recognized as a distinct taxon, but it seems to be isolated geographically from other populations (Howell and Webb 1995) and previous analyses of morphological characters and limited vocal characters (Johnson 1980) suggested its phenotypic distinctiveness (hereafter, referred to as “Guerrero”). With the exception of *E. d. difficilis* (hereafter, *difficilis*) and *E. o. hellmari* (hereafter, *hellmayri*), these taxa are distributed entirely south of the USA–Mexico border, from northern Mexico to western Panama (see Chapter 2 for details on the distributions of *difficilis* and *hellmayri*). *Empidonax o. occidentalis* (hereafter, *occidentalis*) is distributed in mountainous regions of Mexico, in the Sierra Madre Occidental, Sierra Madre Oriental, and the Trans-Mexican Volcanic Belt, extending to the southeast as far as Oaxaca. *Empidonax f. salvinii* (hereafter, *salvinii*) is distributed south of the Isthmus of Tehuantepec, from the highlands in extreme southeastern Oaxaca to eastern Nicaragua, and is allopatric with *occidentalis*. *Empidonax f. flavescens* (hereafter, *flavescens*) is distributed throughout the highlands of Costa Rica and the western portion of Panama, and is allopatric with *salvinii*.

Phylogenetic analysis of mtDNA

I obtained sequence data for the mtDNA ND2 gene for the entire *E. difficilis–occidentalis–flavescens* clade from Dr. John Klicka at the University of Washington, Seattle, Washington, USA (Table C1). This included the six taxa examined here and three additional subspecies with restricted geographical ranges (*E. d. insulicola* from the Channel Islands, CA; *E. d. cineritius* from Baja California del Sur, MX; *E. f. imperturbatus* from the Sierra de los Tuxtlas, Veracruz, MX) that were not included in the present study. *Empidonax flaviventris* (Yellow-bellied Flycatcher) was used as the outgroup for the phylogeny. I used PAUP* Version 4.0b10 (Swofford 2001) to create a maximum parsimony tree based on the ND2 gene. I performed 1000 random addition replicates. I performed a heuristic search with TBR branch swapping. To create

a consensus tree, I performed a strict consensus of the 12 most parsimonious trees. I performed 1000 bootstrap replicates with 5 random addition replicates per bootstrap replicate. I used the mean pairwise uncorrected p-distances between the individuals of the different taxa as a measure of genetic distance between taxa for subsequent analyses.

Bioacoustic data collection

Very few recordings existed of the vocalizations (especially of song) for taxa south of the USA–Mexico border. I made the first recordings of several populations (and of the “Guerrero” taxon). Songs were recorded in May and early June. In all populations, dawn song was delivered for a brief period of 15–30 minutes in the pre-dawn, perhaps explaining why so few recordings existed prior to my study. No song was observed after the dawn song period, although I frequently heard other types of vocalizations (e.g., male position notes).

The dawn-singing performances of all taxa are combinations of three distinct elements (Figure 1). As in Chapter 2, I treated these as three distinct song types – Song 1 (S1), Song 2 (S2), and Song 3 (S3) – that are often delivered in a repetitive 1–2–3 order. In addition to the three song elements, I analyzed a fourth vocalization – the so-called “male position note” (MPN) (Johnson 1980). This is given primarily by males (but not exclusively; ACR, unpublished data). “Position note” aptly describes the function of this vocalization, as it seems to be uttered mainly as a contact call that allows the male to alert the female to its location. It is also uttered in aggressive territorial interactions (ACR, unpublished data), and is sometimes substituted for S1 in less intensive singing bouts that occur outside of the dawn-singing period (in *difficilis* and genetically admixed *difficilis/hellmayri* individuals).

I analyzed 487 vocalizations from recordings of 290 individual birds made at 31 sites (26 sites with >2 vocal samples; mean = 15.7 vocal samples per site; Table 1, Table C2). I recorded the majority of songs (225/290 recordings = 77.6%), but supplemented my recordings by including songs from museum sound archives (Museum of Vertebrate Zoology – MVZ; Macaulay Library of Natural Sounds – ML, Borror Laboratory of Bioacoustics – BL), from the public bird vocalization archive *xeno-canto.org*, and from individual recordists (see Acknowledgements). I recorded songs digitally in linear PCM format on a Marantz PMD671 digital recorder, at 48 kHz, using a Sennheiser ME-66/K6 shotgun microphone. Archival recordings used in the study were recorded on various analogue platforms and digitized at sample rates of 96 kHz (MVZ and ML). Recordings from *xeno-canto.org* and from individual recordists were made on various analogue or digital devices, and provided in various digital formats. See Chapter 2 for details on how sounds were extracted from their source format and saved for measurement using *Signal* (Engineering Design, Berkeley, CA) sound analysis software.

All song measurements were performed in *Raven Pro* (Cornell Laboratory of Ornithology, Ithaca, NY). In Chapter 2, I showed that the spectral characteristics of vocalizations (i.e., the ‘shape’ of the vocal elements) changed between *E. d. difficilis* and *E. o. hellmayri*. This was evident largely in differences in the location of inflection points and in the acuteness of the frequency transitions at the inflection points. As in Chapter 2, I utilized a landmark-based approach that relied on the identification of 10 homologous points that exist across taxa for each vocalization type (Figure 2). This enabled a better estimation of changes in the structure (i.e., spectral characteristics) of songs – i.e., the type of microevolutionary changes that would be expected between closely related taxa.

Landmarks (LMs) were determined based on the start and end points of song spectrograms

and on internal inflection points (Figure 2; Appendix B). While it is impossible to prove homology even in innate vocalizations, the lack of cultural change due to learning and the similar location of inflection points across vocalization types gave me a high degree of confidence that I was scoring homologies in these analyses. Landmarks were often correlated with increases or decreases in amplitude evident in the corresponding waveforms, and these were used as secondary checks for marking landmarks on spectrograms. Song 3 is perhaps the most divergent of the four vocalization types, and the homology with the other three vocalization types may not be immediately apparent. While I have a high degree of confidence that I was able to mark the same landmarks on S3s, I had to compromise with respect to *difficilis*. In the most typical *difficilis* S3s, the part of the song corresponding to the first half of the vocalization in other types is missing. In a minority of songs (approximately 5%–10%), a reduced representation of the normally missing part of the vocalization is present. I chose these types to represent *difficilis* in these analyses, in order to avoid the problem of having missing data in multivariate analyses. This could result in an underestimation of the divergence of *difficilis* S3s from the S3s of the other taxa, but I felt that the alternative (excluding S3s from the study) was worse. For this reason, certain analyses were performed with and without S3 (see below).

Using these LMs, I derived the following variables for each vocalization type: the change in frequency (Δf , in Hz) from LM02 to LM05 (frequency spread of the first part of the first half of the vocalization), Δf LM05 to LM06 (frequency spread of the second part of the first half of the vocalization), the absolute value of Δf LM05 to LM07 (total frequency spread), Δf LM07 to LM09 (frequency spread of the second half of the vocalization), the total time length of the vocalization (duration), the *relative* change in time (Δt) from LM02 to LM05 (i.e., $(\Delta t \text{ LM05 to LM02}) \div \text{duration}$), relative Δt LM05 to LM06, and relative Δt LM05 to LM06, the slope between LM02 and LM05, and the slope between LM05 and LM06. I used the frequency at LM05 (the frequency peak of the vocalization) and the derived variables described above (11 variables total) to analyze variation in *each of the four vocalization types* across the six taxa.

Bioacoustic data analysis

I analyzed song measurement data using two different approaches. In the first approach, I examined song variation across taxa using taxon song as the unit of comparison. In this way, I were able to test whether songs differed between taxa and whether the close correlation between song type and genotype that we found in the *E. difficilis*–*E. o. hellmayri* comparison (Chapter 2) was maintained across the entire species complex, or whether mismatches between genetic distance and song distance were evident that could indicate that non-neutral processes have affected song divergence. This analysis provided a measure of song divergence against which to compare song playback responses. In the second approach, I examined song divergence using the vocalization type as the unit of comparison to test whether there is a trend toward increased intra-repertoire differentiation in some taxa.

Taxon song differences. To determine whether the combined song repertoires of the six taxa were distinct, I first performed a separate Principal Components Analysis (PCA) of each of the three song types. Input for each PCA were the measurements of the 11 variables described above on the songs of multiple individuals from each taxon (104 per S1, 150 per S2, and 105 per S3; Table 1). To measure variation between the combined song repertoires of the six taxa, I entered the first two PCs from the PCA of each of the three individual song types into a new PCA. This approach allowed me to account for unevenness in the number of samples per song type across taxa, and obviated the need to exclude data from taxa for which I had better sampling

in order to have even numbers for each taxon for the input data. I chose only the first two PCs, because additional PCs (even those with eigenvalues >1) described intra-taxon song variation, and were not useful in separating taxa by song. I plotted the first two PCs that resulted from this second PCA (of combined song repertoire per taxon), and calculated the pairwise Euclidean distance between the six resulting points to create a measure of “taxon song distance” to use in subsequent analyses. I performed a Hierarchical Clustering Analysis (HCA) using taxon song distance to provide an additional way of visualizing song variation among the six taxa. All Principal Components Analyses were performed in JMP 11.2.0 (SAS Institute Inc. 2013). All other analyses were performed in RStudio Version 0.98.1062 (RStudio 2013).

Correlations between taxon song distance, mtDNA distance, and latitude. I used linear regression to examine whether mtDNA divergence could explain song divergence in this clade. In Chapter 2, I established a strong correlation between genotype and song type in *difficilis* and *hellmayri* for both nuclear DNA and mtDNA (haplotype frequency). A broader comparison of song divergence and genetic divergence across multiple taxa could reveal whether this relationship is maintained, or whether selection has acted differently along different branches of the *E. difficilis-occidentalis-flavescens* phylogeny, resulting in different rates of phenotypic change relative to genetic change. The direct genetic mechanisms underlying song divergence are unlikely to be located within the mitochondrial genome. Nevertheless, phylogenetic relationships based on mtDNA can give accurate and useful estimates of the history of divergence in a group of organisms (Kerr et al 2007), and thus provide a useful metric against which to compare the history of phenotypic divergence in a group of organisms (e.g., Price and Lanyon 2007, Weir and Wheatcroft 2010, Weir et al 2012).

Because bird song evolution can be affected by the latitude at which taxa breed (“taxon latitude”), it was necessary to examine whether song divergence was affected by latitude in this clade. First, I used linear regression to examine whether the mtDNA distance and latitudinal distance between taxa were correlated. For taxon latitude I used the latitudes at which the recordings used in this analysis were made. Other studies have used the mean latitude of the entire range of a taxon in analyses of the effect of latitude on phenotypic variation in birds (e.g., Cicero and Johnson 2002, Weir and Wheatcroft 2011). Because song can vary over a latitudinal scale that is much smaller than the geographic ranges of some of the taxa examined here (Collins et al 2009), I felt that it was more conservative to use the latitude at which the actual recordings examined were made. Thus, if selection intensity varied along the latitudinal range of a taxon, I would have a more accurate estimate of its effects. I used linear regression to examine whether taxon latitude could explain taxon song variation. In addition, I plotted histograms of the ratio of pairwise song distance to pairwise genetic distance to illustrate any mismatches between the rates of song divergence and genetic divergence among taxa. I also used linear regression to examine pairwise taxon distance for each of the three song types to determine whether the broader category of taxon song distance masked any closer relationships between individual song types and mtDNA genotype.

Divergence among vocalization types. One of the principal ways in which bird song has been shown to vary with latitude is through an increase in song complexity in migratory taxa breeding at high latitudes relative to related non-migratory taxa breeding at low latitudes (Read and Weary 1992, Price 2008, Weir and Wheatcroft 2011). Songs may be unlikely to diverge between closely related subspecies taxa through the addition of new song elements. Instead, I assessed complexity as increased “syllable diversity” – i.e., increased differentiation among the existing vocalizations that comprise the vocal repertoire of each taxon. Thus, the use of the word

“syllable” in this case refers to each of the three song types and the MPN, which I include as an additional homologous vocalization (128 MPN samples analyzed; Table 1). Because MPN is not a song type, I refer to the three song types plus MPN as the vocal repertoire (differentiating it from the song repertoire, comprised of the three song types). If these four vocalization types become increasingly differentiated from one another within a taxon, I assess this as an increase in syllable diversity.

In order to examine whether the songs of the higher latitude taxa in this clade exhibit increased syllable diversity, I employed an approach that, to my knowledge, has not been used in previous analyses of bird vocalizations. Because I measured the same homologous landmarks on all four vocalization types of all six taxa (24 unique vocalizations), I was able to perform multivariate analyses in which vocalization type (rather than taxon) was the unit of analysis. I used the mean value for each of the 11 input variables per vocalization type per taxon in a PCA to quantify the relationships between the 24 vocalizations. As in the analysis of taxon song, I used the first two PCs to calculate the pairwise Euclidean distance between the 24 vocalizations to create a measure of “vocal distance”. I calculated the mean pairwise intraspecific vocal distance for each taxon as a measure of syllable diversity.

Correlations between vocal distance and latitude. To test whether vocal repertoire complexity was higher in the taxa breeding at higher latitudes, I used linear regression to examine the relationship between syllable diversity and latitudinal distance (i.e., pairwise differences in taxon latitude). Syllable diversity could result from divergence among existing vocalization types within taxa. To examine how that divergence has taken place, and whether syllable diversity is driven by varying rates of evolutionary change among vocalizations, I compared the relative change in the four different vocalization types (i.e., the pairwise distance between vocalization types) across taxa with latitude. Due to the problems with including *difficilis* S3s detailed above, I also performed the same analyses of syllable diversity with S3 excluded for comparison.

Song playback experiments.

I performed 144 unique song playback (hereafter, “PB”) experiments across five taxa for this study (Table 1). This resulted in 536 trials for use in these analyses. These data were augmented with 196 trials that I performed for the study outlined in Chapter 2 (therefore, 732 total trials for the present study). Because I had already performed extensive tests of the responses of *difficilis* to *hellmayri* song (and vice versa), I did not perform those comparisons again for this study. Instead, I included data for those comparisons from Chapter 2 (including additional responses to conspecific song) in the present study. I felt justified in doing this as the individual experiment and the order in which the stimuli were presented were shown to have no effect on the responses of these taxa in Chapter 2.

PB experiments occurred during the breeding season for each taxon, and tested the responses of males of five taxa to six song types. I used song stimuli from all taxa for which I analyzed song in this study, but I did not perform PB experiments in *flavescens* populations. Taxa were tested with the songs of their nearest taxonomic relatives within the clade, as well as with the song of their own taxon (Table 2). Stimulus songs were created primarily from my own field recordings, but were supplemented with recordings from MVZ, ML, *xeno-canto.org*, and individual recordists.

Detailed methods for how song playback experiments were conducted are outlined in Chapter 2. The following changes were implemented in this series of experiments. Because of

the difficulty in performing experiments in several of the areas I visited, I analyze trials independently, rather than as part of a complete repeated measures experiment (i.e., the results of partially completed experiments in which a focal individual was tested with a subset of the designated stimulus songs were retained). Because the protocol for these experiments closely followed that outlined in Chapter 2, I feel that I can safely rule out the effect of stimulus order, stimulus exemplar, and calendar day on which the experiment was conducted (as these were shown to have no effect on responses in two of the focal taxa). In addition, because approach was shown to be so informative in the analyses in Chapter 2, and because song only occurred in experiments in *difficilis* populations, I monitored approach as the only response variable. The PB response data tended to be skewed toward low or high values, and thus tended to be non-normal. For that reason, I used Mann-Whitney-Wilcoxon Tests to examine pairwise differences in approach response to different song types in each focal taxon, including a Bonferroni correction for 51 comparisons (i.e., a significance value of $p = 0.001$). In addition, I used linear regression to test whether approach responses could best be explained by the mtDNA distance between the focal taxon and the song stimulus taxon or by song distance (taxon song distance) between the focal taxon and the song stimulus taxon.

RESULTS

Phylogenetic analysis of mtDNA

Phylogenetic analysis of ND2 retained 12 most parsimonious trees (tree length = 193, CI = 0.829, RI = 0.948) (Figure 3). Of 1041 characters, 886 characters were constant, 144 variable characters were parsimony informative, and 11 characters were parsimony uninformative. The strict consensus tree illustrated some interesting relationships among taxa, namely in the polyphyletic status of *E. occidentalis*. The Guerrero population is basal to the entire clade, exhibiting relatively high levels of mtDNA distance from both *occidentalis* (5.70%) and *hellmayri* (5.19%) (Table 2). Furthermore, *occidentalis* exhibits a relatively high level of mtDNA distance from *hellmayri* (3.49%). These relationships within *E. occidentalis* have not been elucidated in previous analyses of the phylogenetic relationships within this clade (Johnson 1980, Johnson and Cicero 2002). The sister status of *difficilis* and *hellmayri* and of *salvinii* and *flavescens* agrees with earlier analyses.

Bioacoustic analysis

Taxon song variation. PCA of the three individual song types showed that S2 was the most effective in separating taxa, although it separated taxa into three rather than six groups (Figure 4; Table E1). Not surprisingly, these groups agree with the traditional taxonomy of this group, much of which was based on phenotypic characters. The songs of *difficilis* tended to be the most distinct, and *difficilis* individuals tended to form a unique cluster in the plots of each song type. This was especially evident with respect to S2, in which *difficilis* individuals form a distinct and divergent cluster. By contrast, there is a high level of overlap between *hellmayri*, *occidentalis*, and “Guerrero”, and these taxa together form a fairly distinct cluster, especially in the S2 plot. The closely related *salvinii* and *flavescens* together form another fairly distinct cluster in each PCA, especially in the PCA of S2. The two taxa overlap considerably in the PCA of S3 and of MPN (for which there is only one sample for *flavescens*).

Both PCA (Figure 5) and HCA (Figure 6) separate taxa based on combined song repertoire and showed a congruent set of relationships. PCA of taxon song resulted in two PC

axes with eigenvalues >1 , that together explained 87.1% of the variation in song among taxa. PC1 had an eigenvalue of 2.79 and explained 46.5% of the variation, and PC2 had an eigenvalue of 2.44 and explained 40.6% of the variation (Table E2).

Correlations between taxon song distance and mtDNA distance. Mitochondrial genetic distance was not correlated with latitude ($R^2 = 0.04$; $p = 0.20$). Thus, both mtDNA distance and latitudinal distance can be treated as independent metrics against which song variation can be compared. Neither taxon song distance nor pairwise taxon distance for any of the three individual song types were correlated with mtDNA distance (taxon song distance: $R^2 = 0.02$, $p = 0.62$; S1: $R^2 = 0.03$, $p = 0.20$; S2: $R^2 = 0.01$, $p = 0.27$; S3: $R^2 = 0.01$, $p = 0.73$) (Figure 7). This reflects a mismatch between song divergence and genetic divergence evident in the relatively high level of divergence in song over a relatively small amount of mtDNA divergence in the comparison of *difficilis* and *hellmayri* and the relative stasis in song across much greater mtDNA divergence in comparisons of “Guerrero”, *occidentalis*, and *hellmayri* (Figure 8). This is evident in the histogram of the ratio of song distance to mtDNA distance, which highlights both of these trends (Figure 8). MPN distance was correlated with mtDNA distance ($R^2 = 0.17$, $p = 0.04$). This is notable considering that MPN is not a part of the song repertoire, and may not be acted on by the same selective forces.

Correlations between taxon song distance and latitude. Song distance was correlated with latitudinal distance, both at the level of taxon song ($R^2 = 0.32$; $p = 0.01$; Figure 7), and for each of the three song types (S1: $R^2 = 0.35$, $p = 0.003$; S2: $R^2 = 0.43$, $p = 0.001$; S3: $R^2 = 0.33$, $p = 0.004$; Figure 9). S1 and S2 show positive correlations with latitude, but S3 is negatively correlated with latitude. This is likely a result of the relatively divergent S3 of *salvinii*. MPN distance was correlated with latitude as well ($R^2 = 0.40$; $p = 0.001$).

Divergence among vocalization types. PCA of the four vocalization types of the six taxa (24 unique vocalizations) highlighted (i) a great deal of overlap among S2s and MPNs of most taxa, suggesting the close relationship of these vocal types; (ii) the uniqueness of the S3s; and (iii) the extreme divergence of the *difficilis* S2 (Figure 10). The first three PCs had eigenvalues >1 , and together explained 83.2% of the variation among song types (PC1 = 52.1%, PC2 = 17.7%, PC3 = 13.4%; Table E3). The close clustering of many of the vocal types highlights their structural similarity. The similarity among vocal types between *hellmayri*, *occidentalis*, and “Guerrero” is notable given the relatively high levels of mtDNA divergence between them, and points to relative stasis in song divergence among these taxa (the vocalizations of these taxa are very difficult to differentiate auditorily). The close relationship between *flavescens* and *salvinii* is evident as well, as well as the structural similarity between the S1s and MPNs of these taxa (also difficult to differentiate auditorily). These close relationships among vocalizations stand in contrast to the vocalizations of *difficilis*. While the S1, S3, and MPN are not particularly divergent from the corresponding vocalizations in the other five taxa, the *difficilis* S2 shows a level of divergence from any other vocalization that is greater than any other pairwise comparison of vocalizations. Comparison of the positions of the four vocalization types in Figure 10 also highlights the high level of distance between vocalizations within *difficilis* relative to intra-repertoire distances within other taxa, and indicates a higher level of syllable diversification in *difficilis* relative to the other taxa.

For the PCA of the three vocalizations per taxon (S3 excluded; 18 unique vocalizations), the first three PCs had eigenvalues >1 and together explained 83.1% of the variation among song types (PC1 = 49.1%, PC2 = 19.6%, PC3 = 14.4%). This analysis confirmed the patterns evident in the PCA of the four vocalizations, and highlights the higher level of syllable diversification

(higher syllable diversity) in *difficilis* to an even greater extent, once again, due largely to the highly divergent S2 in *difficilis*.

Correlations between vocal distance and latitude. Syllable diversity for the comparison of the *four* vocalization types was not correlated with latitude ($R^2 = 0.08$, $p = 0.29$; Figure 11). This seems to be a result of the relatively high level of divergence in *salvinii* S3s relative to the S3s of other taxa. Among the pairwise comparisons of the *four* individual vocal types, only S1–S2 distance was correlated with latitude ($R^2 = 0.68$, $p = 0.03$). This trend was driven by the relatively high level of distance between the *difficilis* S2 and S1. With S3 removed, syllable diversity shows a strong correlation with latitude ($R^2 = 0.68$, $p = 0.03$; Figure 11), and S1–S2 distance was even more strongly correlated with latitude ($R^2 = 0.84$, $p = 0.01$). As in the analysis of four vocal types, the other pairwise comparisons of individual vocal types showed no correlation with latitude.

Analysis of song playback experiments

Approach response to song playback was correlated with mtDNA distance between the taxon of the focal individual and the taxon of the stimulus song ($R^2 = 0.31$, $p = 0.002$), and even more highly with the distance between the song of the taxon of the focal individual and the taxon of the stimulus song ($R^2 = 0.64$, $p \ll 0.001$; Figure 12). Despite these correlations, pairwise comparison of responses to homotypic vs. heterotypic songs (Mann-Whitney-Wilcoxon Tests) showed some unexpected results (Figure 13, Table 3). Despite the high level of similarity between the songs of *hellmayri* and *occidentalis*, *occidentalis* discriminated between the two. Somewhat anomalously, the difference in response of *occidentalis* to homotypic song and *flavescens* song was not significant, despite showing strong discrimination between homotypic song and the song of the more closely related *salvinii* (which is very similar to *flavescens*). Although *hellmayri* responded more to its own song type than to *occidentalis* or to *difficilis*, the differences were not significant. Moreover, despite the high level of song distance between *hellmayri* and *difficilis* and the low level of song distance between *hellmayri* and *occidentalis*, reactions by *hellmayri* did not differ to the two song types ($p = 0.71$). “Guerrero” did not respond differently to any song type, but the number of experiments in this population was relatively low relative to other populations. *Salvinii* responded more to its own song than to any other song type. *Difficilis* responded more to its own song type than to any other.

DISCUSSION

Divergence in taxon songs

The relationships among the taxa in the *E. difficilis*–*occidentalis*–*flavescens* clade illustrated in the ND2 phylogeny generated for this study depart in important ways from the prevailing understanding of the relationships in this group (e.g., Brodkorb 1949, Johnson 1980) and provide an interesting context within which to examine patterns of vocal diversification and its effects on lineage divergence (Figure 3). The principal difference in the understanding of the evolutionary relationships among these taxa that emerges from the present study is the polyphyly of *E. o. occidentalis*.

Vocalizations are distinct in this group, but not always at the level of taxon (Figure 4). Rather than a distinct cluster for each of the six taxa, PCA of each of the four vocalizations tends to separate individuals into three main geographic groups that correspond to a Pacific Slope group (*E. d. difficilis*), a group occupying the interior western North American mountain ranges

(i.e., *E. occidentalis* as it is currently recognized, in the Rocky Mountains and associated ranges in the USA and in the Sierra Madre in Mexico), and a Central American group (*flavescens* and *salvini*; although these two taxa show a greater level of separation than the three constituent taxa of *E. occidentalis*). It is interesting that although the ND2 tree shows *E. occidentalis* (*hellmayri*, *occidentalis*, and “Guerrero” in this study) to be polyphyletic, the three taxa show a high level of vocal affinity. Future research should examine the relationships in this group and the pattern of historical divergence within this group in greater detail, ideally using multiple genetic loci. Nevertheless, analysis of the combined song repertoire (taxon song) did separate all taxa (Figure 5, Figure 6), and I was able to use the level of divergence between song repertoires (song distance) to test hypotheses regarding the process of vocal diversification in this group.

Among all of the taxa, *difficilis* is the most divergent in its vocalizations. This is perhaps most evident in the comparison with its sister taxon, *hellmayri*. Over the least amount of mtDNA distance (0.8%) between any two taxa in this study, *difficilis* shows a very high level of divergence from *hellmayri* in song repertoire (song distance = 4.7; Table 2, Figure 8). Song divergence in this group in general is driven primarily by the distinctiveness of S2 (Figure 4), which creates the greatest amount of separation among the three taxonomic groups mentioned above. This is especially true in *difficilis*, in which the S2 has become highly distinct relative to the S2 of other members of the clade (Figure 10), and appears as an outlier in all of the comparisons of S2 with mtDNA or latitudinal distance. This stands in contrast to the high level of vocal similarity evident in the polyphyletic *E. occidentalis* group, which, with genetic distances much larger than that between *difficilis* and *hellmayri* (range = 3.2%–5.7%), shows much lower differentiation in song (song distance range = 0.6–1.4).

Given these disparities, it is perhaps not surprising that song distance was not correlated with mtDNA distance (Figure 7). In Chapter 2, I showed a close correlation between both mitochondrial and nuclear genetic divergence and song divergence in the comparison of *difficilis*, *hellmayri*, and genetically admixed individuals. This correlation is due largely to the effects of admixture on two divergent song types. In a broader phylogenetic context, this correlation breaks down, and in fact, the high level of song divergence per genetic divergence between *difficilis* and *hellmayri* is a major reason for the lack of a correlation. Like any phenotypic trait, the degree to which a correlation exists between trait divergence and genetic divergence depends to a large extent on whether selection is acting on the trait. The mismatches between song divergence and genetic divergence evident in this clade suggest that song divergence has not been a strictly neutral process.

Divergence in vocal repertoires

The structure of the *difficilis* S2 is distinct from the other taxa due to a higher peak frequency (LM05) that results in a much more sharply peaked vocalization (vs. the more rounded peaks of the other S2s), by an amplitude break at the end of the first part of the vocalization (evident as a gap in the spectrogram tracing), and by an additional upward frequency sweep in the second half of the song (Figure 1, Figure 2). The break in the sound indicates that the *difficilis* S2 has become a non-linear vocalization. Because vocalizations that span large frequency ranges are difficult to produce as continuous sounds, the voice has a tendency to “crack”, or produce amplitude breaks in the sound – i.e., to become non-linear (Dooling 2004). Non-linear vocalizations exist in both birds and mammals (Fee et al 1998, Wilden et al 1998, Banta Lavanex 1999, Fitch et al 2002, Dooling 2004), and are thought to be energetically expensive sounds to produce (Lambrechts 1996, ten Cate et al 2002), and therefore potentially

honest signals of condition (Zahavi 1975, 1977, Collins 2004). Songs that are difficult to produce can serve as index signals of the actual fighting ability of singing males (Searcy and Beecher 2009).

Non-linear vocalizations are often associated with bird songs with higher frequency ratios among song elements. Birds are particularly sensitive to vocal frequency in general (Nelson 1988, Slabbekoorn and ten Cate 1998, Dooling 2004), but the frequency *contrast* in particular (evident as greater frequency ratios among song elements) has been shown to have more important effects on song salience (ten Cate et al 2002). For example, in Eurasian Collared-Doves (*Streptopelia decaocto*), heavier males produce larger frequency jumps, and territorial males respond more aggressively to playback of vocalizations that have greater frequency ratio contrasts among song elements than to vocalizations without frequency modulations (ten Cate et al 2002). In this case, it is the *change* in frequency among vocal elements rather than the overall highest frequency that elicits the greatest reactions (ten Cate et al 2002). Thus, selection for songs with greater frequency ratios can result in the evolution of non-linear vocalizations as the mechanism whereby greater frequency ratios are achieved.

An effect of greater frequency ratios in songs is a trend to toward simplification of other aspects of the song in order to accommodate the difficult non-linear vocalization. Lambrechts (1996) showed that songs with “song frequency plasticity” (SFP), or the abrupt modification of frequency between consecutive song elements, were difficult to produce in Great Tits (*Parus major*), and hypothesized that “when individuals deviate from individual-specific (or species-specific) frequencies to increase within-repertoire variation, they must change certain singing characteristics to sing with a minimum of plasticity. Males can thus decrease either the percentage of time spent singing, the rate of note repetition, or the intensity of sound at an extreme frequency” (pp. 317–318). Thus, the difficulty of producing a song with a non-linear vocalization and a high frequency ratio among elements can result in compromises in other aspects of song as energetic or biomechanical necessities. Simplification of the structure of elements of the song that accompany a non-linear vocalization seems like another possible response to SFP.

Among the taxa in the *E. difficilis*–*occidentalis*–*flavescens* clade, there seems to be a trend toward upward frequency sweeps in vocalizations, and, to some extent, a trend toward non-linear vocalizations. Figure 1 shows multiple vocalizations characterized by upward frequency sweeps spanning relatively large frequency bandwidths: *difficilis* S2 (peak frequency and an additional upward sweep at the end); *difficilis* MPN; *hellmayri*, *occidentalis*, and “Guerrero” S2 and MPN; *salvini* S1 and MPN. This suggests that frequency sweeps may affect signal salience in these taxa in general. In *difficilis*, a trend toward greater frequency sweeps and greater frequency ratios among song elements seems to have had important effects on the structure of the song repertoire. The exaggerated peak of the non-linear *difficilis* S2 creates a higher S2:S1 frequency ratio than in other taxa (Figure 1). It is more difficult to calculate the S2:S3 frequency ratio because I have used S3s that include an additional peak not evident in many *difficilis* S3s (Figure 1). In addition, it is possible to argue that S1 and S3 have become simplified in *difficilis*, perhaps due to pressure to accommodate the non-linear S2. S1 is not extremely different from the S1 of *hellmayri*, *occidentalis*, and “Guerrero”, but is noticeably truncated in comparison with *salvini* and *flavescens*. With respect to S3, the typical *difficilis* S3 has lost an entire segment of the song relative to the other taxa (Figure 1). Further research is needed to determine the importance of frequency ratios in these taxa (especially in *difficilis*), but the changes that have occurred in the repertoire of *difficilis* are consistent with selection for greater frequency ratios

among song elements and the concomitant changes in other aspects of song that often accompany increased frequency ratios. It is interesting that in the taxa in which S1 exhibits a greater frequency range (*salvini* and *flavescens*), S2 seems to have become simplified (seen, e.g., in the compressed frequency range). It is important to note that the vocal changes in *difficilis* are very unlikely to be simply a function of body size. There is substantial overlap in body size between *difficilis*, “Guerrero”, *salvini*, and *flavescens* (Johnson 1980), but far less overlap in vocal characters.

The *difficilis* S2 has become a much more divergent and complex song type relative any other vocalization in the clade. Each inflection point is exaggerated or “stretched” in the *difficilis* S2 resulting in a multi-parted, structurally complex sound. By measuring the same homologous landmarks on each vocalization type, I could compare intra-repertoire distance using multiple vocalization types (i.e., the three song types and MPN) to determine the relative rate at which overall vocal repertoire has diverged among taxa. Two main things stand out in these analyses: (1) the *difficilis* S2 is an outlier in the level of divergence from other vocalization types (Figure 10), and (2) largely (but not entirely) due to the divergent nature of the S2, intra-repertoire distance among four vocalization types (or among three vocal types when the S3 is excluded) is much greater in *difficilis* than in any of the other taxa (Table 2). In other words, syllable diversity, as seen in the increasing intra-taxon diversification among vocal elements is much greater in *difficilis*. MPNs show some similar patterns as songs in terms of trends toward upward frequency sweeps and the development of non-linear vocalizations (Figure 1). Non-linear MPNs occur in *hellmayri*, *occidentalis*, and “Guerrero”, none of which has non-linear song types. Future research should investigate the function of the MPN and its relation to song behavior.

An increase in syllable diversity is one metric that has been used to measure song repertoire evolution, and in particular, the evolution of increased song repertoire complexity (Read and Weary 1992, Weir and Wheatcroft 2011). Here I expand this to a broader view of *vocal* repertoire. The principal ways in which repertoire can change or become more complex in oscines are through the addition of new song elements, through performance of multiple song types, or through development of increased syllable diversity (Read and Weary 1992). Due to the much lower rate at which innate songs change, the addition of new vocal elements to an existing repertoire is likely to occur over much greater spans of time and may not be the type of change that is evident between closely related taxa such as those examined in the present study. Switching between song types is unlikely because species with innate song tend to have a single simple song (although perhaps multiple vocalization types). An increase in syllable diversity occurring through the modification of the structure of existing vocal elements seems a more likely way that innate song repertoires could diverge among closely related taxa. I argue that repertoire has diverged in this way in this clade, and is most evident in the relatively high level of syllable diversity that has evolved in *difficilis* relative to other members of the clade. Syllable diversity has increased in *difficilis* over a rapid evolutionary time frame, as seen in the increased distance between vocal elements relative to other members of the clade, and especially relative to its sister taxon, *hellmayri*. This provides a unique opportunity to observe how vocal repertoires can evolve in birds. This case may or may not be typical of how this process occurs in birds, but because song changes occur so rapidly relative to genetic change in birds with learned song, studies of avian repertoire evolution are typically limited in their ability to trace the evolution in any particular case (Hailman and Ficken 1996). The present study, with its utilization of homologous landmarks across vocalizations and across taxa provides a unique opportunity to

observe how an avian vocal repertoire can evolve, and how, via the development of increased syllable diversity, it can become more complex.

Mechanisms of vocal divergence

Sexual selection is an important force in the evolution of bird songs (Payne 1983, Andersson 1994, Ritchie 2007). This is especially true with respect to repertoire evolution (Catchpole 1982, Read and Weary 1992). The context within which sexual selection acts on song repertoire depends on the life history and the natural history of the taxon. Higher levels of extra-pair mating, higher levels of parental care, and migratory behavior are all correlated with latitude and are correlated with more complex repertoires (Kunkel 1974, Read and Weary 1992). This can lead to differences in repertoire complexity even among members of the same genus (Read and Weary 1992). Recent studies (Collins et al 2009, Weir and Wheatcroft 2011) have highlighted elevated rates of signal diversification and song complexity in higher latitude migratory species. An abbreviated and accelerated breeding season in higher latitude migratory species is thought to increase the selective pressure on song because female mate choice, based at least in part on song type, must take place within a shorter period of time (Read and Weary 1992, Irwin 2000, Weir and Wheatcroft 2011). This is in contrast to tropical species, in which the breeding season is protracted, and pair bonds between mates often persist beyond a single breeding season (Kunkel 1974, Stutchbury and Morton 2001).

Because I have been able to characterize changes in repertoire over a clade of closely related species with both migratory and non-migratory taxa, and with a combined distribution spanning over 50° of latitude, the *E. difficilis-occidentalis-flavescens* clade provides an interesting test case for the effects of latitude on song divergence. Although taxon song divergence was not correlated with mtDNA divergence, it was correlated with latitudinal distance (Figure 7). This is reflective of the three main clusters that resulted from the analyses of the four vocal types per taxon – i.e., there is a latitudinal pattern of song divergence from *E. flavescens* through the *E. occidentalis* group to *E. difficilis* evident for the combined taxon repertoire or for each individual vocalization type, that is not congruent with the phylogenetic relationships. This pattern is suggestive of an effect of latitude on song divergence, but does not provide information on the effects of latitude on repertoire complexity *per se*. The effects of latitude on syllable diversity, the metric of repertoire complexity that I utilize in this study, are more mixed. Examination of syllable diversity using pairwise distances between all four vocalization types does not show a correlation with latitude. When S3 is removed, the correlation was significant ($R^2 = 0.68$, $p = 0.03$). While, the difference between these two comparisons cannot be ignored, I attribute the difference largely to the necessity of including *difficilis* S3s that were more similar to the other taxa (see Methods) and thus result in an underestimate of syllable diversity in *difficilis*. The comparison is also affected by the relatively divergent S3 of *salvini*, and I cannot rule out without further study that different song types affect the salience of the overall song repertoire across taxa. Importantly, syllable diversity in *difficilis* is driven by S2, and S2 changes unambiguously with latitude (Figure 11). Thus, these results are consistent with studies that have found increased selection for repertoire complexity in migratory species breeding at high latitudes. A broader study, perhaps of song diversification within the entire *Empidonax* genus, could better address this.

Song playback experiments

With the exception of *difficilis* and *hellmayri*, there is no known geographic contact between any of the taxa in this analysis (moreover, the three more restricted subspecies that I excluded from the analysis are also allopatric). I used PB experiments to simulate contact between taxa in order to examine the functional effects of varying levels of song divergence and varying levels of repertoire complexity on taxon recognition.

As in the analyses in Chapter 2, the results of the PB experiments indicated an overall linear decrease in the magnitude of approach response with increasing song distance (Figure 12). There are some important exceptions to this, however, evident in the pairwise comparisons of responses to homotypic to heterotypic songs (Table 3, Figure 13). Similar to Chapter 2, *hellmayri* did not show a high level of discrimination among song types; responses to homotypic song were not significantly greater than responses to either *difficilis* or *occidentalis* song, and responses to those two divergent song types did not differ. In contrast, *occidentalis* exhibited a higher level of discrimination among song types, as approach was significantly greater to homotypic song than to either of the very similar songs of *hellmayri* or “Guerrero”. “Guerrero” exhibited slightly higher approaches to homotypic song than to the songs of its closest relatives, but the differences were not significant. Thus, whether the small song distances that characterize *hellmayri*, *occidentalis*, and “Guerrero” are salient varies among those taxa. For *occidentalis*, the slight differences seem to be salient signals of taxon identity. On the other hand, the slight differences do not seem to be adequate to result in taxon recognition in *hellmayri* or “Guerrero”. Fewer experiments were performed in “Guerrero” than in other taxa, so additional experiments would help to determine whether this taxon truly exhibits lower levels of song discrimination.

For *hellmayri*, this could be an indication of a broader preference function that might be more typical of some higher latitude migratory species (see below). There is no evidence that the more complex repertoire of *difficilis*, perhaps the result of sexual selection, is salient across taxa. As seen in Chapter 2, *difficilis* exhibited a high level of discrimination among song types. The lowest latitude taxon in which I performed PB experiments, *salvini*, also showed a high level of discrimination among song types. This suggests that smaller changes in song may be adequate taxon recognition cues for lower latitude species. In higher latitude taxa, more extreme differences might be necessary, perhaps in response to pressure to choose a mate in a limited amount of time. More subtle cues might function as adequate taxon recognition signals in lower latitude taxa, due to the protracted breeding season and pair bonds that occur in many lower latitude bird species (Kunkel 1974, Stutchbury and Morton 2001). Additional PB experiments performed in *flavescens* populations (the lowest latitude taxon) would add to a more complete understanding of the effects of latitude on taxon recognition in this clade.

An important caveat in the assessment of song differences as potential behavior barriers in this group is that, according to present knowledge, only *difficilis* and *hellmayri* meet in geographic contact. In Chapter 2, I showed that despite the apparently high level of discrimination among song types by *difficilis*, a high level of admixture has occurred between these taxa (although gene flow from the contact zone to parental *difficilis* populations is limited). I point out in that study that whether divergent song types result in behavioral barriers to gene flow depends on the larger behavioral and ecological context that exists inside and outside of the area of contact. Thus, for the taxa with highest level of song divergence per genetic distance in this group, hybridization and admixture has occurred in secondary contact. We do not know what the outcome of secondary contact between other taxa in the group would be, due to mitigating factors such as the density of conspecific mates relative to heterospecific mates in the area of

contact, or differences in other behaviors related to reproduction and territorial defense (e.g., asymmetric aggression levels, as seen in Chapter 2).

Even if song divergence did not result in behavioral isolation and reproductive isolation between *difficilis* and *hellmayri*, that does not indicate that selection is not affecting song, nor that latitude has not affected vocal evolution in this clade. An abbreviated breeding season could affect both song divergence and taxon recognition. If the cost of mate searching is elevated in migratory taxa due to a shorter breeding season, these taxa might exercise less discrimination – i.e., they might be willing to recognize individuals exhibiting a broader range of phenotypes as conspecifics and potential competitors or mates (Price 2008). This could be especially evident in secondary contact zones where conspecific mates can be rare due to low population densities. This could lead to the evolution of more complex songs to aid in mate choice, and because there is no trait optimum imposed by natural selection, could result in divergent song types between populations (West-Eberhard 1983). In this way, the decreased amount of time available for mate choice might drive the evolution of more divergent song types in migratory taxa. Whether these divergent song types act as behavioral barriers to hybridization depends on the interaction between increased sexual selection for complex song and the cost of mate searching in populations with shortened breeding seasons (Price 2008). The divergent song of *difficilis* may have evolved because it aids in mate choice, but song divergence may not have proceeded to the point that it prevents hybridization with *hellmayri*. This illustrates the advantages presented by studies of secondary contact zones between taxa when these types of questions are being investigated, as the full scope of factors that affect the outcome of secondary contact are difficult to anticipate.

CONCLUSION

The *E. difficilis–occidentalis–flavescens* clade provides an interesting opportunity to examine patterns of vocal divergence in birds. Rates of song divergence vary across taxa, complicating the relationship between song type and genotype that has long been assumed for suboscine birds (e.g., Isler and Isler et al 2005, but see Raposo and Höfling 2003), and pointing out the need to test hypotheses regarding the patterns and function of vocal divergence on lineage diversification with in depth field-based studies. Rates of vocal evolution differ markedly across taxa in this clade, suggesting a role for sexual selection in trait divergence. Song divergence is more closely correlated with the latitudinal distance between taxa than with the genetic (mtDNA) distance. Song complexity seems to be correlated with latitude as well. On the other hand, at least some of the lower latitude taxa seem to be capable of using very small differences to discriminate between homotypic and heterotypic song. Taken together, these findings suggest that small differences in song may be adequate taxon recognition cues for lower latitude species, whereas higher latitude taxa may require more extreme differences, perhaps in response to pressure to choose a mate in a limited amount of time. This study provides a unique view into how vocal repertoires can evolve in birds, and how this relates to lineage diversification, and points out the critical importance of examining song divergence within the proper ecological context to fully understand how divergent song types affect taxon recognition.

TABLES & FIGURES

Table 1. Numbers and locations of genetic samples, samples of different vocalization types, and song playback experiments (“PB”) used in this study.

Table 2. Pairwise distances in latitudinal range, mtDNA (ND2) sequence, and song for six taxa examined in this study. Also, “intra-rep distance”, or mean pairwise Euclidean distance between conspecific vocalization types that comprise the vocal repertoire of each taxon (also referred to in the text as “syllable diversity”) calculated from the first two PCs of a PCA of individual vocalization types. Intra-rep distance is shown for both the analysis using four vocalizations and for the analysis using three vocalizations. Also listed are the numbers of PB experiments testing the song of Taxon 2 in populations of Taxon 1.

Table 3. Comparisons from song playback experiments tested with Mann-Whitney-Wilcoxon Tests. All comparisons except for one are between responses to homotypic song and one of the heterotypic song types (the exception is *hellmayri:difficilis* vs. *hellmayri:occidentalis*). P-values in boldfaced type are significant after a Bonferroni correction for multiple comparisons ($p = 0.003$). All significant p-values indicate a greater response to homotypic song.

Figure 1. Sound spectrograms of song and MPN for the six taxa examined in this study. The left column shows the three-part song repertoire and the right column shows MPNs.

Figure 2. Plots of the landmarks used to derive the variables used in the analysis of vocalizations in this study. Each landmark plotted here represents the mean value for frequency and time (relative to the beginning of the song) per vocalization type per taxon. The lines connecting the landmark points are not necessarily characters used in this analysis, but are present to facilitate the comparison with the spectrograms of the vocalizations in Figure 1.

Figure 3. Strict consensus tree from a maximum parsimony analysis of the ND2 gene. Bootstrap values for the nodes are based on 1000 replicates with 5 random addition replicates per bootstrap replicate.

Figure 4. Plot of the first two principal components from a PCA of each of four vocalization types (six taxa). In each plot, axes show the proportion of the variance explained by PC1 and PC2. Colors indicate the following taxa: red = *difficilis*, orange = *hellmayri*, green = *occidentalis*, black = “Guerrero”, blue = *salvinii*, and purple = *flavescens*.

Figure 5. Plot of the first two principal components from a Principal Components Analysis performed of the combined song repertoire (three song types) for each of six taxa. This is referred to as “taxon song” in the text. In each plot, axes show the proportion of the variance explained by PC1 and PC2. Letters indicate the following taxa: D = *difficilis*, H = *hellmayri*, O = *occidentalis*, G = “Guerrero”, S = *salvinii*, and F = *flavescens*.

Figure 6. Dendrogram from a Hierarchical Clustering Analysis of the combined song repertoire (taxon song) for each of six taxa.

Figure 7. Left: linear model of song distance as a function of mtDNA distance between taxa ($R^2 = 0.02$, $p = 0.62$). Right: linear model of song distance as a function of latitudinal distance between taxa ($R^2 = 0.32$; $p = 0.01$).

Figure 8. Histogram of the ratio of song distance to mitochondrial distance for each pairwise comparison of 6 taxa. Headings at the top of the graph show the particular comparison corresponding to that value. Comparisons including *difficilis* are clustered at the right of the plot (high values), while comparisons including “Guerrero” tend to be clustered toward the left of the plot (low values). Abbreviations indicate the following taxa: diff = *difficilis*, hell = *hellmayri*, occi = *occidentalis*, guer = “Guerrero”, salv = *salvinii*, flav = *flavescens*.

Figure 9. Plots of linear models of the pairwise distance between six taxa for the four vocalization types as functions of latitude. S1: $R^2 = 0.35$, $p = 0.003$; S2: $R^2 = 0.43$, $p = 0.001$; S3: $R^2 = 0.33$, $p = 0.004$; MPN: $R^2 = 0.40$, $p = 0.001$.

Figure 10. Left: Plot of the first two principal components from a Principal Components Analysis performed of 24 vocalization types (four vocalization types for six taxa). Right: Plot of the first two principal components from a Principal Components Analysis performed of 18 vocalization types (three vocalization types for six taxa). In each plot, axes show the proportion of the variance explained by PC1 and PC2. Symbols indicate the following vocalization types: 1 = Song 1, 2 = Song 2, 3 = Song 3, M = MPN. Colors indicate the following taxa: red = *difficilis*, orange = *hellmayri*, green = *occidentalis*, black = “Guerrero”, blue = *salvinii*, and purple = *flavescens*.

Figure 11. Left column: Linear models of syllable diversity (mean pairwise Euclidean distance among vocalization types per taxon) as a function of latitude, based on distances between four vocalization types (top; $R^2 = 0.08$, $p = 0.29$) and three vocalization types (bottom; $R^2 = 0.68$, $p = 0.03$). Right: Linear models of mean Song 1–Song 2 distance per taxon as a function of latitude, based on distances between four vocalization types (top; $R^2 = 0.68$, $p = 0.03$) and three vocalization types (bottom; $R^2 = 0.84$, $p = 0.01$).

Figure 12. Approach response from song playback experiments as a function of mtDNA distance (left; $R^2 = 0.31$, $p = 0.002$) and song distance (right; $R^2 = 0.64$, $p \ll 0.001$).

Figure 13. Approach response to six song stimulus types by five taxa from song playback experiments. Error bars show 95% confidence interval.

Table 1.

TAXON	SITE	COUNTRY	ST/PROV	COUNTY	LAT	LONG	SONG1	SONG2	SONG3	MPN	PB
<i>E. d. difficilis</i>	Haida Gwaii	Canada	British Columbia	-	53.6	-132.2	1	-	5	2	-
<i>E. d. difficilis</i>	Skagit River	USA	Washington	Skagit, Whatcom	48.6	-121.4	1	-	2	-	-
<i>E. d. difficilis</i>	Olympic Peninsula	USA	Washington	Clallum	47.9	-123.0	1	1	1	1	-
<i>E. d. difficilis</i>	Western Washington	USA	Washington	Pierce	46.9	-121.6	-	2	1	2	-
<i>E. d. difficilis</i>	Coos Bay	USA	Oregon	Coos	43.4	-124.2	2	-	2	1	-
<i>E. d. difficilis</i>	N California Coast	USA	California	Humboldt	40.9	-124.0	-	1	-	3	-
<i>E. d. difficilis</i>	Elk Creek	USA	California	Glenn	39.8	-122.7	-	2	-	2	-
<i>E. d. difficilis</i>	N San Francisco Bay	USA	California	Marin, Alameda	37.9	-122.7	4	11	2	3	20
<i>E. d. difficilis</i>	Yosemite	USA	California	Mariposa	37.7	-119.8	-	-	-	3	-
<i>E. d. difficilis</i>	S San Francisco Bay	USA	California	Santa Clara	37.2	-122.3	7	11	5	9	11
<i>E. d. difficilis</i>	Carmel Valley	USA	California	Monterey	36.4	-121.6	4	4	2	2	2
<i>E. d. difficilis</i>	Santiago Oaks	USA	California	Orange	33.8	-117.8	-	2	-	-	-
<i>E. o. hellmayri</i>	San Francisco Peaks	USA	Arizona	Coconino	35.3	-111.7	4	6	4	4	9
<i>E. o. hellmayri</i>	White Mountains	USA	Arizona	Apache	33.9	-109.1	-	2	-	-	-
<i>E. o. hellmayri</i>	Black Range	USA	New Mexico	Grant	32.9	107.8	4	8	2	5	-
<i>E. o. hellmayri</i>	Pinaleno Mtns	USA	Arizona	Graham	32.6	-109.8	5	4	4	6	19
<i>E. o. hellmayri</i>	Santa Catalina Mtns	USA	Arizona	Pima	32.4	-110.8	-	1	2	1	-
<i>E. o. hellmayri</i>	Guadalupe Mtns	USA	Texas	Culbertson	31.9	-104.8	2	2	3	2	-
<i>E. o. hellmayri</i>	Chiricahua Mtns	USA	Arizona	Cochise	31.9	-109.2	4	5	5	9	-
<i>E. o. hellmayri</i>	Davis Mtns	USA	Texas	Jeff Davis	30.7	-104.1	1	2	1	3	-
<i>E. o. occidentalis</i>	Creel	Mexico	Chihuahua	-	27.7	-107.6	2	2	2	-	-
<i>E. o. occidentalis</i>	Durango Highway	Mexico	Sinaloa	-	23.5	-106.5	-	-	-	1	-
<i>E. o. occidentalis</i>	Xilitla	Mexico	Queretaro	-	21.2	-99.2	-	-	-	1	-
<i>E. o. occidentalis</i>	Tlanchinol	Mexico	Hidalgo	-	21.0	-98.7	1	1	-	-	-
<i>E. o. occidentalis</i>	Xalapa	Mexico	Veracruz	-	19.5	-96.9	16	26	17	24	37
<i>E. o. occidentalis</i>	Cuernavaca	Mexico	Morelos	-	19.0	-99.2	-	-	-	3	-
<i>E. o. occidentalis</i>	Pollo Nino	Mexico	Oaxaca	-	17.1	-96.6	1	1	1	1	-
<i>E. o. occidentalis</i> (Guerrero)	Omiltemi	Mexico	Guerrero	-	17.6	-99.7	20	22	20	16	14
<i>E. f. salvinii</i>	San Cristobal de las Casas	Mexico	Chiapas	-	16.7	-92.7	8	13	10	15	22
<i>E. f. salvinii</i>	Antigua	Guatemala	Sacatepéquez	-	14.5	-90.7	12	17	10	8	10
<i>E. f. flavescens</i>	Talamanca Mtns	Costa Rica	San José	-	9.6	-83.7	4	4	4	1	-
TOTAL							104	150	105	128	144

Table 2.

Taxon 1	Taxon 2	Latitudinal distance	mtDNA distance (%)	Song distance	Taxon 1 intra-rep distance (4 vocals)	Taxon 1 intra-rep distance (3 vocals)	No. PB trials testing Txn2 song on Txn1
<i>E. d. difficilis</i>	<i>difficilis</i>	-	-	-	7.4	8.8	83*
	<i>hellmayri</i>	6	0.77	4.72			44**
	<i>occidentalis</i>	18	3.49	5.95			32
	Guerrero	20	5.25	5.79			20
	<i>salvinii</i>	23	4.87	5.73			31
	<i>flavescens</i>	30	4.90	6.28			-
<i>E. o. hellmayri</i>	<i>difficilis</i>	6	0.77	4.72	5.2	5.0	40**
	<i>hellmayri</i>	-	-	-			90***
	<i>occidentalis</i>	12	3.23	1.31			29
	Guerrero	14.4	5.19	1.41			-
	<i>salvinii</i>	17	4.42	4.64			28
	<i>flavescens</i>	23	4.45	4.18			-
<i>E. o. occidentalis</i>	<i>difficilis</i>	18	3.49	5.95	4.4	4.4	18
	<i>hellmayri</i>	12	3.23	1.31			35
	<i>occidentalis</i>	-	-	-			36
	Guerrero	2.4	5.70	0.59			17
	<i>salvinii</i>	5	4.35	4.68			18
	<i>flavescens</i>	11	4.39	3.92			16
<i>E. o. occidentalis</i> (Guerrero)	<i>difficilis</i>	20	5.25	5.79	4.3	3.4	-
	<i>hellmayri</i>	14.4	5.19	1.41			12
	<i>occidentalis</i>	2.4	5.70	0.59			13
	Guerrero	-	-	-			13
	<i>salvinii</i>	2.6	5.16	4.09			13
	<i>flavescens</i>	9	4.93	3.33			-
<i>E. f. salvinii</i>	<i>difficilis</i>	23	4.87	5.73	6.3	2.3	18
	<i>hellmayri</i>	17	4.42	4.64			18
	<i>occidentalis</i>	5	4.35	4.68			32
	Guerrero	2.6	5.16	4.09			14
	<i>salvinii</i>	-	-	-			29
	<i>flavescens</i>	6	1.13	1.17			33
<i>E. f. flavescens</i>	<i>difficilis</i>	30	4.90	6.28	5.1	3.4	-
	<i>hellmayri</i>	23	4.45	4.18			-
	<i>occidentalis</i>	11	4.39	3.92			-
	Guerrero	9	4.93	3.33			-
	<i>salvinii</i>	6	1.13	1.17			-
	TOTAL TRIALS						

*Includes 50 trials performed for Chapter 2 analyses

**Trials performed for Chapter 2 analyses

***Includes 62 trials performed for Chapter 2 analyses

Table 3.

PB Comparison 1		PB Comparison 2		Mann-Whitney-Wilcoxon Test	
Taxon 1	Taxon 2	Taxon 1	Taxon 2	W statistic	p-value
<i>E. d. difficilis</i>	<i>difficilis</i>	<i>difficilis</i>	<i>hellmayri</i>	2247.5	1.83E-07
<i>E. d. difficilis</i>	<i>difficilis</i>	<i>difficilis</i>	<i>occidentalis</i>	2031.5	4.86E-08
<i>E. d. difficilis</i>	<i>difficilis</i>	<i>difficilis</i>	Guerrero	1142.5	8.01E-05
<i>E. o. hellmayri</i>	<i>hellmayri</i>	<i>hellmayri</i>	<i>difficilis</i>	1743	0.015
<i>E. o. hellmayri</i>	<i>hellmayri</i>	<i>hellmayri</i>	<i>occidentalis</i>	1231	0.053
<i>E. o. hellmayri</i>	<i>difficilis</i>	<i>hellmayri</i>	<i>occidentalis</i>	549.5	0.712
<i>E. o. occidentalis</i>	<i>occidentalis</i>	<i>occidentalis</i>	<i>hellmayri</i>	1026.5	4.93E-06
<i>E. o. occidentalis</i>	<i>occidentalis</i>	<i>occidentalis</i>	Guerrero	484	0.001
<i>E. o. occidentalis</i>	<i>occidentalis</i>	<i>occidentalis</i>	<i>salvinii</i>	607	1.55E-07
<i>E. o. occidentalis</i>	<i>occidentalis</i>	<i>occidentalis</i>	<i>flavescens</i>	440	0.0026
Guerrero	Guerrero	Guerrero	<i>hellmayri</i>	104.5	0.1474
Guerrero	Guerrero	Guerrero	<i>occidentalis</i>	97.5	0.5147
Guerrero	Guerrero	Guerrero	<i>salvinii</i>	104.5	0.3097
<i>E. f. salvinii</i>	<i>salvinii</i>	<i>salvinii</i>	<i>occidentalis</i>	887	1.68E-10
<i>E. f. salvinii</i>	<i>salvinii</i>	<i>salvinii</i>	Guerrero	382.5	2.64E-06
<i>E. f. salvinii</i>	<i>salvinii</i>	<i>salvinii</i>	<i>flavescens</i>	784.5	1.41E-05

Figure 1.

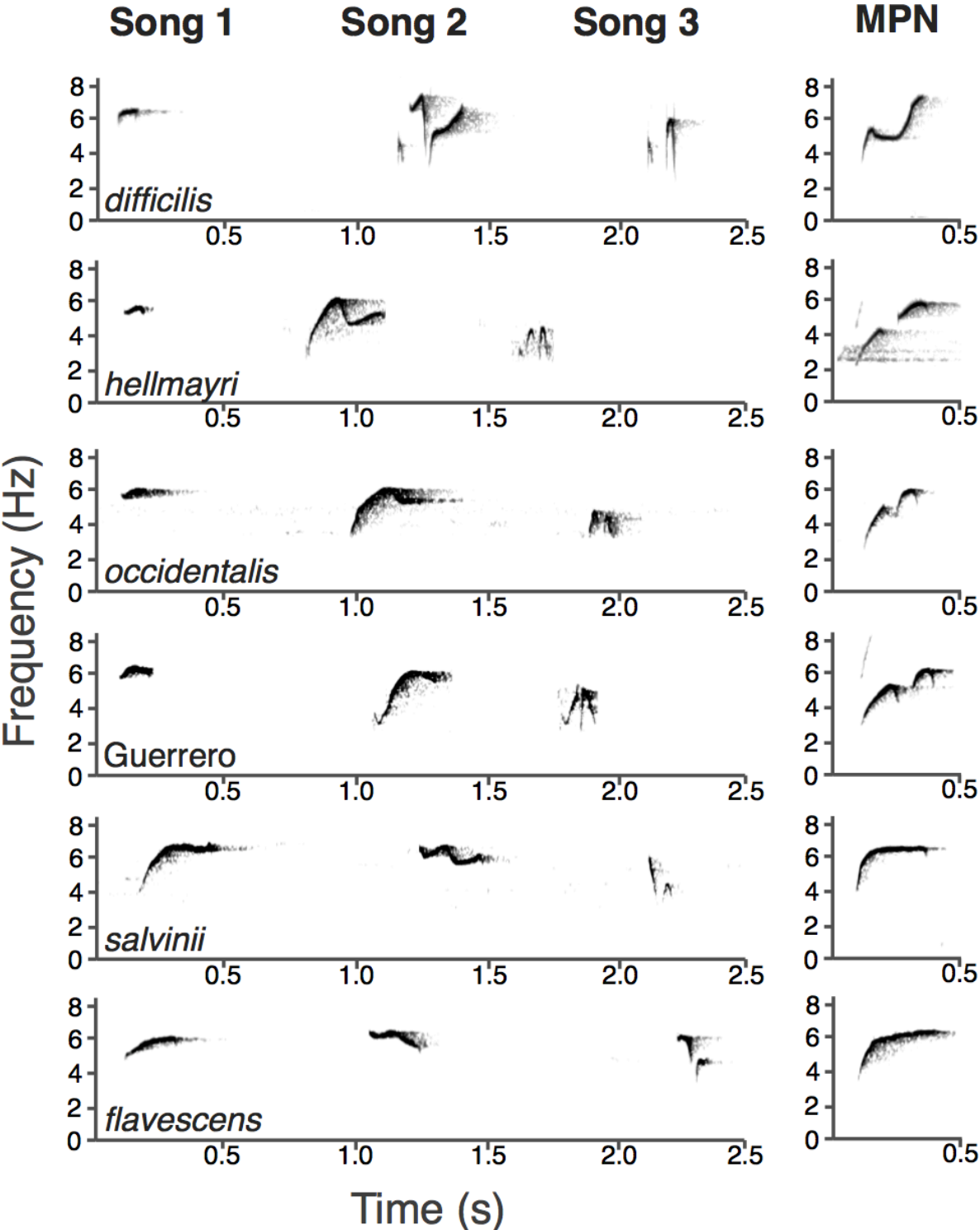


Figure 2.

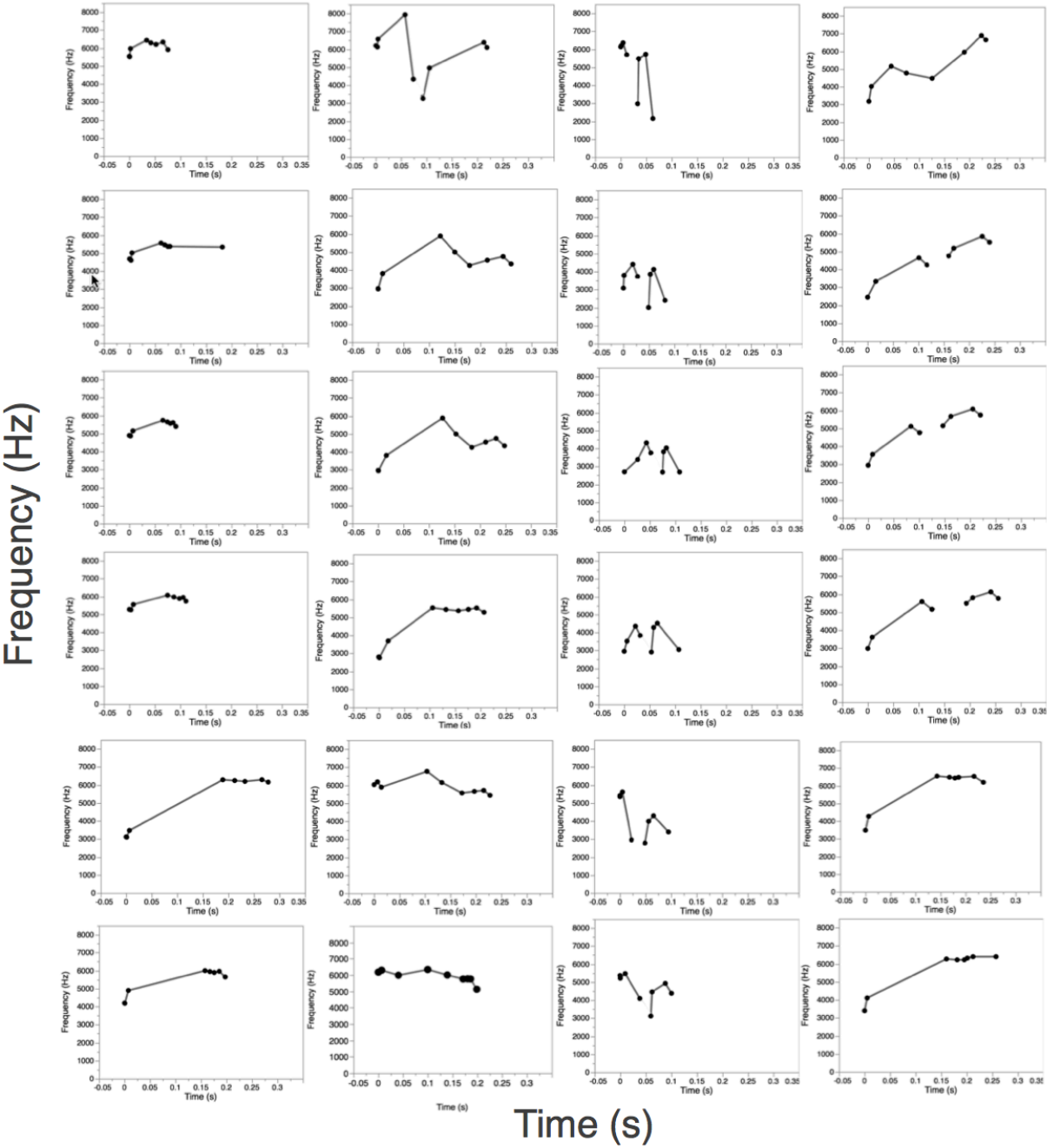


Figure 3.

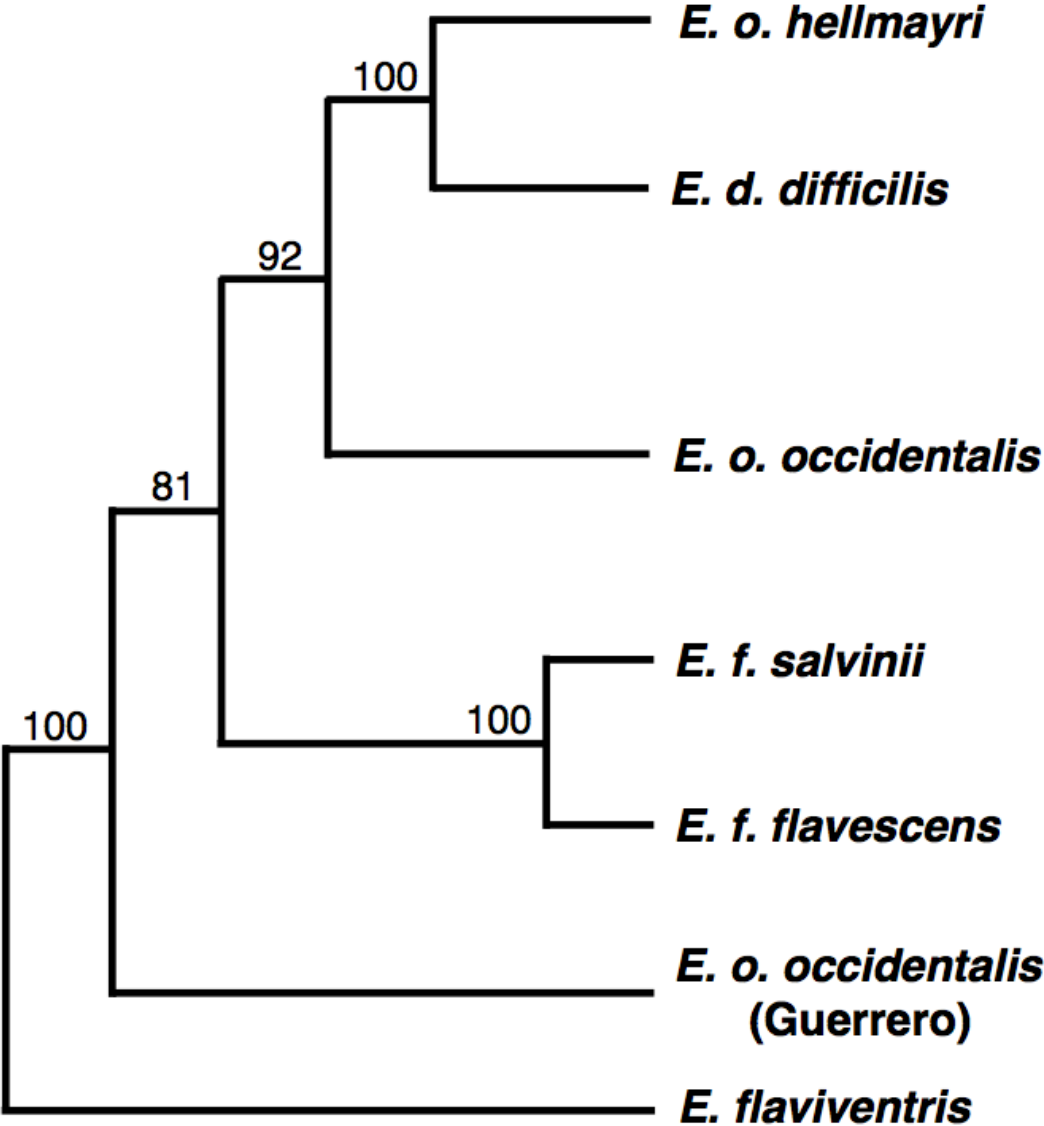


Figure 4.

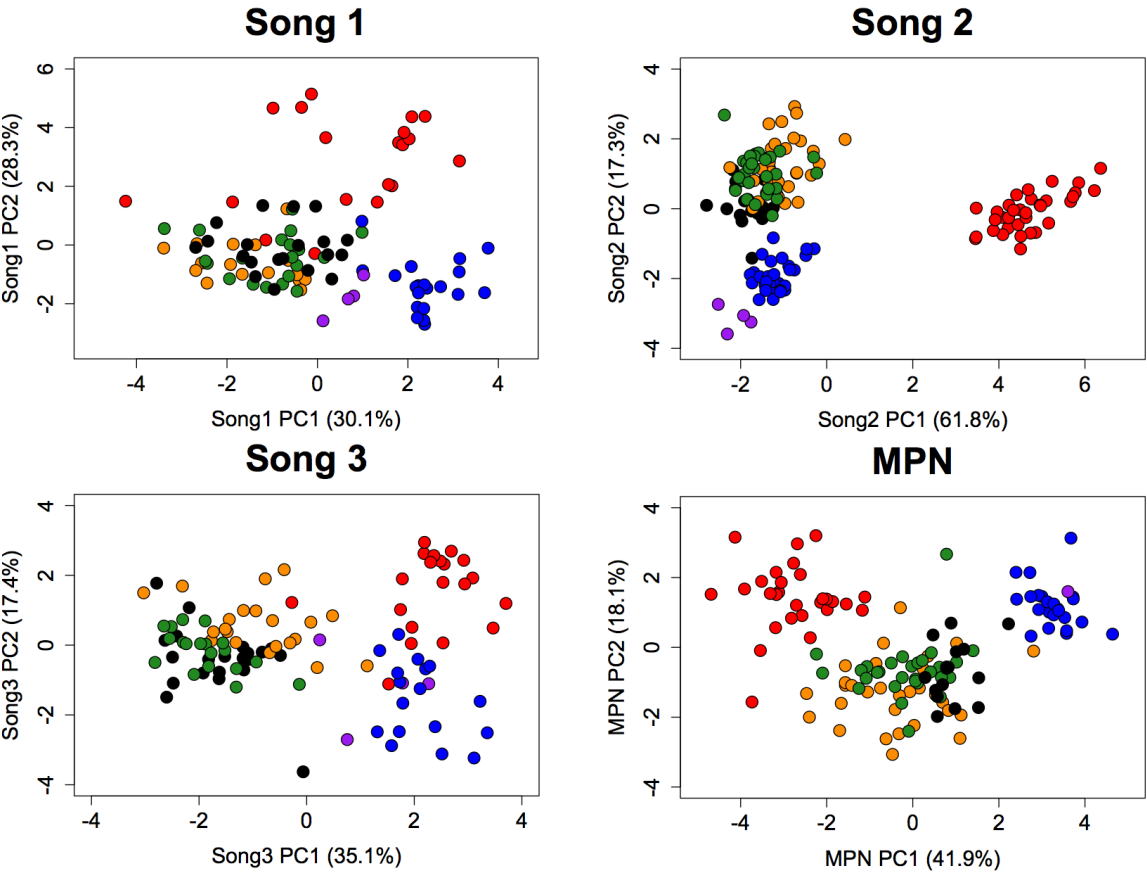


Figure 5.

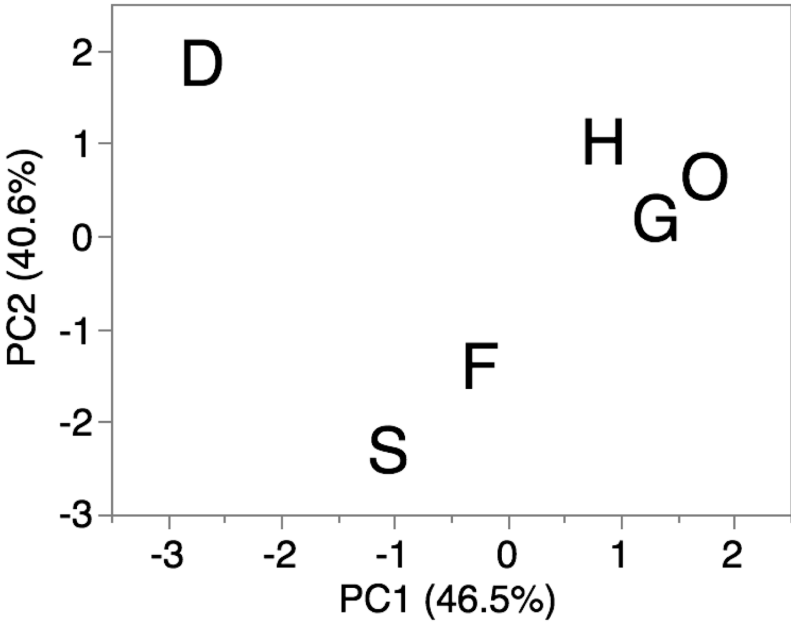


Figure 6.

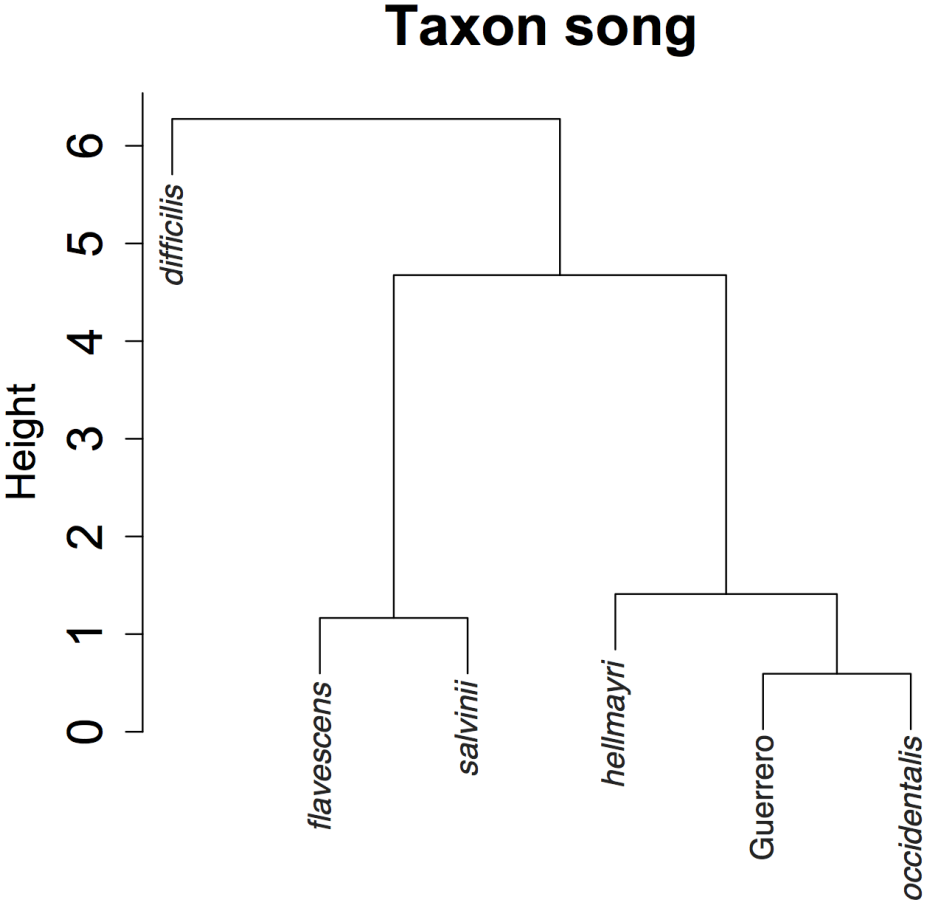


Figure 7.

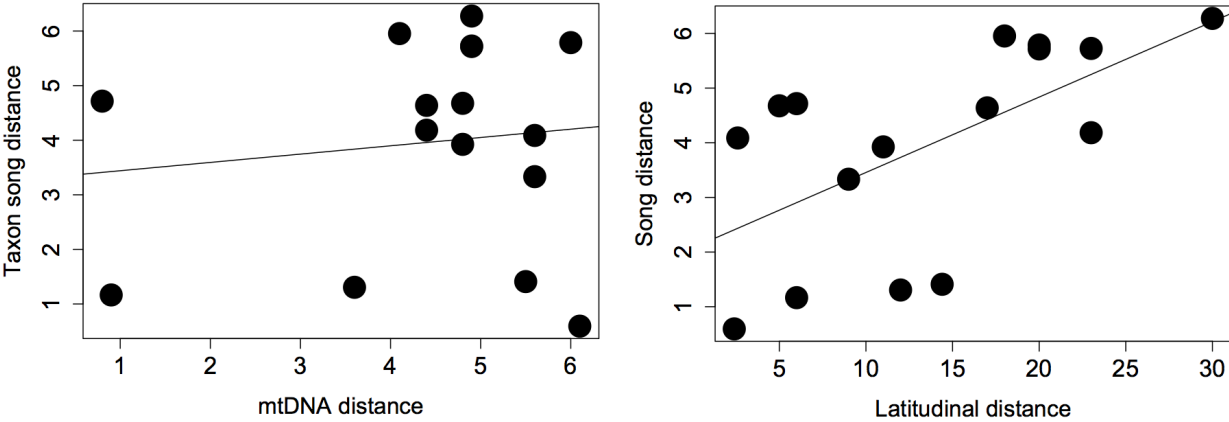


Figure 8.

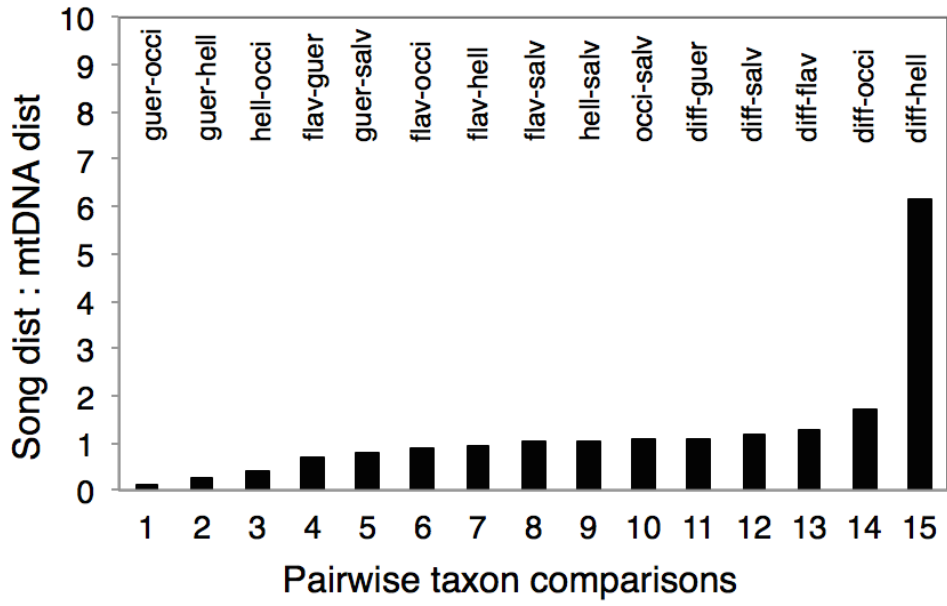


Figure 9.

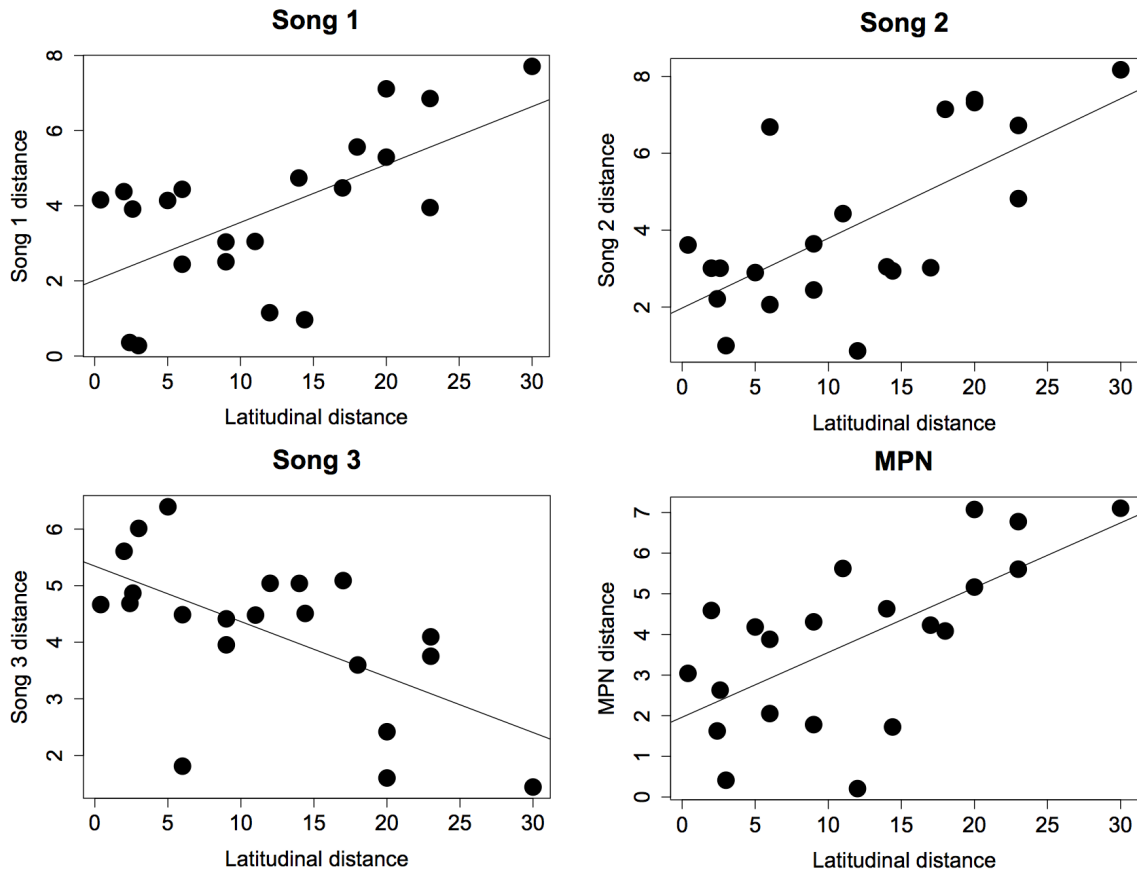


Figure 10.

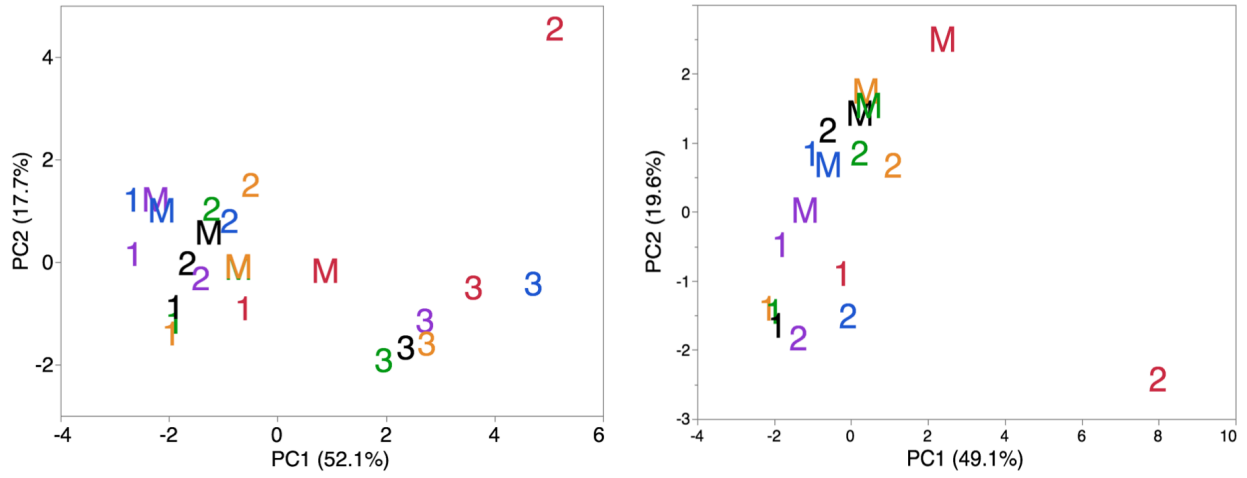


Figure 11.

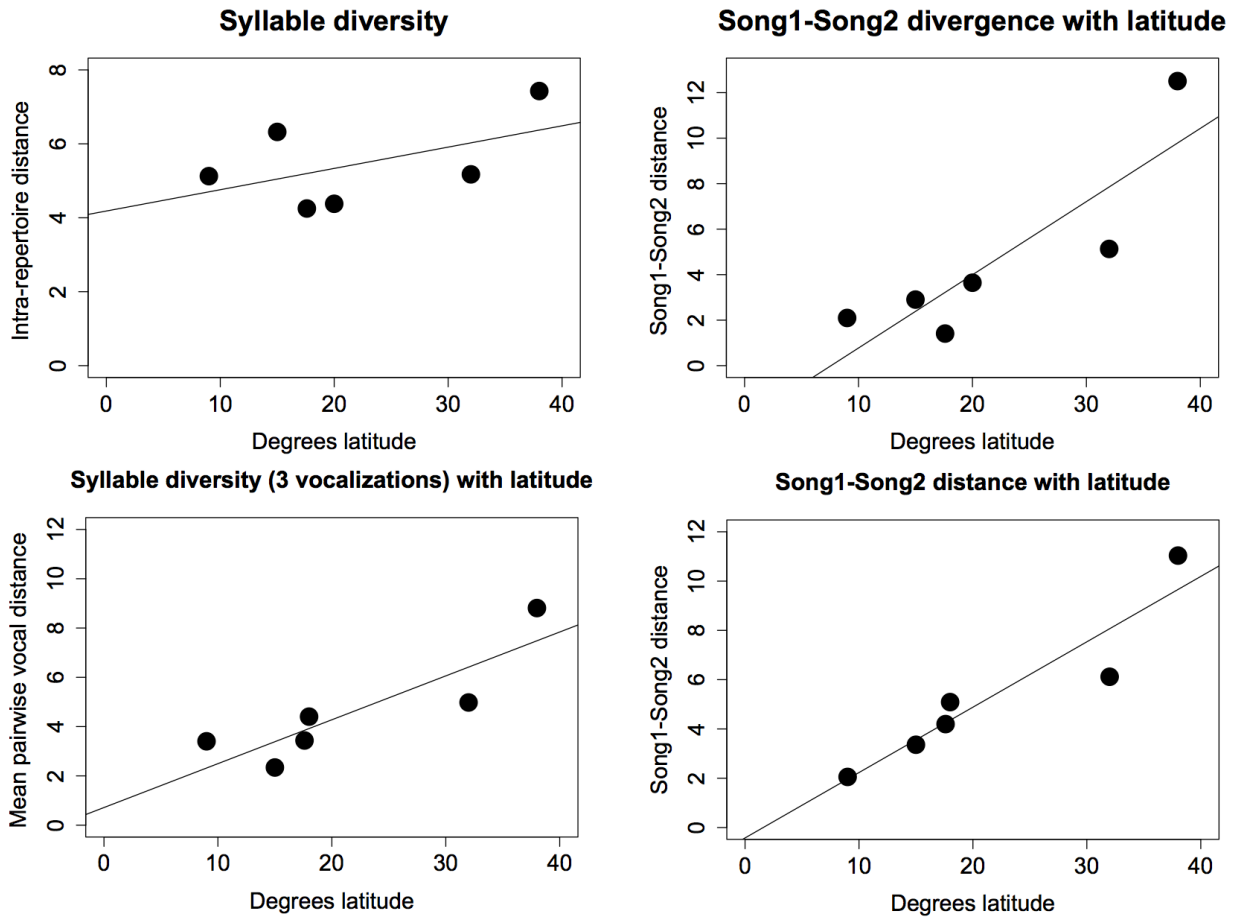


Figure 12.

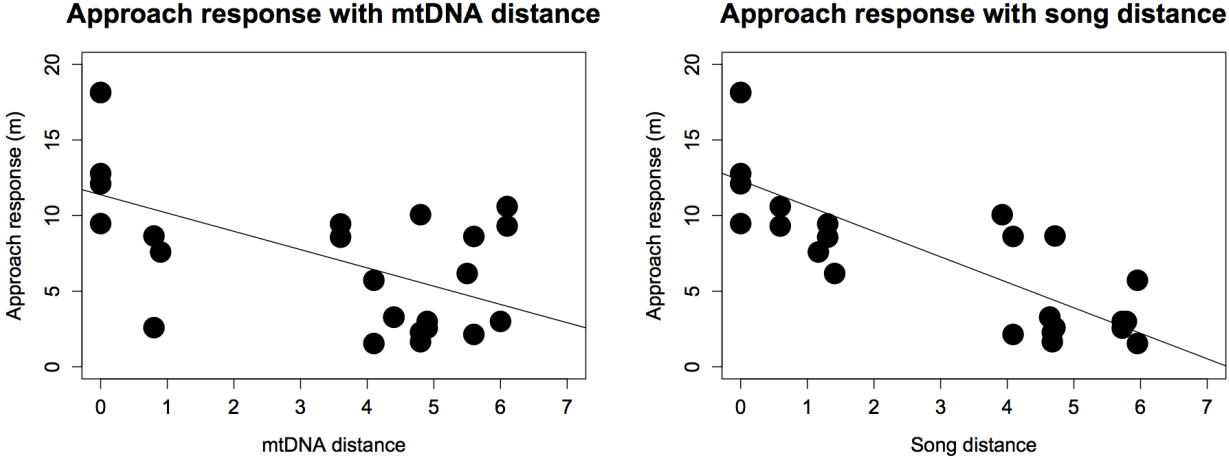
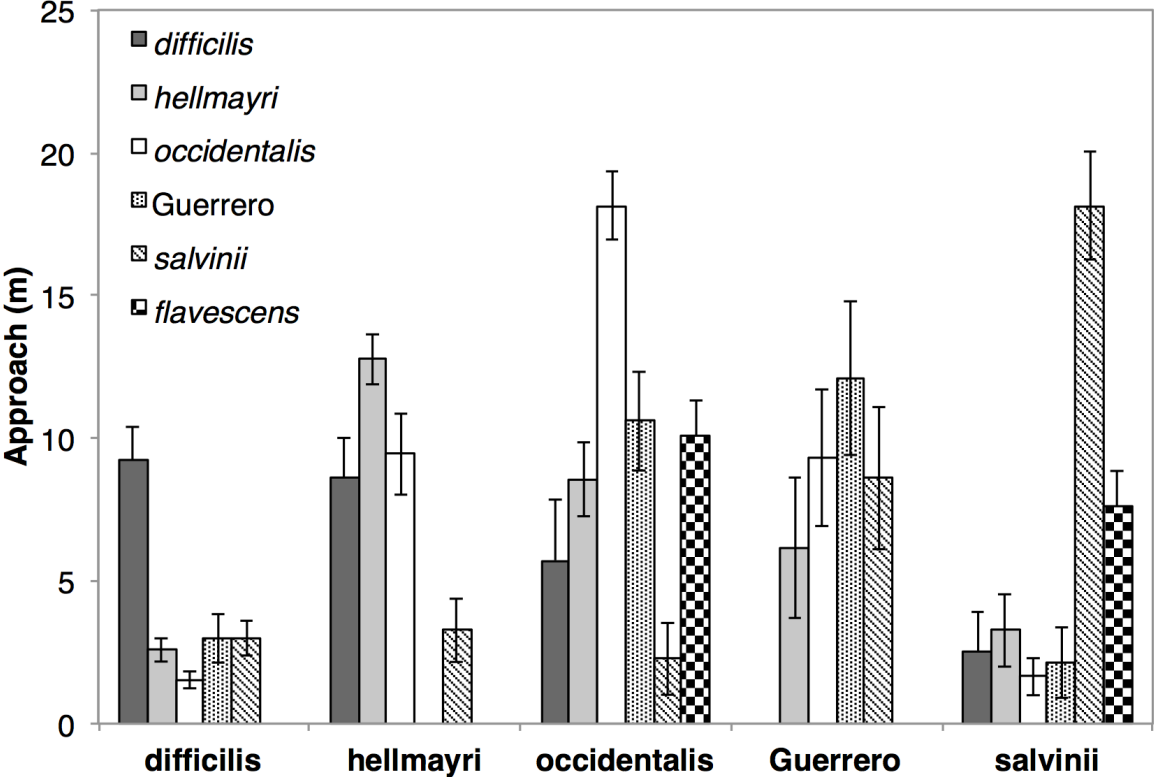


Figure 13.



CONCLUSION

The research outlined in this dissertation provides an integrated view of how the evolution of acoustic signals has contributed to and has been affected by lineage divergence in the *Empidonax difficilis-occidentalis-flavescens* species complex. This research provides one of the broadest examinations of the interaction between genotype and innate song type yet conducted. In addition, this study highlights some more general issues regarding research into the interaction of phenotypic divergence and genetic divergence in lineage diversification. In particular, it highlights: (i) the effectiveness of combining fine scale analyses of population-level divergence with broader phylogenetic analyses in understanding evolutionary patterns and processes, (ii) the utility of integrating intensive field-based research (and especially field-based experiments) with data derived from museum collections, (iii) the necessity to question long-held assumptions about the function of different phenotypic traits as isolating mechanisms (e.g., song, and in particular, suboscine song), and (iv) the utility of (the often-overlooked) suboscine passerines for examinations of song evolution and function in birds.

Some key findings emerge from my dissertation research. First, I establish that widespread population admixture has occurred between Pacific-slope and Cordilleran Flycatchers over a relatively broad geographic area in northwestern North America, and that the pattern of admixture is asymmetrical. I connect this pattern to differences in song and in song response behavior. I demonstrate that the songs of Pacific-slope and Cordilleran Flycatchers are distinct and highly correlated with nuclear genotype, and that the songs of admixed individuals exhibit spectral characteristics intermediate to the parental species. Pacific-slope Flycatchers seem to discriminate among song types to a greater degree than Cordilleran Flycatchers, but Cordilleran Flycatchers respond to song more aggressively. I assess these results in combination with the geographical patterns of song variation and genetic variation to hypothesize a historical scenario for secondary contact between these taxa in which aggressive hybridization led by Cordilleran males drove widespread population admixture. In the broader phylogenetic analysis, I provide a unique view into how vocal repertoires can evolve in birds, and how this relates to lineage diversification. I find varying rates of song divergence across taxa and across latitude, with songs distinct between some taxa, but not others. Song complexity seems to be higher in higher latitude migratory taxa (due primarily to the highly divergent Pacific-slope song type), and I find at least preliminary evidence that lower latitude species are able to use more subtle vocal cues in taxon recognition than higher latitude migratory taxa.

Thus, to return to the questions presented in the Introduction, innate song does seem to be able to function as a strong isolating mechanism, but this depends on ecological and behavioral contexts. That is, the abbreviated breeding seasons at higher latitudes might drive more extreme reproductive behaviors such as singing and territorial defense compared to lower latitude taxa. This can affect the effectiveness as song as a taxon discrimination trait, by affecting both the rate of evolution of song and the behavioral context in which the song is performed.

Second, I found that patterns of song divergence correlate closely to patterns of genetic divergence in the comparison of song divergence and admixture between Pacific-slope and Cordilleran Flycatchers, but that varying rates of song divergence relative to genetic divergence in the broader phylogenetic comparison make this correlation weaker. Song seems capable of a high level of consistency across relatively large genetic distances or a high level of divergence over relatively small genetic distances, indicating that in at least some of these taxa, song divergence has been driven by selection. Claiming that I have evidence that song differences drove lineage diversification would be premature, but song differences do seem to have important roles in maintaining taxon boundaries. Despite the high level of admixture between

Pacific-slope and Cordilleran Flycatchers at interior sites, gene flow does not occur at any significant level into core Pacific-slope populations. Multiple lines of evidence suggest that Pacific-slope song has become highly derived, relative to the songs of other taxa in this clade and that it has a greater, or at least more varied, role in reproduction. Thus, attributing an important role to song differences in decreasing gene flow from Cordilleran populations into core Pacific-slope populations seems reasonable. Moreover, the high level of discrimination in lower latitude taxa among very similar song types suggests that song could be an effective cue for assortative mating in these taxa. This comes with the caveat that the context that often exist in secondary contact zones, such as low population density, can promote hybridization in taxa that would likely mate assortatively if the cost of mate searching were lower.

Taxonomy

The research outlined here requires a reassessment of the current taxonomy in the *Empidonax difficilis-occidentalis-flavescens* complex. A strict adherence to the Biological Species Concept and the criterion of reproductive isolation (Mayr 1982) would inevitably require lumping Pacific-slope and Cordilleran Flycatchers, and presumably resurrect the “Western Flycatcher” (the former species name before Pacific-slope and Cordilleran Flycatchers were split). This would undoubtedly bring immense satisfaction to many western North American bird identification enthusiasts, not a few of whom reside in the Pacific Northwest, and experience the duress of classifying birds from the Pacific-slope–Cordilleran contact zone in a particularly acute way.

I feel that the situation is complex and does not fit well with existing taxonomic schemes. There seems to be an asymmetric species collapse between Pacific-slope and Cordilleran Flycatchers, with Pacific-slope Flycatchers remaining effectively isolated from Cordilleran Flycatchers, but Cordilleran populations likely to experience continued gene flow from Pacific-slope populations. This may seem to contradict the finding of widespread population admixture in the interior Pacific Northwest. I would concede that although Pacific-slope Flycatchers may not have remained reproductively isolated from Cordilleran Flycatchers following secondary contact, current *coastal* Pacific-slope populations may be effectively reproductively isolated, and may remain so. As outlined in Chapter 2, this is likely due to a combination of divergence in song, divergence in migration times, and the ecological barrier created by the mountain ranges that comprise Pacific Slope. Thus, the Pacific-slope Flycatcher, as a genetically and phenotypically distinct entity, may persist indefinitely, while the northern subspecies of the Cordilleran Flycatcher (i.e., *E. o. hellmayri*) may not. While it may be easy to dismiss the isolation of Pacific-slope populations as an ephemeral situation that will inevitably dissolve with the passing of time, I would point out that all species designations are provisional if we use the criterion of the impermanence of current isolation, and so in this respect, retaining the Pacific-slope Flycatcher as a species should pose no particular problem.

As for the Mexican and Central American taxa, no decision seems warranted without further genetic analysis (especially nuclear DNA) and further examination of potential areas where geographic contact could occur between taxa. Unfortunately, at the present time, fieldwork in some of the most crucial areas is difficult due to criminal activity and political insecurity. *Empidonax o. occidentalis* and *E. f. salvinii* show levels of discrimination between homotypic song and the songs of closely related (and currently conspecific) taxa that are similar to those observed in recently diverged species. This is a particularly important line of evidence given the key role of song in reproduction. Thus, a search for areas of contact between the

apparently allopatric taxa in this group should be performed to determine whether or not they meet and how song differences affect hybridization and gene flow in the area of sympatry. If the necessary fieldwork is performed and the Mexican and Central American taxa are found to be allopatric, given the complicated outcome of secondary contact in Pacific-slope and Cordilleran Flycatchers, it may be most conservative to classify them as allospecies.

Suggestions for future research

As the research outlined in dissertation unfolded, it raised numerous additional questions that should be addressed in future research. Each of the avenues of research proposed below would make important contributions to understanding the history of diversification in this complex, and to understanding current patterns of gene flow. Addressing these questions would not only clarify the evolutionary history and taxonomy of the *Empidonax difficilis-occidentalis-flavescens* complex, but would utilize the advantages of this study system to address questions of continuing interest to studies of avian speciation and to studies of speciation and lineage diversification in general.

Multilocus investigation of gene flow among taxa. A more formal analysis of gene flow dynamics, using the latest genomic techniques in combination with coalescent methods is necessary to provide a more complete view of the history of population divergence and admixture in this complex. This is important for a better understanding of the history of contact and admixture between Pacific-slope and Cordilleran Flycatchers, and especially in understanding current patterns of gene flow. Additional genetic analyses are also necessary in the Mexican and Central American taxa, for which a more complete understanding of gene flow using a multilocus dataset (including broad sampling of the nuclear genome) is crucial for a more complete understanding of song divergence and lineage diversification, and for elucidating current or historical population admixture.

Patterns of niche divergence. Niche divergence has been proposed as a key mechanism through which the taxa in this clade and in the genus *Empidonax* in general have diversified (Johnson 1980, Johnson and Cicero 2002). An investigation of niche divergence would be invaluable in understanding the dynamics of gene flow into and out of the contact zone between Pacific-slope and Cordilleran Flycatchers, and may be reflective of patterns in numerous passerine taxa pairs that meet in similar Pacific Slope–Great Basin contact zones (Johnson 1978, Swenson 2004).

The effects of difference in migration timing on gene flow. Recent studies have highlighted the important but understudied impact of divergent migratory behaviors on gene flow between closely related taxa (e.g., Ruegg & Smith 2002, Bearhop et al. 2005, Delmore et al 2012, Ruegg et al. 2012, 2014, Toews et al 2014). Temporal isolation could occur if arrival times to breeding territories in a contact zone differ between sister taxa, or if gene flow out of the contact zone were negatively affected by differences in arrival times between parental and contact zone populations. Pacific-slope populations arrive to the breeding grounds nearly two months earlier than Cordilleran populations (Johnson 1973). Thus, potential migrants from interior to coastal populations could experience some level of temporal isolation, while migrants from coastal to interior populations might experience an advantage in establishing territories due to their earlier arrival to interior breeding sites. This could interact with differences in song to create significant barriers to gene flow, and could help to explain the greatly reduced level of gene flow into Pacific-slope populations described in Chapter 2.

Further investigations of reproductive behavior. Studies of mate choice and territorial behavior in these taxa have been conducted in the past (Davis et al 1963, Beaver and Baldwin 1975, Ainsley 1992), but much remains unknown, especially as it relates to hybridization and gene flow. For example, detailed investigations of the phenology of territoriality and mate choice could be important in understanding the dynamics of gene flow between Pacific-slope and Cordilleran populations, and could illuminate important contrasts between higher and lower latitude taxa that help to explain the evolution and function of song. Related research on asymmetries in aggressive territorial behavior between taxa could help to determine if a more general pattern exists of more aggressive interior and less aggressive Pacific Slope taxa that is an effect of environment-based selection for adaptation to harsher environments in interior taxa (*cf.* Pearson and Rohwer 2000). This could have important effects on hybridization and gene flow in multiple avian contact zones in western North America.

Further investigation of vocalizations. Much research remains to be done on the vocalizations of these and related taxa. For example, future research could examine in depth the function of the geographically variable male position note (MPN). Playback experiments could be used to test its effects in intra- and interspecific interactions. In addition, important research remains to be done on the effects of environment-based selection on vocalizations in *Empidonax*. Many of the studies that have looked at the effect of the environment on bird songs have focused on relatively rapidly evolving oscine songs (e.g., Morton 1975, Nottebohm 1975, Slabbekoorn 2004). The variation that exists in songs and MPN across such a broad latitudinal and environmental range in the *Empidonax difficilis-occidentalis-flavescens* complex suggest that this would be a particularly interesting group in which to investigate this. In addition, the energetics of vocalizations (including song rates and the use of non-linear vocalizations) could be a rich avenue of research. Finally, this would be an ideal system in which to analyze the genetic mechanisms underlying song and song divergence, given the apparent absence of the confounding effects of learning.

In many ways, this dissertation was inspired by the work of Ned K. Johnson (Johnson 1980, 1994, Johnson and Marten 1988, Cicero and Johnson 2002, Johnson and Cicero 2002). Johnson's work on the *Empidonax difficilis-occidentalis-flavescens* complex was exhaustive, and provided a baseline of information and ideas that most subsequent studies have used as a departure point to one degree or another (e.g., Sakai and Noon 1991, Ainsley 1992, Howell and Cannings 1992, Lowther 2000). The conclusions that I reach differ from Johnson's in some important ways, but Johnson's 1980 monograph served as a reference point again and again as I pursued this work. Even as my findings seemed to depart from, and in many ways, to contradict Johnson's findings and conclusions, my repeated consultation of his work and his ideas did not diminish. In that sense, this dissertation builds upon and refines the invaluable body of research that resulted from Johnson's dedicated work.

LITERATURE CITED

- Ainsley, D. T. J. 1992. Vocalizations and nesting behaviour of the Pacific-slope flycatcher, *Empidonax difficilis*. MSc thesis. Univ. of Victoria, Canada.
- Alatalo, R.V., Eriksson, D., Gustafsson, L. and Lundberg, A. 1990. Hybridization between pied and collared flycatchers—sexual selection and speciation theory. *Journal of Evolutionary Biology*. 3: 375–389.
- American Ornithologists' Union. 1989. Thirty-seventh supplement to the American Ornithologists' Union check-list of North American birds. *The Auk*. 106: 532–538.
- Andersson, M. 2004. *Sexual Selection*. Princeton University Press, Princeton, NJ.
- Avise, J. C. 2004. Molecular markers, natural history, and evolution. Sinauer Ass., Sunderland.
- Baker, M.C. and Baker, A.E.M. 1990. Reproductive behavior of female buntings: Isolating mechanisms in a hybridizing pair of species. *Evolution*. 44: 332–338.
- Baker, M.C., Bjerke, T.K., Lampe, H.U., and Espmark, Y.O. 1987. Sexual response of female yellowhammers to differences in regional song dialects and repertoire sizes. *Animal Behaviour*. 35: 395–401.
- Baker, M.C. & Boylan, J.T. 1999. Singing behavior, mating associations and reproductive success in a population of hybridizing lazuli and indigo buntings. *Condor*. 101: 493–504.
- Baker, M.C., Spittle-Nabors, K.J., and Bradly, D.C. 1981. Early experience determines song dialect responsiveness of female sparrows. *Science*. 214: 819–821.
- Ballard, J. W. and Whitlock, M. C. 2004. The incomplete natural history of mitochondrial DNA. *Molecular Ecology*. 13: 729–44.
- Baptista, L.F. 1975. Song dialects and demes in sedentary populations of the white-crowned sparrow. *University of California Publications in Zoology*. University of California Press, Berkeley, CA.
- Ballentine, B., J. Hyman, and S. Nowicki. 2004. Vocal performance influences female response to male bird song: an experimental test. *Behavioral Ecology* 15:163–168.
- Barrowclough, G. F. 1980. Genetic and phenotypic differentiation in a wood warbler (genus *Dendroica*) hybrid zone. *The Auk*. 97: 655–668.
- Barton, N.H. and Gale, K.S. 1993. Genetic analysis of hybrid zones. In: Harrison, R.G. (ed). *Hybrid zones and the evolutionary process*. Oxford University Press, New York, pp. 13–45.
- Barton, N. H. and Hewitt, G. M. 1981. Hybrid zones and speciation. In: Atchley, W. R. and Woodruff, D. S. (eds). *Evolution and Speciation: Essays in Honor of M. J. D. White*. Cambridge Univ. Press, Cambridge, pp. 109–145.
- Barton, N. H., and Hewitt, G.M. 1985. Analysis of hybrid zones. *Annual Reviews of Ecology and Systematics*. 16:113-148.
- Barton, N. H. and Hewitt, G. M. 1989. Adaptation, speciation and hybrid zones. *Nature*. 341: 497-503.
- Beaver, D.L. and Baldwin, P.H. 1975. Ecological overlap and the problem of competition and sympatry in the Western and Hammond's Flycatchers. *Condor*. 77: 1–13.
- Bensch, S. and Åkesson, M. 2005. Ten years of AFLP in ecology and evolution: why so few animals? *Molecular Ecology*. 14: 2899–2914.
- Bolger, A.M., Lohse, M., Usadel, B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 30: 2114–2120.
- Bowie, R. C. K., Fjeldså, J., Hackett, S. J. and Crowe, T. M. 2004. Molecular evolution in space

- and through time: mtDNA phylogeography of the olive sunbird (*Nectarina olivacea/obscura*) throughout continental Africa. *Molecular Phylogenetics and Evolution*. 33: 56–74.
- Braune, P., Schmidt, S., Zimmermann, E. 2008. Acoustic divergence in the communication of cryptic species of nocturnal primates (*Microcebus* spp.). *BMC Biology*. 6: 19.
- Brelsford, A., and D.E. Irwin. 2009. Incipient speciation despite little assortative mating: the yellow-rumped warbler hybrid zone. *Evolution*. 63: 3050–3060.
- Brodkorb, P. 1949. Variation in the North American forms of the western flycatcher. *Condor*. 51:35-39
- Brodsky, L.M., Ankney, C.D., and Dennis, D.G. 1988. The influence of male dominance on social interaction in black ducks and mallards. *Animal Behaviour*. 36: 1371–1378.
- Bronson, C.L., Grubb, Jr., T.C., Sattler, G.D., and Braun, M.J. 2003. Mate preference: a possible causal mechanism for a moving hybrid zone. *Animal Behaviour*. 65: 489–500.
- Brunsfeld, S., Sullivan, J., Soltis, D., and Soltis, P. 2001. Comparative phylogeography of northwestern North America: A synthesis. In: Silvertown, J. and Antinovic, J. (eds.) *Integrating ecological and evolutionary processes in a spatial context*. Blackwell Science, Oxford, pp. 319–339.
- Burnham, K.P., and Anderson, D.R. 2002. *Model Selection and Inference: A Practical Information-theoretic Approach, Second Edition*. Springer-Verlag, New York.
- Campbell, R. W., Dawe, N., McTaggart-Cowan, I., Cooper, J., Kaiser, G., McNall, M. and Smith, G.1997. *The Birds of British Columbia*. UBC Press, Vancouver.
- Carling M. and Brumfield R. 2008. Haldane’s rule in an avian system: using cline theory and divergence population genetics to test for differential introgression of mitochondrial, autosomal and sex-linked loci across the *Passerina* bunting hybrid zone. *Evolution*. 62: 2600–2615.
- Catchpole, C.K. 1980. Sexual selection and the evolution of complex songs among European warblers of the genus *Acrocephalus*. *Behaviour*. 74: 149–166.
- Catchpole, C.K. 1982. The evolution of bird sounds in relation to mating and spacing behavior. Pages 297–319 in *Acoustic Communication in Birds* (D.E. Kroodsma and E.H. Miller, Eds.). Academic Press, London, UK.
- Catchpole, C.K. 1986. Song repertoires and reproductive success in the great reed warbler, *Acrocephalus arundinaceus*. *Behavioural Ecology and Sociobiology*. 19: 439–445.
- Catchpole, C. 1987. Bird song, sexual selection, and female choice. *Trends in Ecology and Evolution*. 2: 94–97.
- Catchpole, C.K. and Slater, P.J.B. 1995. *Bird Songs: Biological Themes and Variations*. Cambridge University Press, Cambridge.
- Chesser, R.T. 2004. Molecular systematics of New World suboscine birds. *Molecular Phylogenetics and Evolution*. 32: 11–24.
- Cicero, C. 2004. Barriers to sympatry between avian sibling species (Paridae: *Baeolophus*) in tenuous secondary contact. *Evolution*. 58: 1573–1587.
- Cicero, C. and Johnson, N.K. 1998. Molecular phylogeny and ecological diversification in a clade of New World songbirds (genus *Vireo*). *Molecular Ecology*. 7: 1359–1370.
- Cicero, C. and Johnson, N. 2002. Phylogeny and character evolution in the Empidonax group of tyrant flycatchers (Aves: Tyrannidae): A test of W. E. Lanyon’s hypothesis using mtDNA sequences. *Molecular Phylogenetics and Evolution*. 22: 289–302.

- Clayton, N.S. 1990a. Assortative mating in zebra finch subspecies, *Taeniopygia guttata guttata* and *T. g. castanotis*. *Philosophical Transactions of the Royal Society of London, B*. 330: 351–370.
- Clayton, N.S. 1990b. Suspecies recognition and song learning in zebra finches. *Animal Behaviour*. 40: 1009–1017.
- Clayton, N.S. 1990c. Mate choice and pair formation in Timor and Australian mainland Zebra finches. *Animal Behaviour*. 39: 474–480.
- Clement, M., Posada, D. and Crandall, K. A. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology*. 9: 1657–1659.
- Collins, S. 2004. Vocal fighting and flirting: The functions of bird song. In: Marler, P. and Slabbekoorn, H. (eds.). *Nature's Music: The Science of Birdsong*. Elsevier Academic Press, San Diego, pp. 39–79
- Collins, S.A., de Kort, S.R., Perez-Tris, J., and Telleria, J.L. 2009. Migration strategy and divergent sexual selection on bird song. *Proceedings of the Royal Society London Series B*. 278: 1713–1720.
- Corander, J., and Marttinen, P. 2006. Bayesian identification of admixture events using multilocus molecular markers. *Molecular Ecology*. 15: 2833–2843.
- Corander, J., Marttinen, P., Siren, J., Tang, J. 2008. Enhanced Bayesian modeling in BAPS software for learning genetic structures of populations. *BMC Informatics*. 9: 539.
- Coyne, J.A. and Orr, H.A. 2004. *Speciation*. Sinauer Associates, Inc., Sunderland.
- Davis, J., Fislser, G.F., and Davis, B.S. 1963. The breeding biology of the Western Flycatcher. *Condor*. 65: 337–382.
- den Hartog, P.M., de Kort, S.R., and ten Cate, C. 2007. Hybrid vocalizations are effective within, but not outside, an avian hybrid zone. *Behavioral Ecology*. 18: 608–614.
- Derryberry, E.P. 2007. Evolution of bird song affects signal efficacy: and experimental test using historical and current signals. *Evolution*. 61: 1938–1945.
- Derryberry, E. P., Seddon, N., Claramunt, S., Tobias, J. A., Baker, A., Aleixo, A., and Brumfield, R. T. 2012. Correlated evolution of beak morphology and song in the Neotropical woodcreeper radiation. *Evolution* 66: 2784–2797.
- Dooling, R.J. 2004. Audition: can birds hear everything they sing? Pages 206–225 in *Nature's Music: The Science of Birdsong* (P. Marler and H. Slabbekoorn, Eds.). Elsevier Academic Press, San Diego.
- Dooling, R.J. and Searcy, M.H. 1980. Early perceptual selectivity in the swamp sparrow. *Developmental Psychobiology*. 13: 499–506.
- Drovetski, S. V., Zink, R. M., Rohwer, S., Fadeev, I. V., Nesterov, E. V., Karagodin, I., Koblik, E. A. and Red'kin Y. A. 2004. Complex biogeographic history of a Holarctic passerine. *Proceedings of the Royal Society B*. 271: 545–551.
- DuBois, A.L., Nowicki, S., and Searcy, W.A. 2009. Swamp sparrows modulate vocal performance in an aggressive context. *Biology Letters*. 5: 163–165.
- Duckworth, R. A. 2008. Adaptive dispersal strategies and the dynamics of a range expansion. *American Naturalist*. 172: S4–S17.
- Duckworth, R.A. and Badyaev, A.V. 2007. Coupling of dispersal and aggression facilitates the rapid range expansion of a passerine bird. *Proceedings of the National Academy of Sciences*. 104: 15017–15022.
- Dunn, J.L., and Alderfer, J. 2011. *Field Guide to the Birds of North America*. National Geographic Society, Washington D.C.

- Edwards, S. V., Kingan, S. B., Calkins, J. D., Balakrishnan, C. N., Jennings, W. B., Swanson, W. J. and Sorenson, M. D. 2005. Speciation in birds: genes, geography, and sexual selection. *Proceedings of the National Academy of Sciences*. 102: 6550–6557.
- Elias, D.O., Mason, A.C., and Hebets, E.A. 2010. A signal-substrate match in the substrate-borne component of a multimodal courtship display. *Current Zoology*. 56: 370–378.
- Emlen, S.T., Rising, J.D., and Thompson, W.L. 1975. A behavioral and morphological study of sympatry in the Indigo and Lazuli Buntings of the Great Plains. *Wilson Bulletin*. 87: 145–179.
- Endler, J. A. 1977. *Geographic Variation, Speciation, and Clines*. Princeton University Press, New Jersey.
- Excoffier, L., Foll, M., and Petit, R.J. 2009. Genetic consequences of range expansions. *Annual Review of Ecology Evolution and Systematics*. 40: 481–501.
- Excoffier, L., Laval, G. and Schneider, S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics*. Online 1: 47–50.
- Falush, D., Stephens, M. and Pritchard, J. K. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*. 7: 574–578.
- Fitzpatrick, J., Bates, J. Bostwick, K. Caballero, I., Clock, B., Farnsworth, A., Hosner, P., Joseph, L., Langham, G., Lebbin, D., Mobley, J., Robbins, M., Scholes, E., Tello, J., Walther, B., and Zimmer, K. 2004. Family Tyrannidae (Tyrant-flycatchers). Pages 170–462 in *Handbook of the Birds of the World–Volume 9* (del Hoyo, J., Elliott, A., and Christie, D.A., Eds.). Lynx Edicions, Barcelona.
- Fu, Y.X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*. 133: 693–709.
- García-Moreno, J. 2004. Is there a universal mtDNA clock for birds? *Journal of Avian Biology*. 35: 465–468.
- Good, J. M., Hird, S., Reid, N., Demboski, J. R., Stepan, S. J., Martin-Nims, T. R. and Sullivan, J. 2008. Ancient hybridization and mitochondrial capture between two species of chipmunks. *Molecular Ecology*. 17: 1313–1327.
- Grant, P.R. and Grant, R. 1997. Hybridization, sexual imprinting, and mate choice. *American Naturalist*. 149: 1–28.
- Grant, B.R. & Grant, P.R. 1998. Hybridization and speciation in Darwin’s finches: The role of sexual imprinting on a culturally transmitted trait. In: Howard, D.J. and Berlocher, S.H. (eds.). *Endless Forms: Species and Speciation*. Oxford University Press, New York, pp. 404–422.
- Greenwood, P.J. 1980. Mating systems, philopatry, and dispersal in birds and mammals. *Animal Behavior*. 28: 1140–1162.
- Greenwood, P.G. 1987. Inbreeding, philopatry, and optimal outbreeding in birds. In: Cooke, F. and Buckley, P.A. (eds.). *Avian Genetics*. New York: Academic Press, pp. 207–222.
- Groenen, M.A., Wahlberg, P., Foglio, M., Cheng, H.H., Megens, H.J., Crooijmans, R.P., Besnier, F., Lathrop, M., Muir, W.M., Wong, G.K., Gut, I., Andersson, L. 2009. A high density SNP based linkage map of the chicken genome reveals sequence features correlated with recombination rate. *Genome Research*. 19: 510–519.
- Hackett, S. J. 1996. Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). *Molecular Phylogenetics and Evolution*. 5: 368–382.

- Hailman, J.P. and Ficken, M.S. 1996. Comparative analysis of vocal repertoires, with reference to chickadees. Pages 136–159 in *Ecology and Evolution of Acoustic Communication in Birds* (D.E. Kroodsma and E.H. Miller, Eds.). Cornell University Press, Ithaca.
- Harrison, R.G. 1993. *Hybrid Zones and the Evolutionary Process*. Oxford University Press, New York.
- Hewitt, G. M. 1988. Hybrid zones – natural laboratories for evolutionary studies. *Trends in Ecology and Evolution*. 3: 158–167.
- Howell, S.N., and Webb, S.W. 1995. *The Birds of Mexico and Northern Central America*. Oxford University Press, Oxford.
- Hubbard, J. P. 1969. The relationships and evolution of the *Dendroica coronata* complex. *The Auk* 86: 393–432.
- Irwin, D.E. 2000. Song variation in an avian ring species. *Evolution*. 54: 998-1010.
- Irwin, D. E. 2002. Phylogeographic breaks without geographic barriers to gene flow. *Evolution*. 56: 2383–2394.
- Irwin, D. E., Alström, P., Olsson, U. and Benowitz-Fredericks, Z. M. 2001a. Cryptic species in the genus *Phylloscopus* (Old World leaf warblers). *Ibis*. 143: 233–247.
- Irwin, D. E., Bensch, S., Irwin, J. H. and Price, T. D. 2005. Speciation by distance in a ring species. *Science*. 307: 414–416.
- Irwin D. E., Bensch, S. and Price, T. D. 2001. Speciation in a ring. *Nature*. 409: 333–337.
- Irwin, D.E., Brelsford, A., Toews, D., MacDonald, C., and Phinney, M. 2009. Extensive hybridization in a contact zone between MacGillivray’s and mourning warblers (*Oporornis tolmiei* and *O. philadelphia*) detected using molecular and morphometric analyses. *Journal of Avian Biology*. 40: 539–552.
- Irwin, D.E. and Price, T.D. 1999. Sexual imprinting, learning, and speciation. *Heredity*. 82: 347–354.
- Isler, M.L., Isler, P.R., and Brumfield, R.T. 2005. Clinal variation in vocalizations of an antbird (Thamnophilidae) and implications for defining species limits. *The Auk*. 122: 433–444.
- Jetz, W., Thomas, G.H., Joy, J.B., Hartmann, K. and Mooers, A.O. 2012. The global diversity of birds in space and time. *Nature* 491: 444–448.
- Johnson, N. 1963. Biosystematics of sibling species of flycatchers in the *Empidonax hammondi-oberholseri-wrightii* complex. University of California Press, Berkeley.
- Johnson, N.K. 1973. Spring migration of the Western Flycatcher, with notes on seasonal changes in sex and age ratios. *Bird-Banding*. 44: 205–220.
- Johnson, N.K. 1980. *Character evolution and evolution of sibling species in the Empidonax difficilis-flavescens complex (Aves: Tyrannidae)*. University of California Press, Berkeley, CA.
- Johnson, N. K. 1994. Old-school taxonomy versus modern biosystematics: species-level decision in *Stelgidopteryx* and *Empidonax*. *The Auk*. 111: 773–780.
- Johnson, N.K., and Cicero, C. 2002. The role of ecologic diversification in sibling speciation of *Empidonax* flycatchers (Tyrannidae): multigene evidence from mtDNA. *Molecular Ecology*. 11: 2065–2081.
- Johnson, N.K. and Cicero, C. 2004. New mitochondrial DNA data affirm the importance of Pleistocene speciation in North American Birds. *Evolution*. 58: 1122-1130.
- Johnson, N. K. and Marten, J. A. 1988. Evolutionary genetics of flycatchers. II. Differentiation in the *Empidonax difficilis* complex. *The Auk*. 105: 177–191.

- Kallioinen, R.U.O., Hughes, J.M., and Mather, P.B. 1995. Significance of back colour in territorial interactions in the Australian magpie. *Australian Journal of Zoology*. 43: 665–673.
- Kenyon, H.L., Toews, D. and Irwin, D.E. 2011. Can song discriminate between MacGillivray’s and mourning warblers in a narrow hybrid zone? *The Condor*. 113: 655–663.
- Kerr, K.C.R., Stoeckle, M.Y., Dove, C.J., Weigt, L.A., Francis, C.M., Hebert, P.D.N. 2007. Comprehensive DNA barcode coverage of North American birds. *Molecular Ecology Notes*. 7: 535–543.
- Kirkpatrick, M. and Ravigné, V. 2002. Speciation by natural and sexual selection: models and experiments. *The American Naturalist* 159: 22–35.
- Klicka, J., Spellman, G.M., Winker, K., Chua, V., and Smith, B.T. 2011. A phylogeographic and population genetic analysis of a widespread, sedentary North American bird: The Hairy Woodpecker (*Picoides villosus*). *Auk*. 128: 346–362.
- Kofler, R., Orozco-terWengel, P., De Maio, N., Pandey, R.V., Nolte, V., Futschik, A., Kosiol, C., Schlotterer, C. 2011. PoPoolation: A toolbox for population genetic analysis of next generation sequencing data from pooled individuals. *PLoS ONE*. 6: e15925.
- Kroodsma, D.E. 1984. Song of the alder flycatcher (*Empidonax alnorum*) and the willow flycatcher (*Empidonax traillii*) are innate. *Auk*. 101: 13–24.
- Kroodsma, D.E. 1985. Development and use of two song forms by the eastern phoebe. *Wilson Bulletin*. 97: 21–29.
- Kroodsma, D. E., Albano, D. J., Houlihan, P. W. and Wells, J. A. 1995. Song development by black-capped chickadees (*Parus atricapillus*) and Carolina chickadees (*P. carolinensis*). *The Auk*. 112: 29–43.
- Kroodsma, D.E. and Konishi, M. 1991. A subsong bird (Eastern Phoebe, *Sayornis phoebe*) develops normal song without auditory feedback. *Animal Behaviour* 42: 477–487.
- Krosby, M. and Rohwer, S.A. 2000 km genetic wake yields evidence for northern glacial refugia and hybrid zone movement in a pair of songbirds. *Proceedings of the Royal Society B*. 276: 615–621.
- Kulba, B. and McGillivray, W. 2000. The distribution and habitat preferences of the “Western flycatcher” in Alberta. [WWW document, 365 kb]. URL <http://www.pma.edmonton.ab.ca/vpub/wefl/index.htm>
- Kunkel, P. 1974. Mating systems of tropical birds: the effects of weakness or absence of external reproduction-timing factors, with special reference to prolonged pair bonds. *Zeitschrift für Tierpsychologie*. 34: 265–307.
- Lachlan, R.F. and Servedio, M.R. 2004. Song learning accelerates allopatric speciation. *Evolution*. 58: 2049–2063.
- Lanyon, W.E. 1978. Revision of the *Myiarchus* flycatchers of South America. *Bulletin of the American Museum of Natural History*. 161: 427–628.
- Li, H. 2011. Improving SNP discovery by base alignment quality. *Bioinformatics*. 27: 1157–1158.
- LI-COR Biosciences. 2003. Applications manual: model 4300 DNA analyzer. LI-COR Biosci., Lincoln.
- Lovette, I. J. 2004. Mitochondrial dating and mixed-support for the “2% rule in birds”. *The Auk*. 121: 1–6.

- Lowry D., Modliszewski, J., Wright, K., Wu, C. and Willis, J. 2008. The strength and genetic basis of reproductive isolating barriers in flowering plants *Philosophical Transactions of the Royal Society B*. 363: 3009–3021.
- Lowther, P.E. 2000. Pacific-slope flycatcher (*Empidonax difficilis*) and Cordilleran flycatcher (*Empidonax occidentalis*). In: Poole, A. and Gill, F. (eds). The birds of North America, no. 556. The birds of North America, Inc., Philadelphia.
- Luther, D.A., and Derryberry, E.P. 2012. Birdsongs keep pace with city life: changes in song over time in an urban songbird affects communication. *Animal Behaviour*. 83: 1059–1066.
- Lynch, A. 1996. The population memetics of birdsong. In: Kroodsma, D.E and Miller, E.H. (eds.). *Ecology and Evolution of Acoustic Communication in Birds*. Cornell University Press, Ithaca, pp. 181–197.
- Mack, R.N., Rutter, N.W., Bryant, Jr., V.M. and Valastro, S. 1978. Reexamination of postglacial vegetation history in northern Idaho: Hager Pond, Bonner Co. *Quaternary Research*. 10: 241–255.
- Mack, R.N., Rutter, N.W., Valastro, S., and Bryant, Jr., V.M. 1978. Late Quaternary vegetation history at Waits Lake, Colville River Valley, Washington. *Botanical Gazette*. 139: 499–506.
- Maddison, D. R. and Maddison, W. P. 2003. MacClade 4: Analysis of phylogeny and character evolution. Version 4.06. Sinauer Ass., Sunderland.
- Marler, P. and Peters, S. 1980. Birdsong and speech: Evidence for special processing. Pages 75–112 in *Perspectives on the Study of Speech* (P. Eimas and J. Miller, Eds.). Lawrence Erlbaum, Hillsdale.
- Marler, P. and Slabbekoorn, H. *Nature's Music: The Science of Birdsong*. Elsevier Academic Press, San Diego.
- Marshall, D. B., Hunter, M. G. and Contreras, A. L. 2003. *Birds of Oregon*. Oregon State University Press, Corvallis.
- Martens, J. 1996. Vocalizations and speciation of Palearctic birds. Pages 221–240 in *Ecology and Evolution of Acoustic Communication in Birds* (D.E. Kroodsma and E.H. Miller, Eds.). Cornell University Press, Ithaca.
- Mayr, E. 1942. *Systematics and the Origin of Species*. Columbia University Press, New York.
- McCarthy, E. M. 2006. *Handbook of Avian Hybrids of the World*. Oxford Univeristy Press, New York.
- McDonald, D.B., Clay, R.P., Brumfield, R.T., and Braun, M.J. 2001. Sexual selection on plumage and behavior in an avian hybrid zone: Experimental tests of male-male interactions. *Evolution*. 55: 1443–1451.
- McDonald, J.H. and Kreitman, M. 1991. Adaptive protein evolution at the Adh locus in *Drosophila*. *Nature*. 354: 114–116.
- Mendelson, T.C. and Shaw, K.L. 2002. Genetic and behavioral components of the cryptic species boundary between *Laupala cerasina* and *L. kohalensis* (Orthoptera: Gryllidae). *Genetica*. 116: 301–310.
- Miller, A. 1956. Ecologic factors that accelerate formation of races and species of terrestrial vertebrates. *Evolution*. 10: 262–277.
- Moore, W.S. 1977. An evaluation of narrow hybrid zones in vertebrates. *Quarterly Review of Biology*. 52: 263–277.
- Moore, W. S. and Price, J. T. 1993. Nature of selection in the northern flicker hybrid zone and its

- implications for speciation theory. In: Harrison, R.G. (ed.). *Hybrid Zones and the Evolutionary Process*. Oxford Univ. Press, pp. 196–225.
- Morton, E. S. 1975. Ecological sources of selection on avian sounds. *American Naturalist* 109: 17–34.
- Nelson, D.A. 1998. Geographic variation in song of Gambel's white-crowned sparrow. *Behaviour*. 135: 321–342.
- Nelson, D.A. 1988. Feature weighting in species song recognition by the field sparrow (*Spizella pusilla*). *Behaviour*. 106: 158–182.
- Newton, I. 2003. *The Speciation and Biogeography of Birds*. Academic Press, London.
- Nottebohm, F. 1975. Continental patterns of song variability in *Zonotrichia capensis*: Some possible ecological correlates. *American Naturalist*. 109: 605–624.
- Olson, D.H., and McDowell, M.K. 1983. A comparison of white-bearded manakin (*Manacus manacus*) populations and lek systems in Suriname and Trinidad. *Auk*. 100: 739–742.
- Panhuis, T.M., Butlin, R., Zuk, M., & Tregenza, T. 2001. Sexual selection and speciation. *Trends in Ecology & Evolution*, 16: 364–371.
- Parish, R., Coupé, R. and Lloyd, D. 1996. *Plants of Southern Interior British Columbia and the Inland Northwest*. Lone Pine Publishing, Vancouver.
- Parker, G.A. and Partridge, L. 1998. Sexual conflict and speciation. *Philosophical Transactions of the Royal Society B*. 353: 261–274.
- Patten, M.A., Rotenberry, J.T., and Zuk, M. 2004. Habitat selection, acoustic adaptation, and the evolution of reproductive isolation. *Evolution* 58: 2144–2155.
- Patton, J. L. and Smith, M. F. 1994. Paraphyly, polyphyly, and the nature of species boundaries in pocket gophers (genus *Thomomys*). *Systematic Biology*. 43:11–26.
- Payne, R.B. 1983. Bird songs, sexual selection, and female mating strategies. In: Waser, S.K. (ed.). *Social behavior of female vertebrates*. Academic Press, New York, pp. 55–90.
- Payne, R.B. 1986. Bird songs and avian systematics. In: Johnston, R.F. (ed.). *Current Ornithology Vol. 3*. Plenum Press, New York, pp. 87–126.
- Payne, R.B. 1996. Song traditions in indigo buntings: origin, improvisation, dispersal, and extinction in cultural evolution. Pages 198–220 in *Ecology and Evolution of Acoustic Communication in Birds* (D.E. Kroodsma and E.H. Miller, Eds.). Cornell University Press, Ithaca.
- Pearson, S.F. and Rohwer, S. 2000. Asymmetries in male aggression across an avian hybrid zone. *Behavioral Ecology*. 11: 93–101.
- Pielou, E.C. 1991. *After the Ice Age: The return of life to glaciated North America*. The University of Chicago Press, Chicago.
- Podos, J. 1997. A performance constraint on the evolution of trilled vocalizations in a songbird family (Passeriformes: Emberizidae). *Evolution* 51:537–551.
- Podos, J. and Warren, P.S. 2007. The evolution of geographic variation in birdsong. *Advances in the Study of Behavior* 37: 403–458.
- Poole, A. (ed.). 2005. The Birds of North America Online: <http://bna.birds.cornell.edu/BNA/>. Cornell Laboratory of Ornithology, Ithaca, NY.
- Price, J.J. and Lanyon, S.M. 2002. Reconstructing the evolution of complex bird song in the oropendolas. *Evolution*. 56: 1514–1529.
- Price, J.J., Friedman, N.R., and Omland, K.E. 2007. Song and plumage evolution in the New World orioles (*Icterus*) show similar lability and convergence in patterns. *Evolution*. 61: 850–863.

- Price, T.D. 1998. Sexual selection and natural selection in bird speciation. *Philosophical Transactions of the Royal Society of London, B*. 353: 251–260.
- Price, T.D. 2008. *Speciation in birds*. Roberts and Company Publishers, Greenwood Village.
- Price, T. D. and Bouvier, M. M. 2002. The evolution of F₁ postzygotic incompatibilities in birds. *Evolution*. 56: 2083–2089.
- Pritchard, J. K., Stephens, M. and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*. 155: 945–959.
- Pyle, P. 1997. *Identification Guide to North American Birds*. Slate Creek Press, California.
- Qvarnstrom, A., Haavie, J. Saether, S.A. Eriksson, D. and Part, T. 2006. Song similarity predicts hybridization in flycatchers. *Journal of Evolutionary Biology*. 19: 1202–1209.
- R Development Core Team. 2011. R: A language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>
- Raposo, M.A. and Höfling, E. 2003. Overestimation of vocal characters in Suboscine taxonomy (Aves: Passeriformes: Tyranni): causes and implications. *Lundiana*. 4: 35–42.
- Ratcliffe, L. M. and Grant, P. R. 1985. Species recognition in Darwin’s finches (*Geospiza*, Gould): III. Male responses to playback of different song types, dialects and heterospecific songs. *Animal Behaviour*. 33: 290–307.
- Read, A.F. and Weary, D.M. 1992. The evolution of bird song: comparative analyses. *Philosophical Transactions of the Royal Society of London, B*. 338: 165–187.
- Ribeiro, A.M., Lloyd, P., Feldheim, K.A., and Bowie, R.C.K. 2012. Microgeographic sociogenetic structure of an African cooperative breeding passerine revealed: Integrating behavioural and genetic data. *Molecular Ecology*. 21: 662–672.
- Ricklefs, R.E. 2002. Splendid isolation: historical ecology of the South American passerine fauna. *Journal of Avian Biology*. 33: 207–211.
- Rising, J. D. 1996. The stability of the oriole hybrid zone in western Kansas. *Condor*. 98: 658–663.
- Ritchie, M.G. 2007. Sexual selection and speciation. *Annual Reviews of Ecology, Evolution and Systematics* 38: 79–102.
- Rohwer, S.R. 1982. The evolution of reliable and unreliable badges of fighting ability. *American Zoology* 22: 531–546.
- Rozas, J., Sánchez-DelBarrio, J. C., Messeguer, X. and Rozas, R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*. 19: 2496–2497.
- Rohwer, S., Bermingham, E. and Wood, C. 2001. Plumage and mitochondrial DNA haplotype variation across a moving hybrid zone. *Evolution*. 55: 405–422.
- RStudio 2012. RStudio: Integrated development environment for R (Version 0.96.122). Boston, MA.
- Ruegg, K. 2007a. Genetic, morphological, and ecological characterization of a hybrid zone that spans a migratory divide. *Evolution*. 62: 452–466.
- Ruegg, K. 2007b. Divergence between subspecies groups of Swainson’s Thrush (*Catharus ustulatus ustulatus* and *C. u. swainsoni*). *Ornithological Monographs*. 363: 3009–3021.
- Rush, A.C., Cannings, R.J. and Irwin, D.E. 2009. Analysis of multilocus DNA reveals introgressive hybridization in a contact zone between *Empidonax* flycatchers. *Journal of Avian Biology*. 40: 614–624.
- Ryan, M.J. and Rand, A.S. 1993. Species recognition and sexual selection as a unitary problem in animal communication. *Evolution*. 647–657.

- Safran, R.J., Scordato, E., Symes, L., Rodriguez, R. and Mendelson, T. 2013. Contributions of natural and sexual selection to the evolution of pre-mating reproductive isolation: a research agenda. *Trends in Ecology & Evolution*. 28: 643–650.
- Schemske, D. 2010. Adaptation and *The Origin of Species*. *The American Naturalist*. 176–S1: S4–S25.
- Searcy, W.A. and Andersson, M. 1986. Sexual selection and the evolution of song. *Annual Reviews of Ecology, Evolution and Systematics* 17: 507–533.
- Searcy, W.A. and Beecher, M.D. 2009. Song as an aggressive signal in songbirds. *Animal Behaviour*. 78: 1281–1292.
- Searcy, W.A., and Nowicki, S. 2005. *The Evolution of Animal Communication: Reliability and Deception in Signaling Systems*. Princeton University Press, Princeton, NJ.
- Searcy, W.A., Nowicki, S., and Hughes, M. 1997. The response of male and female song sparrows to geographic variation in song. *Condor*. 99: 651–657.
- Searcy, W.A. and Yasukawa, K. 1990. Use of the song repertoire in intersexual and intrasexual contexts by male red-winged blackbirds. *Behavioral Ecology and Sociobiology*. 27: 123–128.
- Searcy, W.A. and Yasukawa, K. 1996. Song and female choice. Pages 454–473 in *Ecology and Evolution of Acoustic Communication in Birds* (D.E. Kroodsma and E.H. Miller, Eds.). Cornell University Press, Ithaca.
- Seddon, N. 2005. Ecological adaptation and species recognition drives vocal evolution in Neotropical suboscine birds. *Evolution*. 59: 200–215.
- Seddon, N. and Tobias, J.A. 2007. Song divergence at the edge of Amazonia: an empirical test of the peripatric speciation model. *Biological Journal of the Linnean Society* 90: 173–188.
- Sedgwick, J. A. 1993. Dusky Flycatcher (*Empidonax oberholseri*). In: Poole, A. and Gill, F. (eds.). *The birds of North America No. 78*. The Birds of North America, Inc., Philadelphia.
- Sedgwick, J. A. 1994. Hammond's Flycatcher (*Empidonax hammondi*). In: Poole, A. and Gill, F. (eds.). *The birds of North America No. 109*. The Birds of North America, Inc., Philadelphia.
- Sedgwick, J.A. 2001. Geographic variation in the song of willow flycatchers: Differentiation between *Empidonax traillii adastus* and *E. t. extimus*. *The Auk*. 118: 366–379.
- Seehausen, O., Butlin, R.K., Keller, I., Wagner, C., Boughman, J.W., Hohenlohe, Peichel, C.L., Saetre, G-P., Bank, C., Brannstrom, A., Brelsford, A., Clarkson, C.S., Eroukhmanoff, F., Feder, J.L., Fischer, M.C., Foote, A.D., Franchini, P., Jiggins, C.D., Jones, F.C., Lindholm, A.K., Lucek, K., Maan, M.E., Marques, D.A., Martin, S.H., Matthews, B., Meier, J.I., Most, M., Nachman, M.W., Nonaka, E., Rennison, D.J., Schwarzer, J., Watson, E.T., Westram, A.M. and Wildmer, A. 2014. Genomics and the origin of species. *Nature Reviews Genetics*. 270: 53–59.
- Seutin, G., White, B. N. and Boag, P. T. 1991. Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology*. 69: 82–90.
- Sibley, D. 2014. *The Sibley Guide to Birds*. Alfred A. Knopf, New York.
- Simpson, J.T., Wong, K., Jackman, S.D., Schein, J.E., Jones, S.J., and Birol, I. 2009. ABySS: a parallel assembler for short read sequence data. *Genome Research*. 19: 1117–1123.
- Slabbekoorn, H. 2004. Singing in the wild: The ecology of bird-song. In *Nature's Music: The Science of Birdsong*, P. Marler and H. Slabbekoorn, eds. Academic Press, San Diego. pp. 178–205.

- Slabbekoorn, H. and Smith, T.B. 2002. Bird song, ecology, and speciation. *Philosophical Transactions of the Royal Society, B*. 357: 493–503.
- Slabbekoorn, H. and ten Cate, C. 1998. Perceptual tuning to frequency characteristics of territorial signals in collared doves. *Animal Behaviour*. 56: 847–857.
- Slater, P.J.B. 1989. Bird song learning: causes and consequences. *Ethology Ecology and Evolution*. 1: 19–46.
- Sobel, J., Chen, G., Watt, L. and Schemske, D. 2009. The biology of speciation. *Evolution*. 64: 295–315
- Soha, J., Nelson, D.A., and Parker, P.G. 2002. Genetic analysis of song dialect populations in Puget Sound white-crowned sparrows. *Behavioral Ecology*. 15: 636–646.
- Stein, A.C., and Uy, J.A.C. 2006. Unidirectional introgression of a sexually selected trait across an avian hybrid zone: A role for female choice? *Evolution*. 60: 1476–1485.
- Stein, R.C. 1963. Isolating mechanisms between populations of Traill’s Flycatchers. *Proceedings of the American Philosophical Society* 107: 21–50.
- Stutchbury, B.J.M. and Morton, E.S. 2001. *Behavioral Ecology of Tropical Birds*. Elsevier Academic Press, San Diego.
- Sullivan, B.L., Wood, C.L., Iliff, M.J., Bonney, R.E., Fink, D., and Kelling, S. 2009. eBird: a citizen-based bird observation network in the biological sciences. *Biological Conservation*. 142: 2282–2292.
- Swenson, N.G. and Howard, D.J. 2005. Clustering of contact zones, hybrid zones, and phylogeographic breaks in North America. *American Naturalist*. 166: 581–591.
- Swofford, D.L. 2001. PAUP*: Phylogenetic Analyses Using Parsimony (* and other methods). Version 4.0b10. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.
- ten Cate, S. 2004. Birdsong and evolution. Pages 296–317 in *Nature’s Music: The Science of Birdsong* (P. Marler and H. Slabbekoorn, Eds.). Elsevier Academic Press, San Diego.
- ten Cate, C., Slabbekoorn, H. Ballintijn, M.R. 2002. Birdsong and male-male competition: Causes and consequences of vocal variability in the collared dove (*Streptopelia decaocto*). *Advances in the Study of Behavior*. 31: 31–75.
- ten Cate, C., and Vos, D. R. 1999. Sexual imprinting and evolutionary processes in birds: A reassessment. *Advances in the Study of Behavior*. 28: 1–31.
- Tobias, J.A., Brawn, J.D., Brumfield, R.T., Derryberry, E.P., Kirschel, A.N.G, and Seddon, N. 2012. The importance of suboscine birds as study systems in ecology and evolution. *Ornitologia Neotropical* 23: 261–274.
- Toews D.P. and Brelsford, A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology*. 16: 3907–3930.
- Toews, D., Brelsford, A., and Irwin, D.E. 2011. Hybridization between Townsend’s and black-throated green warblers in an avian suture zone. *Journal of Avian Biology*. 42: 434–446.
- Toews, D. and Irwin, D.E.. 2008. Cryptic speciation in a Holarctic passerine revealed by genetic and bioacoustic analyses. *Molecular Ecology*. 17: 2691–2705.
- Uy, J.A.C., Moyle, R.G., and Filardi, C.E. 2009. Plumage and song differences mediate species recognition between incipient flycatcher species of the Solomon Islands. *Evolution*. 63: 153–164.
- Vallender, R., Robertson, R. J., Friesen, V. L. and Lovette, I. J. 2007. Complex hybridization dynamics between golden-winged and blue-winged warblers (*Vermivora chrysoptera* and *Vermivora pinus*) revealed by AFLP, microsatellite, intron and mtDNA markers *Molecular Ecology*. 16: 2017–2029.

- Veen, T., Borge, T., Griffith, S.C., Sætre, G.-P., Bures, S. Gustafsson, L. & Sheldon, B.C. 2001. Hybridization and adaptive mate choice in flycatchers. *Nature*. 411: 45–50.
- Vehrencamp, S.L., Yantachka, J., Hall, M.L., and de Kort, S.R. 2013. Trill performance components vary with age, season, and motivation in banded wren. *Behavioral Ecology and Sociobiology*. 67: 409–419.
- Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research*. 23: 4405–4414.
- Wahl, T. R., Tweit, B. and Mlodinow, S. G. (eds). 2005. *Birds of Washington*. Oregon State University Press, Corvallis.
- Wake, D.B. and Schneider, C.J. 1998. Taxonomy of the Plethodontid salamander genus *Ensatina*. *Herpetologica*. 54: 279–298.
- Wang, Z., Baker, A. J., Hill, G. E. and Edwards, S. V. 2003. Reconciling actual and inferred population histories in the house finch (*Carpodacus mexicanus*) by AFLP analysis. *Evolution*. 57: 2852–2864.
- Weir, J. and Schluter, D. 2004. Ice sheets promote speciation in boreal birds. *Proceedings of the Royal Society London Series B*. 271: 1881–1887.
- Weir, J. T. and Schluter, D. 2008. Calibrating the avian molecular clock. *Molecular Ecology*. 17: 2321–2328.
- Weir, J.T. and Wheatcroft, D. 2011. A latitudinal gradient in rates of evolution of avian syllable diversity and song length. *Proceedings of the Royal Society London Series B*. 278: 1713–1720.
- Weir, J.T., Wheatcroft, D., and Price, T.D. 2012. The role of ecological constraint in driving the evolution of avian song frequency across a latitudinal gradient. *Evolution*. 66: 2773–2783.
- West-Eberhard, M.J. 1983. Sexual selection, social competition, and speciation. *Quarterly Review of Biology* 58: 155–183.
- Winker, K. 1994. Divergence in the mitochondrial DNA of *Empidonax traillii* and *E. alnorum*, with notes on hybridization. *The Auk*. 111: 710–713.
- Zahavi, A. 1975. Mate selection: A selection for a handicap. *Journal of Theoretical Biology*. 53: 205–214.
- Zahavi, A. 1977. The cost of honesty (further remarks on the handicap principle). *Journal of Theoretical Biology*. 67: 603–605.
- Zink, R. M. and Barrowclough, G. F. 2008. Mitochondrial DNA under siege in avian phylogeography. *Molecular Ecology*. 17: 2107–2121.

APPENDIX A – Sample lists for Chapter 2

Table A1. List of samples used for mtDNA (ND2) analysis in Chapter 2. Source abbreviations: ACR = Andrew C. Rush, JK = John Klicka/Univ. Washington Burke Museum, MVZ = Museum of Vertebrate Zoology/Univ. California Berkeley, RAM = Royal Alberta Museum.

Catalog	Source	Site	County	State/Prov	Country
GMS1587	JK	Chiricahua Mtns	Cochise	AZ	USA
GMS1592	JK	Chiricahua Mtns	Cochise	AZ	USA
RB272	JK	Chiricahua Mtns	Cochise	AZ	USA
RB273	JK	Chiricahua Mtns	Cochise	AZ	USA
RB274	JK	Chiricahua Mtns	Cochise	AZ	USA
RB275	JK	Chiricahua Mtns	Cochise	AZ	USA
RB358	JK	Chiricahua Mtns	Cochise	AZ	USA
RB359	JK	Chiricahua Mtns	Cochise	AZ	USA
RB360	JK	Chiricahua Mtns	Cochise	AZ	USA
RB364	JK	Chiricahua Mtns	Cochise	AZ	USA
RB365	JK	Chiricahua Mtns	Cochise	AZ	USA
RB366	JK	Chiricahua Mtns	Cochise	AZ	USA
DHB3042	JK	Hualapai Mtns	Mohave	AZ	USA
RB356	JK	Hualapai Mtns	Mohave	AZ	USA
RB357	JK	Hualapai Mtns	Mohave	AZ	USA
RB361	JK	Hualapai Mtns	Mohave	AZ	USA
RB362	JK	Hualapai Mtns	Mohave	AZ	USA
RB363	JK	Hualapai Mtns	Mohave	AZ	USA
JK09540	JK	Pinaleno Mtns	Graham	AZ	USA
JK09542	JK	Pinaleno Mtns	Graham	AZ	USA
JK09547	JK	Pinaleno Mtns	Graham	AZ	USA
JK09551	JK	Pinaleno Mtns	Graham	AZ	USA
RB178	JK	Pine Vly Mtns	Washington	UT	USA
RB179	JK	Pine Vly Mtns	Washington	UT	USA
RB180	JK	Pine Vly Mtns	Washington	UT	USA
RB181	JK	Pine Vly Mtns	Washington	UT	USA
RB182	JK	Pine Vly Mtns	Washington	UT	USA
RB183	JK	Pine Vly Mtns	Washington	UT	USA
RB184	JK	Pine Vly Mtns	Washington	UT	USA
RB185	JK	Pine Vly Mtns	Washington	UT	USA
RB282	JK	Rocky Mtn NP	Larimer	CO	USA
RB283	JK	Rocky Mtn NP	Larimer	CO	USA
RB284	JK	Rocky Mtn NP	Larimer	CO	USA
RB285	JK	Rocky Mtn NP	Larimer	CO	USA
RB286	JK	Rocky Mtn NP	Larimer	CO	USA
RB287	JK	Rocky Mtn NP	Larimer	CO	USA
RB288	JK	Rocky Mtn NP	Larimer	CO	USA
RB289	JK	Rocky Mtn NP	Larimer	CO	USA
RB290	JK	Rocky Mtn NP	Larimer	CO	USA
RB291	JK	Rocky Mtn NP	Larimer	CO	USA
RB292	JK	Rocky Mtn NP	Larimer	CO	USA
JK04601	JK	Sacramento Mtns	Lincoln	NM	USA
RB276	JK	Sacramento Mtns	Lincoln	NM	USA
RB277	JK	Sacramento Mtns	Lincoln	NM	USA
RB278	JK	Sacramento Mtns	Lincoln	NM	USA
RB279	JK	Sacramento Mtns	Lincoln	NM	USA
RB280	JK	Sacramento Mtns	Lincoln	NM	USA
RB281	JK	Sacramento Mtns	Lincoln	NM	USA
RB316	JK	Sacramento Mtns	Lincoln	NM	USA

RB317	JK	Sacramento Mtns	Lincoln	NM	USA
RB318	JK	Sacramento Mtns	Lincoln	NM	USA
RB319	JK	Sacramento Mtns	Lincoln	NM	USA
RB320	JK	Sacramento Mtns	Lincoln	NM	USA
RB321	JK	Sacramento Mtns	Lincoln	NM	USA
RB322	JK	Sacramento Mtns	Lincoln	NM	USA
RB323	JK	Sacramento Mtns	Lincoln	NM	USA
RB230	JK	San Francisco Peaks	Coconino	AZ	USA
RB231	JK	San Francisco Peaks	Coconino	AZ	USA
RB232	JK	San Francisco Peaks	Coconino	AZ	USA
RB233	JK	San Francisco Peaks	Coconino	AZ	USA
RB234	JK	San Francisco Peaks	Coconino	AZ	USA
RB235	JK	San Francisco Peaks	Coconino	AZ	USA
RB237	JK	San Francisco Peaks	Coconino	AZ	USA
RB238	JK	San Francisco Peaks	Coconino	AZ	USA
RB239	JK	San Francisco Peaks	Coconino	AZ	USA
RB240	JK	San Francisco Peaks	Coconino	AZ	USA
168529	MVZ	Snake Range	White Pine	NV	USA
168530	MVZ	Snake Range	White Pine	NV	USA
168531	MVZ	Snake Range	White Pine	NV	USA
168532	MVZ	Snake Range	White Pine	NV	USA
168533	MVZ	Snake Range	White Pine	NV	USA
168534	MVZ	Snake Range	White Pine	NV	USA
168535	MVZ	Snake Range	White Pine	NV	USA
168536	MVZ	Snake Range	White Pine	NV	USA
168537	MVZ	Snake Range	White Pine	NV	USA
168538	MVZ	Snake Range	White Pine	NV	USA
RB218	JK	Taos	Taos	NM	USA
RB219	JK	Taos	Taos	NM	USA
RB220	JK	Taos	Taos	NM	USA
RB221	JK	Taos	Taos	NM	USA
RB222	JK	Taos	Taos	NM	USA
RB223	JK	Taos	Taos	NM	USA
RB224	JK	Taos	Taos	NM	USA
RB225	JK	Taos	Taos	NM	USA
RB226	JK	Taos	Taos	NM	USA
RB227	JK	Taos	Taos	NM	USA
RB228	JK	Taos	Taos	NM	USA
167071	MVZ	Wet Mtns	Custer	CO	USA
167072	MVZ	Wet Mtns	Custer	CO	USA
167073	MVZ	Wet Mtns	Custer	CO	USA
167074	MVZ	Wet Mtns	Custer	CO	USA
167091	MVZ	Wet Mtns	Custer	CO	USA
167092	MVZ	Wet Mtns	Custer	CO	USA
167093	MVZ	Wet Mtns	Custer	CO	USA
167094	MVZ	Wet Mtns	Custer	CO	USA
ACR010	ACR	Hope	-	BC	CA
ACR012	ACR	Hope	-	BC	CA
ACR068	ACR	Hope	-	BC	CA
ACR069	ACR	Hope	-	BC	CA
ACR070	ACR	Hope	-	BC	CA
ACR094	ACR	Hope	-	BC	CA
ACR095	ACR	Hope	-	BC	CA
ACR096	ACR	Hope	-	BC	CA
BTS06196	JK	Central Oregon Cst	Coos	OR	USA
BTS06201	JK	Central Oregon Cst	Coos	OR	USA
RB132	JK	Central Oregon Cst	Lincoln	OR	USA
RB133	JK	Central Oregon Cst	Lincoln	OR	USA

RB134	JK	Central Oregon Cst	Lincoln	OR	USA
RB135	JK	Central Oregon Cst	Lincoln	OR	USA
RB136	JK	Central Oregon Cst	Lincoln	OR	USA
RB137	JK	Central Oregon Cst	Lincoln	OR	USA
RB138	JK	Central Oregon Cst	Lincoln	OR	USA
RB139	JK	Central Oregon Cst	Lincoln	OR	USA
169254	MVZ	Crockett Pk	Lake	CA	USA
169257	MVZ	Crockett Pk	Lake	CA	USA
169258	MVZ	Crockett Pk	Lake	CA	USA
169259	MVZ	Crockett Pk	Lake	CA	USA
169261	MVZ	Crockett Pk	Lake	CA	USA
167003	MVZ	Crockett Pk	Lake	CA	USA
167004	MVZ	Crockett Pk	Lake	CA	USA
167005	MVZ	Crockett Pk	Lake	CA	USA
167006	MVZ	Crockett Pk	Lake	CA	USA
167007	MVZ	Crockett Pk	Lake	CA	USA
167008	MVZ	Crockett Pk	Lake	CA	USA
169257	MVZ	Crockett Pk	Lake	CA	USA
169261	MVZ	Crockett Pk	Lake	CA	USA
RB324	JK	Mendocino	Mendocino	CA	USA
RB325	JK	Mendocino	Mendocino	CA	USA
RB326	JK	Mendocino	Mendocino	CA	USA
RB327	JK	Mendocino	Mendocino	CA	USA
RB346	JK	Mendocino	Mendocino	CA	USA
RB347	JK	Mendocino	Mendocino	CA	USA
RB348	JK	Mendocino	Mendocino	CA	USA
RB349	JK	Mendocino	Mendocino	CA	USA
RB350	JK	Mendocino	Mendocino	CA	USA
RB402	JK	Mendocino	Mendocino	CA	USA
RB343	JK	Monterey	Monterey	CA	USA
RB344	JK	Monterey	Monterey	CA	USA
RB367	JK	Monterey	Monterey	CA	USA
RB368	JK	Monterey	Monterey	CA	USA
RB369	JK	Monterey	Monterey	CA	USA
RB370	JK	Monterey	Monterey	CA	USA
RB371	JK	Monterey	Monterey	CA	USA
RB372	JK	Monterey	Monterey	CA	USA
RB373	JK	Monterey	Monterey	CA	USA
RB374	JK	Monterey	Monterey	CA	USA
RB397	JK	Monterey	Monterey	CA	USA
RB398	JK	Monterey	Monterey	CA	USA
RB399	JK	Monterey	Monterey	CA	USA
RB400	JK	Monterey	Monterey	CA	USA
RB401	JK	Monterey	Monterey	CA	USA
RB333	JK	N California Cst	Del Norte	CA	USA
RB334	JK	N California Cst	Del Norte	CA	USA
RB335	JK	N California Cst	Del Norte	CA	USA
RB336	JK	N California Cst	Del Norte	CA	USA
RB337	JK	N California Cst	Del Norte	CA	USA
RB338	JK	N California Cst	Del Norte	CA	USA
RB339	JK	N California Cst	Del Norte	CA	USA
RB340	JK	N California Cst	Del Norte	CA	USA
RB341	JK	N California Cst	Del Norte	CA	USA
RB342	JK	N California Cst	Del Norte	CA	USA
JK03071	JK	Olympic Pen	Clallum	WA	USA
JK10009	JK	Olympic Pen	Clallum	WA	USA
JK10010	JK	Olympic Pen	Clallum	WA	USA
JK10011	JK	Olympic Pen	Clallum	WA	USA

JK10012	JK	Olympic Pen	Clallum	WA	USA
JK10013	JK	Olympic Pen	Clallum	WA	USA
JK10014	JK	Olympic Pen	Clallum	WA	USA
JK10015	JK	Olympic Pen	Clallum	WA	USA
JK10016	JK	Olympic Pen	Clallum	WA	USA
JK10017	JK	Olympic Pen	Clallum	WA	USA
JK10018	JK	Olympic Pen	Clallum	WA	USA
JK10019	JK	Olympic Pen	Clallum	WA	USA
ACR116	ACR	Rogue Rv	Jackson	OR	USA
ACR117	ACR	Rogue Rv	Jackson	OR	USA
ACR118	ACR	Rogue Rv	Jackson	OR	USA
ACR119	ACR	Rogue Rv	Jackson	OR	USA
ACR120	ACR	Rogue Rv	Jackson	OR	USA
JK10200	JK	San Bernadino Mtns	San Bernadino	CA	USA
JK10201	JK	San Bernadino Mtns	San Bernadino	CA	USA
JK10203	JK	San Bernadino Mtns	San Bernadino	CA	USA
JK10205	JK	San Bernadino Mtns	San Bernadino	CA	USA
RB186	JK	San Bernadino Mtns	San Bernadino	CA	USA
RB187	JK	San Bernadino Mtns	San Bernadino	CA	USA
JK10202	JK	San Jacinto Mtns	Riverside	CA	USA
JK10204	JK	San Jacinto Mtns	Riverside	CA	USA
JK10206	JK	San Jacinto Mtns	Riverside	CA	USA
JK10207	JK	San Jacinto Mtns	Riverside	CA	USA
JK10208	JK	San Jacinto Mtns	Riverside	CA	USA
RB188	JK	Shasta E	Shasta	CA	USA
RB189	JK	Shasta E	Shasta	CA	USA
RB190	JK	Shasta E	Shasta	CA	USA
RB191	JK	Shasta E	Shasta	CA	USA
RB192	JK	Shasta E	Shasta	CA	USA
RB193	JK	Shasta E	Shasta	CA	USA
RB351	JK	Shasta E	Shasta	CA	USA
RB352	JK	Shasta E	Shasta	CA	USA
RB353	JK	Shasta E	Shasta	CA	USA
RB354	JK	Shasta E	Shasta	CA	USA
RB355	JK	Shasta E	Shasta	CA	USA
ACR307	ACR	Shasta W	Shasta	CA	USA
ACR308	ACR	Shasta W	Shasta	CA	USA
ACR309	ACR	Shasta W	Shasta	CA	USA
ACR310	ACR	Shasta W	Shasta	CA	USA
ACR311	ACR	Shasta W	Shasta	CA	USA
ACR312	ACR	Shasta W	Shasta	CA	USA
ACR313	ACR	Shasta W	Shasta	CA	USA
ACR314	ACR	Shasta W	Shasta	CA	USA
168471	MVZ	Shasta W	Shasta	CA	USA
168472	MVZ	Shasta W	Shasta	CA	USA
168473	MVZ	Shasta W	Shasta	CA	USA
168474	MVZ	Shasta W	Shasta	CA	USA
168475	MVZ	Shasta W	Shasta	CA	USA
168476	MVZ	Shasta W	Shasta	CA	USA
168478	MVZ	Shasta W	Shasta	CA	USA
168479	MVZ	Shasta W	Shasta	CA	USA
168480	MVZ	Shasta W	Shasta	CA	USA
168481	MVZ	Shasta W	Shasta	CA	USA
168482	MVZ	Shasta W	Shasta	CA	USA
168483	MVZ	Shasta W	Shasta	CA	USA
168484	MVZ	Shasta W	Shasta	CA	USA
168485	MVZ	Shasta W	Shasta	CA	USA
168486	MVZ	Shasta W	Shasta	CA	USA

168487	MVZ	Shasta W	Shasta	CA	USA
168488	MVZ	Shasta W	Shasta	CA	USA
ACR367	ACR	Skagit	Skagit	WA	USA
ACR368	ACR	Skagit	Skagit	WA	USA
ACR369	ACR	Skagit	Skagit	WA	USA
ACR370	ACR	Skagit	Skagit	WA	USA
ACR372	ACR	Skagit	Skagit	WA	USA
ACR373	ACR	Skagit	Whatcom	WA	USA
ACR374	ACR	Skagit	Whatcom	WA	USA
ACR375	ACR	Skagit	Whatcom	WA	USA
ACR376	ACR	Skagit	Whatcom	WA	USA
ACR074	ACR	Christina Lk	-	BC	CA
ACR075	ACR	Christina Lk	-	BC	CA
ACR076	ACR	Christina Lk	-	BC	CA
ACR101	ACR	Christina Lk	-	BC	CA
ACR102	ACR	Christina Lk	-	BC	CA
ACR103	ACR	Christina Lk	-	BC	CA
ACR104	ACR	Christina Lk	-	BC	CA
ACR105	ACR	Christina Lk	-	BC	CA
ACR106	ACR	Christina Lk	-	BC	CA
ACR107	ACR	Christina Lk	-	BC	CA
ACR029	ACR	Kananaskis	-	AB	CA
ACR030	ACR	Kananaskis	-	AB	CA
ACR032	ACR	Kananaskis	-	AB	CA
ACR033	ACR	Kananaskis	-	AB	CA
ACR034	ACR	Kananaskis	-	AB	CA
ACR077	ACR	Kananaskis	-	AB	CA
ACR078	ACR	Kananaskis	-	AB	CA
ACR079	ACR	Kananaskis	-	AB	CA
ACR080	ACR	Kananaskis	-	AB	CA
ACR081	ACR	Kananaskis	-	AB	CA
ACR082	ACR	Kananaskis	-	AB	CA
RAM33097	RAM	Kananaskis	-	AB	CA
RAM33098	RAM	Kananaskis	-	AB	CA
RAM33099	RAM	Kananaskis	-	AB	CA
RAM33183	RAM	Kananaskis	-	AB	CA
RAM33808	RAM	Kananaskis	-	AB	CA
RAM33809	RAM	Kananaskis	-	AB	CA
ACR083	ACR	Kootenay Lk	-	BC	CA
ACR084	ACR	Kootenay Lk	-	BC	CA
ACR085	ACR	Kootenay Lk	-	BC	CA
ACR086	ACR	Kootenay Lk	-	BC	CA
ACR093	ACR	Lillooet	-	BC	CA
ACR026	ACR	Peace Rv	-	BC	CA
ACR027	ACR	Peace Rv	-	BC	CA
ACR018	ACR	Penticton	-	BC	CA
ACR019	ACR	Penticton	-	BC	CA
ACR061	ACR	Penticton	-	BC	CA
ACR062	ACR	Penticton	-	BC	CA
ACR065	ACR	Penticton	-	BC	CA
ACR066	ACR	Penticton	-	BC	CA
ACR100	ACR	Penticton	-	BC	CA
ACR014	ACR	Princeton	-	BC	CA
ACR017	ACR	Princeton	-	BC	CA
ACR071	ACR	Princeton	-	BC	CA
ACR072	ACR	Princeton	-	BC	CA
ACR073	ACR	Princeton	-	BC	CA
ACR098	ACR	Princeton	-	BC	CA

ACR099	ACR	Princeton	-	BC	CA
ACR087	ACR	Williams Lk	-	BC	CA
ACR088	ACR	Williams Lk	-	BC	CA
ACR089	ACR	Williams Lk	-	BC	CA
ACR090	ACR	Williams Lk	-	BC	CA
ACR091	ACR	Williams Lk	-	BC	CA
ACR108	ACR	Williams Lk	-	BC	CA
ACR109	ACR	Williams Lk	-	BC	CA
ACR273	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR274	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR275	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR276	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR277	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR278	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR279	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR280	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR281	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR282	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR283	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR284	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR295	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR380	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR381	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR382	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR384	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR391	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR393	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR394	ACR	Big Belt Mtns	Broadwater	MT	USA
ACR293	ACR	Big Belt Mtns	Lewis & Clark	MT	USA
ACR294	ACR	Big Belt Mtns	Lewis & Clark	MT	USA
ACR385	ACR	Big Belt Mtns	Lewis & Clark	MT	USA
ACR387	ACR	Big Belt Mtns	Lewis & Clark	MT	USA
ACR388	ACR	Big Belt Mtns	Lewis & Clark	MT	USA
ACR169	ACR	Bitterroot Mtns	Mineral	MT	USA
ACR170	ACR	Bitterroot Mtns	Mineral	MT	USA
ACR172	ACR	Bitterroot Mtns	Mineral	MT	USA
ACR243	ACR	Bitterroot Mtns	Mineral	MT	USA
BHSUX164	JK	Black Hills	Lawrence	SD	USA
BHSUX165	JK	Black Hills	Lawrence	SD	USA
BHSUX167	JK	Black Hills	Lawrence	SD	USA
BHSUX175	JK	Black Hills	Lawrence	SD	USA
JK10213	JK	Black Hills	Lawrence	SD	USA
JK10218	JK	Black Hills	Lawrence	SD	USA
JK10220	JK	Black Hills	Lawrence	SD	USA
JK10221	JK	Black Hills	Lawrence	SD	USA
JK10222	JK	Black Hills	Lawrence	SD	USA
168603	MVZ	Black Hills	Lawrence	SD	USA
168604	MVZ	Black Hills	Lawrence	SD	USA
168605	MVZ	Black Hills	Lawrence	SD	USA
168606	MVZ	Black Hills	Lawrence	SD	USA
168607	MVZ	Black Hills	Lawrence	SD	USA
168608	MVZ	Black Hills	Lawrence	SD	USA
168609	MVZ	Black Hills	Lawrence	SD	USA
168610	MVZ	Black Hills	Lawrence	SD	USA
ACR263	ACR	Blue Mtns N	Columbia	WA	USA
ACR264	ACR	Blue Mtns N	Columbia	WA	USA
ACR265	ACR	Blue Mtns N	Columbia	WA	USA
ACR204	ACR	Blue Mtns S	Umatilla	OR	USA

ACR205	ACR	Blue Mtns S	Umatilla	OR	USA
ACR206	ACR	Blue Mtns S	Umatilla	OR	USA
ACR207	ACR	Blue Mtns S	Umatilla	OR	USA
ACR208	ACR	Blue Mtns S	Umatilla	OR	USA
ACR209	ACR	Blue Mtns S	Umatilla	OR	USA
ACR210	ACR	Blue Mtns S	Umatilla	OR	USA
ACR212	ACR	Blue Mtns S	Umatilla	OR	USA
ACR213	ACR	Blue Mtns S	Umatilla	OR	USA
ACR211	ACR	Blue Mtns S	Union	OR	USA
ACR253	ACR	Clearwater Rv	Idaho	ID	USA
ACR254	ACR	Clearwater Rv	Idaho	ID	USA
ACR255	ACR	Clearwater Rv	Idaho	ID	USA
ACR256	ACR	Clearwater Rv	Idaho	ID	USA
ACR257	ACR	Clearwater Rv	Idaho	ID	USA
ACR258	ACR	Clearwater Rv	Idaho	ID	USA
ACR259	ACR	Clearwater Rv	Idaho	ID	USA
ACR260	ACR	Clearwater Rv	Idaho	ID	USA
ACR261	ACR	Clearwater Rv	Idaho	ID	USA
ACR268	ACR	Clearwater Rv	Idaho	ID	USA
ACR269	ACR	Clearwater Rv	Idaho	ID	USA
ACR270	ACR	Clearwater Rv	Idaho	ID	USA
ACR271	ACR	Clearwater Rv	Idaho	ID	USA
ACR272	ACR	Clearwater Rv	Idaho	ID	USA
ACR350	ACR	Clearwater Rv	Idaho	ID	USA
ACR351	ACR	Clearwater Rv	Idaho	ID	USA
ACR352	ACR	Clearwater Rv	Idaho	ID	USA
ACR353	ACR	Clearwater Rv	Idaho	ID	USA
ACR262	ACR	Clearwater Rv	Nez Perce	ID	USA
ACR156	ACR	Coeur d'Alene	Kootenai	ID	USA
ACR157	ACR	Coeur d'Alene	Kootenai	ID	USA
ACR158	ACR	Coeur d'Alene	Kootenai	ID	USA
ACR159	ACR	Coeur d'Alene	Kootenai	ID	USA
ACR160	ACR	Coeur d'Alene	Kootenai	ID	USA
ACR161	ACR	Coeur d'Alene	Kootenai	ID	USA
ACR162	ACR	Coeur d'Alene	Kootenai	ID	USA
ACR230	ACR	Coeur d'Alene	Kootenai	ID	USA
ACR231	ACR	Coeur d'Alene	Kootenai	ID	USA
ACR232	ACR	Coeur d'Alene	Kootenai	ID	USA
ACR233	ACR	Coeur d'Alene	Kootenai	ID	USA
ACR234	ACR	Coeur d'Alene	Kootenai	ID	USA
ACR235	ACR	Coeur d'Alene	Kootenai	ID	USA
ACR236	ACR	Coeur d'Alene	Kootenai	ID	USA
ACR237	ACR	Coeur d'Alene	Kootenai	ID	USA
ACR163	ACR	Coeur d'Alene	Shoshone	ID	USA
ACR164	ACR	Coeur d'Alene	Shoshone	ID	USA
ACR165	ACR	Coeur d'Alene	Shoshone	ID	USA
ACR166	ACR	Coeur d'Alene	Shoshone	ID	USA
ACR167	ACR	Coeur d'Alene	Shoshone	ID	USA
ACR168	ACR	Coeur d'Alene	Shoshone	ID	USA
ACR238	ACR	Coeur d'Alene	Shoshone	ID	USA
ACR239	ACR	Coeur d'Alene	Shoshone	ID	USA
ACR240	ACR	Coeur d'Alene	Shoshone	ID	USA
ACR241	ACR	Coeur d'Alene	Shoshone	ID	USA
ACR242	ACR	Coeur d'Alene	Shoshone	ID	USA
ACR292	ACR	Crazy Mtns	Sweet Grass	MT	USA
ACR122	ACR	Deschutes	Deschutes	OR	USA
ACR123	ACR	Deschutes	Deschutes	OR	USA
ACR418	ACR	Deschutes	Deschutes	OR	USA

ACR419	ACR	Deschutes	Deschutes	OR	USA
ACR420	ACR	Deschutes	Deschutes	OR	USA
ACR121	ACR	Ft Klamath	Klamath	OR	USA
ACR182	ACR	Hungry Horse	Flathead	MT	USA
JK05299	JK	Jarbidge Mtns	Elko	NV	USA
JK10185	JK	Jarbidge Mtns	Elko	NV	USA
JK10186	JK	Jarbidge Mtns	Elko	NV	USA
JK10187	JK	Jarbidge Mtns	Elko	NV	USA
JK10188	JK	Jarbidge Mtns	Elko	NV	USA
JK10189	JK	Jarbidge Mtns	Elko	NV	USA
JK10190	JK	Jarbidge Mtns	Elko	NV	USA
JK10191	JK	Jarbidge Mtns	Elko	NV	USA
JK10192	JK	Jarbidge Mtns	Elko	NV	USA
JK10193	JK	Jarbidge Mtns	Elko	NV	USA
ACR124	ACR	Kittitas	Kittitas	WA	USA
ACR125	ACR	Kittitas	Kittitas	WA	USA
ACR126	ACR	Kittitas	Kittitas	WA	USA
ACR127	ACR	Kittitas	Kittitas	WA	USA
ACR128	ACR	Kittitas	Kittitas	WA	USA
ACR214	ACR	Kittitas	Kittitas	WA	USA
ACR215	ACR	Kittitas	Kittitas	WA	USA
ACR216	ACR	Kittitas	Kittitas	WA	USA
ACR217	ACR	Kittitas	Kittitas	WA	USA
ACR218	ACR	Kittitas	Kittitas	WA	USA
ACR146	ACR	Lk Pend Orielle	Bonner Co.	ID	USA
ACR147	ACR	Lk Pend Orielle	Bonner Co.	ID	USA
ACR148	ACR	Lk Pend Orielle	Bonner Co.	ID	USA
ACR149	ACR	Lk Pend Orielle	Bonner Co.	ID	USA
ACR150	ACR	Lk Pend Orielle	Bonner Co.	ID	USA
ACR151	ACR	Lk Pend Orielle	Bonner Co.	ID	USA
ACR152	ACR	Lk Pend Orielle	Bonner Co.	ID	USA
ACR153	ACR	Lk Pend Orielle	Bonner Co.	ID	USA
ACR154	ACR	Lk Pend Orielle	Bonner Co.	ID	USA
ACR155	ACR	Lk Pend Orielle	Bonner Co.	ID	USA
ACR173	ACR	Lolo Vly	Ravalli	MT	USA
ACR174	ACR	Lolo Vly	Ravalli	MT	USA
ACR175	ACR	Lolo Vly	Ravalli	MT	USA
ACR176	ACR	Lolo Vly	Ravalli	MT	USA
ACR177	ACR	Lolo Vly	Ravalli	MT	USA
ACR249	ACR	Lolo Vly	Ravalli	MT	USA
ACR250	ACR	Lolo Vly	Ravalli	MT	USA
ACR251	ACR	Lolo Vly	Ravalli	MT	USA
ACR252	ACR	Lolo Vly	Ravalli	MT	USA
ACR377	ACR	Lolo Vly	Ravalli	MT	USA
ACR378	ACR	Lolo Vly	Ravalli	MT	USA
ACR329	ACR	Modoc	Modoc	CA	USA
ACR330	ACR	Modoc	Modoc	CA	USA
ACR331	ACR	Modoc	Modoc	CA	USA
ACR332	ACR	Modoc	Modoc	CA	USA
ACR333	ACR	Modoc	Modoc	CA	USA
ACR334	ACR	Modoc	Modoc	CA	USA
ACR335	ACR	Modoc	Modoc	CA	USA
ACR336	ACR	Modoc	Modoc	CA	USA
ACR337	ACR	Modoc	Modoc	CA	USA
ACR338	ACR	Modoc	Modoc	CA	USA
ACR339	ACR	Modoc	Modoc	CA	USA
ACR340	ACR	Modoc	Modoc	CA	USA
ACR341	ACR	Modoc	Modoc	CA	USA

ACR342	ACR	Modoc	Modoc	CA	USA
ACR343	ACR	Modoc	Modoc	CA	USA
JK10020	JK	Modoc	Modoc	CA	USA
JK10021	JK	Modoc	Modoc	CA	USA
JK10022	JK	Modoc	Modoc	CA	USA
JK10023	JK	Modoc	Modoc	CA	USA
JK10024	JK	Modoc	Modoc	CA	USA
JK10025	JK	Modoc	Modoc	CA	USA
JK10026	JK	Modoc	Modoc	CA	USA
JK10027	JK	Modoc	Modoc	CA	USA
JK10028	JK	Modoc	Modoc	CA	USA
JK10029	JK	Modoc	Modoc	CA	USA
ACR408	ACR	Ochoco Mtns	Crook	OR	USA
ACR409	ACR	Ochoco Mtns	Crook	OR	USA
ACR410	ACR	Ochoco Mtns	Crook	OR	USA
ACR411	ACR	Ochoco Mtns	Crook	OR	USA
ACR412	ACR	Ochoco Mtns	Crook	OR	USA
ACR413	ACR	Ochoco Mtns	Crook	OR	USA
ACR136	ACR	Okanogan E	Okanogan	WA	USA
ACR137	ACR	Okanogan E	Okanogan	WA	USA
ACR138	ACR	Okanogan E	Okanogan	WA	USA
ACR139	ACR	Okanogan E	Okanogan	WA	USA
ACR219	ACR	Okanogan E	Okanogan	WA	USA
ACR220	ACR	Okanogan E	Okanogan	WA	USA
ACR221	ACR	Okanogan E	Okanogan	WA	USA
ACR222	ACR	Okanogan E	Okanogan	WA	USA
ACR223	ACR	Okanogan E	Okanogan	WA	USA
ACR224	ACR	Okanogan E	Okanogan	WA	USA
ACR354	ACR	Okanogan E	Okanogan	WA	USA
ACR355	ACR	Okanogan E	Okanogan	WA	USA
ACR356	ACR	Okanogan E	Okanogan	WA	USA
ACR358	ACR	Okanogan E	Okanogan	WA	USA
ACR359	ACR	Okanogan E	Okanogan	WA	USA
ACR129	ACR	Okanogan W	Okanogan	WA	USA
ACR130	ACR	Okanogan W	Okanogan	WA	USA
ACR131	ACR	Okanogan W	Okanogan	WA	USA
ACR132	ACR	Okanogan W	Okanogan	WA	USA
ACR133	ACR	Okanogan W	Okanogan	WA	USA
ACR134	ACR	Okanogan W	Okanogan	WA	USA
ACR135	ACR	Okanogan W	Okanogan	WA	USA
ACR225	ACR	Okanogan W	Okanogan	WA	USA
ACR226	ACR	Okanogan W	Okanogan	WA	USA
ACR227	ACR	Okanogan W	Okanogan	WA	USA
ACR228	ACR	Okanogan W	Okanogan	WA	USA
ACR229	ACR	Okanogan W	Okanogan	WA	USA
ACR360	ACR	Okanogan W	Okanogan	WA	USA
ACR361	ACR	Okanogan W	Okanogan	WA	USA
ACR362	ACR	Okanogan W	Okanogan	WA	USA
JK08448	JK	Okanogan W	Okanogan	WA	USA
ACR402	ACR	Pattee Creek	Lemhi	ID	USA
ACR403	ACR	Pattee Creek	Lemhi	ID	USA
ACR404	ACR	Pattee Creek	Lemhi	ID	USA
ACR405	ACR	Pattee Creek	Lemhi	ID	USA
ACR406	ACR	Pattee Creek	Lemhi	ID	USA
ACR407	ACR	Pattee Creek	Lemhi	ID	USA
ACR414	ACR	Paulina Lk	Deschutes	OR	USA
ACR415	ACR	Paulina Lk	Deschutes	OR	USA
ACR416	ACR	Paulina Lk	Deschutes	OR	USA

ACR417	ACR	Paulina Lk	Deschutes	OR	USA
ACR266	ACR	Payette	Boise	ID	USA
ACR267	ACR	Payette	Boise	ID	USA
ACR296	ACR	Pocatello	Bannock	ID	USA
ACR297	ACR	Pocatello	Power	ID	USA
ACR298	ACR	Pocatello	Power	ID	USA
ACR299	ACR	Pocatello	Power	ID	USA
ACR300	ACR	Pocatello	Power	ID	USA
ACR301	ACR	Pocatello	Power	ID	USA
ACR344	ACR	Pocatello	Power	ID	USA
ACR345	ACR	Pocatello	Power	ID	USA
ACR346	ACR	Pocatello	Power	ID	USA
ACR347	ACR	Pocatello	Power	ID	USA
ACR348	ACR	Pocatello	Power	ID	USA
ACR349	ACR	Pocatello	Power	ID	USA
ACR285	ACR	Pryor Mtns	Carbon	MT	USA
ACR286	ACR	Pryor Mtns	Carbon	MT	USA
ACR287	ACR	Pryor Mtns	Carbon	MT	USA
ACR288	ACR	Pryor Mtns	Carbon	MT	USA
ACR289	ACR	Pryor Mtns	Carbon	MT	USA
ACR291	ACR	Pryor Mtns	Carbon	MT	USA
ACR186	ACR	Sawtooth Range	Lewis & Clark	MT	USA
ACR188	ACR	Sawtooth Range	Lewis & Clark	MT	USA
ACR183	ACR	Sawtooth Range	Teton	MT	USA
ACR184	ACR	Sawtooth Range	Teton	MT	USA
ACR315	ACR	Siskiyou	Siskiyou	CA	USA
ACR316	ACR	Siskiyou	Siskiyou	CA	USA
ACR317	ACR	Siskiyou	Siskiyou	CA	USA
ACR318	ACR	Siskiyou	Siskiyou	CA	USA
ACR319	ACR	Siskiyou	Siskiyou	CA	USA
ACR320	ACR	Siskiyou	Siskiyou	CA	USA
ACR321	ACR	Siskiyou	Siskiyou	CA	USA
ACR322	ACR	Siskiyou	Siskiyou	CA	USA
ACR323	ACR	Siskiyou	Siskiyou	CA	USA
ACR325	ACR	Siskiyou	Siskiyou	CA	USA
ACR326	ACR	Siskiyou	Siskiyou	CA	USA
ACR327	ACR	Siskiyou	Siskiyou	CA	USA
ACR328	ACR	Siskiyou	Siskiyou	CA	USA
168512	MVZ	Siskiyou	Siskiyou	CA	USA
168513	MVZ	Siskiyou	Siskiyou	CA	USA
168514	MVZ	Siskiyou	Siskiyou	CA	USA
168515	MVZ	Siskiyou	Siskiyou	CA	USA
168516	MVZ	Siskiyou	Siskiyou	CA	USA
168517	MVZ	Siskiyou	Siskiyou	CA	USA
168518	MVZ	Siskiyou	Siskiyou	CA	USA
168519	MVZ	Siskiyou	Siskiyou	CA	USA
168520	MVZ	Siskiyou	Siskiyou	CA	USA
168521	MVZ	Siskiyou	Siskiyou	CA	USA
168522	MVZ	Siskiyou	Siskiyou	CA	USA
168524	MVZ	Siskiyou	Siskiyou	CA	USA
168526	MVZ	Siskiyou	Siskiyou	CA	USA
168527	MVZ	Siskiyou	Siskiyou	CA	USA
168528	MVZ	Siskiyou	Siskiyou	CA	USA
JK01286	JK	Spring Mtns	Clark	NV	USA
JK01287	JK	Spring Mtns	Clark	NV	USA
JK01408	JK	Spring Mtns	Clark	NV	USA
RB314	JK	Spring Mtns	Clark	NV	USA
RB315	JK	Spring Mtns	Clark	NV	USA

ACR140	ACR	Sullivan Lk	Pend Oreille	WA	USA
ACR141	ACR	Sullivan Lk	Pend Oreille	WA	USA
ACR142	ACR	Sullivan Lk	Pend Oreille	WA	USA
ACR143	ACR	Sullivan Lk	Pend Oreille	WA	USA
ACR144	ACR	Sullivan Lk	Pend Oreille	WA	USA
ACR145	ACR	Sullivan Lk	Pend Oreille	WA	USA
ACR178	ACR	Thompson Rv	Sanders	MT	USA
ACR179	ACR	Thompson Rv	Sanders	MT	USA
ACR180	ACR	Thompson Rv	Sanders	MT	USA
ACR181	ACR	Thompson Rv	Sanders	MT	USA
ACR244	ACR	Thompson Rv	Sanders	MT	USA
ACR245	ACR	Thompson Rv	Sanders	MT	USA
ACR246	ACR	Thompson Rv	Sanders	MT	USA
ACR247	ACR	Thompson Rv	Sanders	MT	USA
ACR248	ACR	Thompson Rv	Sanders	MT	USA
ACR395	ACR	Thompson Rv	Sanders	MT	USA
ACR396	ACR	Thompson Rv	Sanders	MT	USA
ACR398	ACR	Thompson Rv	Sanders	MT	USA
ACR399	ACR	Thompson Rv	Sanders	MT	USA
ACR400	ACR	Thompson Rv	Sanders	MT	USA
ACR401	ACR	Thompson Rv	Sanders	MT	USA
ACR112	ACR	Warner Mtns N	Lake	OR	USA
ACR113	ACR	Warner Mtns N	Lake	OR	USA
ACR114	ACR	Warner Mtns N	Lake	OR	USA
ACR115	ACR	Warner Mtns N	Lake	OR	USA
ACR194	ACR	Warner Mtns N	Lake	OR	USA
ACR195	ACR	Warner Mtns N	Lake	OR	USA
ACR196	ACR	Warner Mtns N	Lake	OR	USA
ACR197	ACR	Warner Mtns N	Lake	OR	USA
ACR198	ACR	Warner Mtns N	Lake	OR	USA
ACR199	ACR	Warner Mtns N	Lake	OR	USA
ACR200	ACR	Warner Mtns N	Lake	OR	USA
ACR201	ACR	Warner Mtns N	Lake	OR	USA
ACR202	ACR	Warner Mtns N	Lake	OR	USA

Table A2. List of the SNPs discovered in the genetic analysis performed for Chapter 2. Individuals were genotyped for a subset (67, in boldfaced type) of these SNPs (see Methods).

No.	SNP	Chrom. No.	No.	SNP	Chrom. No.	No.	SNP	Chrom. No.
1	Emdi131579	1	53	Emdi305489	6	105	Emdi256390	13
2	Emdi189733	1	54	Emdi307296	6	106	Emdi286151	13
3	Emdi204427	1	55	Emdi311767	6	107	Emdi316352	13
4	Emdi205431	1	56	Emdi323521	6	108	Emdi324995	13
5	Emdi246215	1	57	Emdi171790	7	109	Emdi119872	14
6	Emdi321905	1	58	Emdi176845	7	110	Emdi146706	14
7	Emdi333653	1	59	Emdi260046	7	111	Emdi289907	14
8	Emdi355627	1	60	Emdi277587	7	112	Emdi300679	14
9	Emdi85355	1A	61	Emdi280733	7	113	Emdi302985	14
10	Emdi86657	1A	62	Emdi344601	7	114	Emdi323266	14
11	Emdi91575	1A	63	Emdi366467	7	115	Emdi327929	14
12	Emdi124883	1A	64	Emdi82209	8	116	Emdi355061	14
13	Emdi178472	1A	65	Emdi173266	8	117	Emdi42579	15
14	Emdi328481	1A	66	Emdi218861	8	118	Emdi207740	15
15	Emdi360731	1A	67	Emdi339884	8	119	Emdi247507	15
16	Emdi376396	1A	68	Emdi350285	8	120	Emdi249095	15
17	Emdi148824	2	69	Emdi371882	8	121	Emdi252118	15
18	Emdi150722	2	70	Emdi372756	8	122	Emdi295057	15
19	Emdi155472	2	71	Emdi42492	9	123	Emdi321886	15
20	Emdi234676	2	72	Emdi62684	9	124	Emdi354599	15
21	Emdi275225	2	73	Emdi123256	9	125	Emdi71070	4A
22	Emdi294871	2	74	Emdi191240	9	126	Emdi73152	4A
23	Emdi329457	2	75	Emdi239152	9	127	Emdi286484	4A
24	Emdi372200	2	76	Emdi304393	9	128	Emdi301656	4A
25	Emdi48765	3	77	Emdi304785	9	129	Emdi312957	4A
26	Emdi64961	3	78	Emdi323930	9	130	Emdi321804	4A
27	Emdi165554	3	79	Emdi98110	10	131	Emdi357895	4A
28	Emdi234724	3	80	Emdi25087	10	132	Emdi178366	17
29	Emdi258790	3	81	Emdi705860	10	133	Emdi182190	17
30	Emdi321884	3	82	Emdi290932	10	134	Emdi212307	17
31	Emdi329352	3	83	Emdi291301	10	135	Emdi217816	17
32	Emdi361023	3	84	Emdi344557	10	136	Emdi230556	17
33	Emdi11085	4	85	Emdi3696360	10	137	Emdi289737	17
34	Emdi189100	4	86	Emdi3705630	10	138	Emdi295554	17
35	Emdi259257	4	87	Emdi461031	11	139	Emdi296833	17
36	Emdi262151	4	88	Emdi646051	11	140	Emdi347965	17
37	Emdi286348	4	89	Emdi689791	11	141	Emdi97298	18
38	Emdi299865	4	90	Emdi299430	11	142	Emdi22317	18
39	Emdi311821	4	91	Emdi302706	11	143	Emdi22825	18
40	Emdi235256	5	92	Emdi313893	11	144	Emdi36151	18
41	Emdi247673	5	93	Emdi65427	12	145	Emdi110506	18
42	Emdi257469	5	94	Emdi95626	12	146	Emdi158198	18
43	Emdi270427	5	95	Emdi222900	12	147	Emdi227328	18
44	Emdi313284	5	96	Emdi260125	12	148	Emdi369300	18
45	Emdi330019	5	97	Emdi289704	12	149	Emdi40666	19
46	Emdi345999	5	98	Emdi321698	12	150	Emdi103484	19
47	Emdi351767	5	99	Emdi325962	12	151	Emdi229147	19
48	Emdi375014	5	100	Emdi350646	12	152	Emdi230575	19
49	Emdi3807	6	101	Emdi35543	13	153	Emdi288059	19
50	Emdi139302	6	102	Emdi56273	13	154	Emdi306968	19
51	Emdi294177	6	103	Emdi80851	13	155	Emdi356871	19
52	Emdi302158	6	104	Emdi181127	13	156	Emdi19743	20

No.	SNP	Chrom. No.	No.	SNP	Chrom. No.
157	Emdi165365	20	209	Emdi57469	Z
158	Emdi287201	20	210	Emdi58873	Z
159	Emdi324117	20	211	Emdi77788	Z
160	Emdi341962	20	212	Emdi87347	Z
161	Emdi347017	20	213	Emdi114281	Z
162	Emdi367709	20	214	Emdi115067	Z
163	Emdi68241	21	215	Emdi120775	Z
164	Emdi172238	21	216	Emdi159958	Z
165	Emdi196676	21	217	Emdi165305	Z
166	Emdi206134	21	218	Emdi182472	Z
167	Emdi208308	21	219	Emdi187972	Z
168	Emdi294693	21	220	Emdi188183	Z
169	Emdi347692	21	221	Emdi188514	Z
170	Emdi354282	21	222	Emdi189368	Z
171	Emdi16750	22	223	Emdi191781	Z
172	Emdi126362	22	224	Emdi199608	Z
173	Emdi207398	22	225	Emdi219423	Z
174	Emdi234921	22	226	Emdi225033	Z
175	Emdi306306	22	227	Emdi231086	Z
176	Emdi343934	22	228	Emdi235393	Z
177	Emdi359817	22	229	Emdi247784	Z
178	Emdi66080	23	230	Emdi250694	Z
179	Emdi68713	23	231	Emdi261576	Z
180	Emdi120253	23	232	Emdi265953	Z
181	Emdi251870	23	233	Emdi287269	Z
182	Emdi253404	23	234	Emdi294077	Z
183	Emdi255237	23	235	Emdi298316	Z
184	Emdi310905	23	236	Emdi303268	Z
185	Emdi336052	23	237	Emdi306688	Z
186	Emdi42501	24	238	Emdi309351	Z
187	Emdi123390	24	239	Emdi309629	Z
188	Emdi189855	24	240	Emdi311766	Z
189	Emdi299685	24	241	Emdi320279	Z
190	Emdi316917	24	242	Emdi335901	Z
191	Emdi320187	24	243	Emdi337408	Z
192	Emdi325982	24	244	Emdi338586	Z
193	Emdi327479	24	245	Emdi339939	Z
194	Emdi30747	25	246	Emdi350503	Z
195	Emdi180680	25	247	Emdi354077	Z
196	Emdi210095	25	248	Emdi355580	Z
197	Emdi337311	25	249	Emdi371082	Z
198	Emdi342358	25	250	Emdi376996	Z
199	Emdi374443	25			
200	Emdi376287	25			
201	Emdi11496	Z			
202	Emdi18542	Z			
203	Emdi40292	Z			
204	Emdi41600	Z			
205	Emdi47118	Z			
206	Emdi50029	Z			
207	Emdi53600	Z			
208	Emdi55737	Z			

Table A3. List of samples used for SNP analysis in Chapter 2. Source abbreviations: ACR = Andrew C. Rush, JK = John Klicka/Univ. Washington Burke Museum, MVZ = Museum of Vertebrate Zoology/Univ. California Berkeley, RAM = Royal Alberta Museum. “Prop. PS” refers to the proportion of Pacific-slope Flycatcher ancestry from *Structure* population assignment test.

Catalog	Source	Site	County	State	Country	Prop. PS
167099	MVZ	Mogollon AZ	Apache	AZ	USA	0.08
167100	MVZ	Mogollon AZ	Apache	AZ	USA	0.07
167101	MVZ	Mogollon AZ	Apache	AZ	USA	0.16
167102	MVZ	Mogollon AZ	Apache	AZ	USA	0.24
167103	MVZ	Mogollon AZ	Apache	AZ	USA	0.18
167104	MVZ	Mogollon AZ	Apache	AZ	USA	0.05
167105	MVZ	Mogollon AZ	Apache	AZ	USA	0.29
167106	MVZ	Mogollon AZ	Apache	AZ	USA	0.20
167107	MVZ	Mogollon AZ	Apache	AZ	USA	0.06
167108	MVZ	Mogollon AZ	Apache	AZ	USA	0.19
GMS1587	JK	Chiricahua Mtns	Cochise	AZ	USA	0.07
GMS1592	JK	Chiricahua Mtns	Cochise	AZ	USA	0.19
RB274	JK	Chiricahua Mtns	Cochise	AZ	USA	0.05
RB275	JK	Chiricahua Mtns	Cochise	AZ	USA	0.17
RB358	JK	Chiricahua Mtns	Cochise	AZ	USA	0.11
RB359	JK	Chiricahua Mtns	Cochise	AZ	USA	0.05
RB360	JK	Chiricahua Mtns	Cochise	AZ	USA	0.04
RB365	JK	Chiricahua Mtns	Cochise	AZ	USA	0.05
RB366	JK	Chiricahua Mtns	Cochise	AZ	USA	0.12
RB230	JK	Coconino	Coconino	AZ	USA	0.13
RB231	JK	Coconino	Coconino	AZ	USA	0.09
RB232	JK	Coconino	Coconino	AZ	USA	0.18
RB233	JK	Coconino	Coconino	AZ	USA	0.09
RB234	JK	Coconino	Coconino	AZ	USA	0.15
RB235	JK	Coconino	Coconino	AZ	USA	0.19
RB237	JK	Coconino	Coconino	AZ	USA	0.05
RB238	JK	Coconino	Coconino	AZ	USA	0.05
RB239	JK	Coconino	Coconino	AZ	USA	0.12
RB240	JK	Coconino	Coconino	AZ	USA	0.15
167109	MVZ	Pinaleno Mtns	Graham	AZ	USA	0.04
167111	MVZ	Pinaleno Mtns	Graham	AZ	USA	0.08
167112	MVZ	Pinaleno Mtns	Graham	AZ	USA	0.13
ACR448	ACR	Pinaleno Mtns	Graham	AZ	USA	0.25
JK09540	JK	Pinaleno Mtns	Graham	AZ	USA	0.26
JK09542	JK	Pinaleno Mtns	Graham	AZ	USA	0.06
JK09547	JK	Pinaleno Mtns	Graham	AZ	USA	0.19
JK09551	JK	Pinaleno Mtns	Graham	AZ	USA	0.04
RB356	JK	Mohave	Mohave	AZ	USA	0.13
RB361	JK	Mohave	Mohave	AZ	USA	0.14
RB362	JK	Mohave	Mohave	AZ	USA	0.13
RB363	JK	Mohave	Mohave	AZ	USA	0.09
167071	MVZ	Wet Mtnsn	Custer	CO	USA	0.10
167073	MVZ	Wet Mtnsn	Custer	CO	USA	0.09
167076	MVZ	Wet Mtnsn	Custer	CO	USA	0.07
167077	MVZ	Wet Mtnsn	Custer	CO	USA	0.13
167079	MVZ	Wet Mtnsn	Custer	CO	USA	0.07
167080	MVZ	Wet Mtnsn	Custer	CO	USA	0.10
167082	MVZ	Wet Mtnsn	Custer	CO	USA	0.26
167083	MVZ	Wet Mtnsn	Custer	CO	USA	0.20

167086	MVZ	Wet Mtns	Custer	CO	USA	0.13
167089	MVZ	Wet Mtns	Custer	CO	USA	0.10
167090	MVZ	Wet Mtns	Custer	CO	USA	0.13
ACR438	ACR	Wet Mtns	Custer	CO	USA	0.06
ACR439	ACR	Wet Mtns	Custer	CO	USA	0.10
ACR440	ACR	Wet Mtns	Custer	CO	USA	0.13
ACR441	ACR	Wet Mtns	Custer	CO	USA	0.12
ACR437	ACR	Gunnison	Gunnison	CO	USA	0.10
RB285	JK	Larimer	Larimer	CO	USA	0.18
RB288	JK	Larimer	Larimer	CO	USA	0.10
RB289	JK	Larimer	Larimer	CO	USA	0.19
RB291	JK	Larimer	Larimer	CO	USA	0.19
RB292	JK	Larimer	Larimer	CO	USA	0.28
ACR442	ACR	Black Range	Grant	NM	USA	0.09
ACR443	ACR	Black Range	Grant	NM	USA	0.19
ACR444	ACR	Black Range	Grant	NM	USA	0.05
ACR446	ACR	Black Range	Grant	NM	USA	0.16
ACR447	ACR	Black Range	Grant	NM	USA	0.13
ACR449	ACR	Black Range	Grant	NM	USA	0.09
ACR450	ACR	Black Range	Grant	NM	USA	0.07
ACR451	ACR	Black Range	Grant	NM	USA	0.10
ACR452	ACR	Black Range	Grant	NM	USA	0.06
JK04601	JK	Sacramento Mtns	Lincoln	NM	USA	0.14
RB277	JK	Sacramento Mtns	Lincoln	NM	USA	0.09
RB278	JK	Sacramento Mtns	Lincoln	NM	USA	0.19
RB279	JK	Sacramento Mtns	Lincoln	NM	USA	0.15
RB280	JK	Sacramento Mtns	Lincoln	NM	USA	0.08
RB281	JK	Sacramento Mtns	Lincoln	NM	USA	0.09
RB316	JK	Sacramento Mtns	Lincoln	NM	USA	0.13
RB317	JK	Sacramento Mtns	Lincoln	NM	USA	0.13
RB318	JK	Sacramento Mtns	Lincoln	NM	USA	0.06
RB319	JK	Sacramento Mtns	Lincoln	NM	USA	0.05
RB320	JK	Sacramento Mtns	Lincoln	NM	USA	0.15
RB321	JK	Sacramento Mtns	Lincoln	NM	USA	0.10
RB218	JK	Taos	Taos	NM	USA	0.19
RB219	JK	Taos	Taos	NM	USA	0.05
RB220	JK	Taos	Taos	NM	USA	0.08
RB221	JK	Taos	Taos	NM	USA	0.10
RB222	JK	Taos	Taos	NM	USA	0.07
RB223	JK	Taos	Taos	NM	USA	0.07
RB224	JK	Taos	Taos	NM	USA	0.06
RB225	JK	Taos	Taos	NM	USA	0.15
RB226	JK	Taos	Taos	NM	USA	0.23
RB227	JK	Taos	Taos	NM	USA	0.13
168529	MVZ	Snake Range	White Pine	NV	USA	0.20
168530	MVZ	Snake Range	White Pine	NV	USA	0.16
168531	MVZ	Snake Range	White Pine	NV	USA	0.11
168532	MVZ	Snake Range	White Pine	NV	USA	0.25
168533	MVZ	Snake Range	White Pine	NV	USA	0.19
168534	MVZ	Snake Range	White Pine	NV	USA	0.07
168535	MVZ	Snake Range	White Pine	NV	USA	0.07
168536	MVZ	Snake Range	White Pine	NV	USA	0.29
168537	MVZ	Snake Range	White Pine	NV	USA	0.18
168538	MVZ	Snake Range	White Pine	NV	USA	0.18
168539	MVZ	Snake Range	White Pine	NV	USA	0.14
168545	MVZ	Snake Range	White Pine	NV	USA	0.07
168546	MVZ	Snake Range	White Pine	NV	USA	0.08
168547	MVZ	Snake Range	White Pine	NV	USA	0.09

168548	MVZ	Snake Range	White Pine	NV	USA	0.31
ACR427	ACR	Uinta	Juab, Utah	UT	USA	0.15
ACR428	ACR	Uinta	Juab, Utah	UT	USA	0.20
ACR429	ACR	Uinta	Juab, Utah	UT	USA	0.18
ACR430	ACR	Uinta	Juab, Utah	UT	USA	0.32
ACR431	ACR	Uinta	Juab, Utah	UT	USA	0.11
ACR432	ACR	Uinta	Juab, Utah	UT	USA	0.08
ACR433	ACR	Uinta	Juab, Utah	UT	USA	0.21
ACR434	ACR	Uinta	Juab, Utah	UT	USA	0.06
ACR435	ACR	Uinta	Juab, Utah	UT	USA	0.27
ACR436	ACR	Uinta	Juab, Utah	UT	USA	0.33
RB179	JK	Washington Co. UT	Washington	UT	USA	0.07
RB180	JK	Washington Co. UT	Washington	UT	USA	0.17
RB181	JK	Washington Co. UT	Washington	UT	USA	0.22
RB182	JK	Washington Co. UT	Washington	UT	USA	0.17
RB183	JK	Washington Co. UT	Washington	UT	USA	0.09
RB184	JK	Washington Co. UT	Washington	UT	USA	0.38
RB185	JK	Washington Co. UT	Washington	UT	USA	0.08
ACR010	ACR	Hope	-	BC	CA	0.88
ACR012	ACR	Hope	-	BC	CA	0.88
ACR069	ACR	Hope	-	BC	CA	0.89
ACR070	ACR	Hope	-	BC	CA	0.92
ACR094	ACR	Hope	-	BC	CA	0.87
ACR095	ACR	Hope	-	BC	CA	0.92
ACR096	ACR	Hope	-	BC	CA	0.85
47300	MVZ	San Pedro Martir	-	BC	MX	0.88
48024	MVZ	San Pedro Martir	-	BC	MX	0.96
48026	MVZ	San Pedro Martir	-	BC	MX	0.91
48027	MVZ	San Pedro Martir	-	BC	MX	0.94
52928	MVZ	San Pedro Martir	-	BC	MX	0.93
314	MVZ	Sitka	Sitka	AK	USA	0.92
329	MVZ	Sitka	Sitka	AK	USA	0.88
9716	MVZ	Sitka	Sitka	AK	USA	0.84
9718	MVZ	Sitka	Sitka	AK	USA	0.87
9719	MVZ	Sitka	Sitka	AK	USA	0.91
RB333	JK	DelNorte	DelNorte	CA	USA	0.92
RB334	JK	DelNorte	DelNorte	CA	USA	0.89
RB335	JK	DelNorte	DelNorte	CA	USA	0.89
RB336	JK	DelNorte	DelNorte	CA	USA	0.86
RB338	JK	DelNorte	DelNorte	CA	USA	0.89
RB339	JK	DelNorte	DelNorte	CA	USA	0.89
RB340	JK	DelNorte	DelNorte	CA	USA	0.94
RB341	JK	DelNorte	DelNorte	CA	USA	0.91
RB342	JK	DelNorte	DelNorte	CA	USA	0.95
AJS88	MVZ	Walker Pass	Kern	CA	USA	0.94
JAC302	MVZ	Walker Pass	Kern	CA	USA	0.93
JAC314	MVZ	Walker Pass	Kern	CA	USA	0.76
JAC316	MVZ	Walker Pass	Kern	CA	USA	0.87
KMCR24	MVZ	Walker Pass	Kern	CA	USA	0.84
167002	MVZ	Lake	Lake	CA	USA	0.87
167003	MVZ	Lake	Lake	CA	USA	0.94
167004	MVZ	Lake	Lake	CA	USA	0.91
167005	MVZ	Lake	Lake	CA	USA	0.91
167006	MVZ	Lake	Lake	CA	USA	0.92
167007	MVZ	Lake	Lake	CA	USA	0.80
167008	MVZ	Lake	Lake	CA	USA	0.85
169255	MVZ	Lake	Lake	CA	USA	0.92
169256	MVZ	Lake	Lake	CA	USA	0.94

169257	MVZ	Lake	Lake	CA	USA	0.87
169258	MVZ	Lake	Lake	CA	USA	0.94
169259	MVZ	Lake	Lake	CA	USA	0.89
169260	MVZ	Lake	Lake	CA	USA	0.93
169261	MVZ	Lake	Lake	CA	USA	0.87
25589	MVZ	Mariposa	Mariposa	CA	USA	0.88
25591	MVZ	Mariposa	Mariposa	CA	USA	0.92
40745	MVZ	Mariposa	Mariposa	CA	USA	0.93
RB324	JK	Mendocino	Mendocino	CA	USA	0.91
RB325	JK	Mendocino	Mendocino	CA	USA	0.93
RB326	JK	Mendocino	Mendocino	CA	USA	0.83
RB327	JK	Mendocino	Mendocino	CA	USA	0.94
RB346	JK	Mendocino	Mendocino	CA	USA	0.87
RB347	JK	Mendocino	Mendocino	CA	USA	0.90
RB349	JK	Mendocino	Mendocino	CA	USA	0.94
RB402	JK	Mendocino	Mendocino	CA	USA	0.77
169269	MVZ	Monterey	Monterey	CA	USA	0.81
169270	MVZ	Monterey	Monterey	CA	USA	0.90
169271	MVZ	Monterey	Monterey	CA	USA	0.70
169272	MVZ	Monterey	Monterey	CA	USA	0.90
169273	MVZ	Monterey	Monterey	CA	USA	0.88
169274	MVZ	Monterey	Monterey	CA	USA	0.92
169275	MVZ	Monterey	Monterey	CA	USA	0.94
169277	MVZ	Monterey	Monterey	CA	USA	0.79
169278	MVZ	Monterey	Monterey	CA	USA	0.93
169279	MVZ	Monterey	Monterey	CA	USA	0.92
169282	MVZ	Monterey	Monterey	CA	USA	0.88
170158	MVZ	Monterey	Monterey	CA	USA	0.94
170159	MVZ	Monterey	Monterey	CA	USA	0.87
ACR303	ACR	Monterey	Monterey	CA	USA	0.87
ACR304	ACR	Monterey	Monterey	CA	USA	0.82
ACR305	ACR	Monterey	Monterey	CA	USA	0.94
ACR306	ACR	Monterey	Monterey	CA	USA	0.85
JK10202	JK	San Jacinto Mtns	Riverside	CA	USA	0.85
JK10204	JK	San Jacinto Mtns	Riverside	CA	USA	0.91
JK10206	JK	San Jacinto Mtns	Riverside	CA	USA	0.84
JK10208	JK	San Jacinto Mtns	Riverside	CA	USA	0.91
JK10200	JK	San Bernadino Mtns	San Bernadino	CA	USA	0.52
JK10201	JK	San Bernadino Mtns	San Bernadino	CA	USA	0.68
JK10203	JK	San Bernadino Mtns	San Bernadino	CA	USA	0.95
JK10205	JK	San Bernadino Mtns	San Bernadino	CA	USA	0.91
RB186	JK	San Bernadino Mtns	San Bernadino	CA	USA	0.92
RB187	JK	San Bernadino Mtns	San Bernadino	CA	USA	0.92
RB404	JK	SantaBarbara	SantaBarbara	CA	USA	0.84
RB188	JK	Shasta-East	Shasta	CA	USA	0.91
RB189	JK	Shasta-East	Shasta	CA	USA	0.84
RB190	JK	Shasta-East	Shasta	CA	USA	0.87
RB191	JK	Shasta-East	Shasta	CA	USA	0.94
RB192	JK	Shasta-East	Shasta	CA	USA	0.70
RB193	JK	Shasta-East	Shasta	CA	USA	0.78
RB351	JK	Shasta-East	Shasta	CA	USA	0.81
RB352	JK	Shasta-East	Shasta	CA	USA	0.92
RB353	JK	Shasta-East	Shasta	CA	USA	0.88

RB354	JK	Shasta-East	Shasta	CA	USA	0.75
RB355	JK	Shasta-East	Shasta	CA	USA	0.91
168471	MVZ	Shasta-West	Shasta	CA	USA	0.83
168472	MVZ	Shasta-West	Shasta	CA	USA	0.88
168473	MVZ	Shasta-West	Shasta	CA	USA	0.89
168474	MVZ	Shasta-West	Shasta	CA	USA	0.94
168475	MVZ	Shasta-West	Shasta	CA	USA	0.92
168476	MVZ	Shasta-West	Shasta	CA	USA	0.92
168477	MVZ	Shasta-West	Shasta	CA	USA	0.87
168478	MVZ	Shasta-West	Shasta	CA	USA	0.90
168479	MVZ	Shasta-West	Shasta	CA	USA	0.86
168480	MVZ	Shasta-West	Shasta	CA	USA	0.92
168481	MVZ	Shasta-West	Shasta	CA	USA	0.74
168482	MVZ	Shasta-West	Shasta	CA	USA	0.82
168483	MVZ	Shasta-West	Shasta	CA	USA	0.83
168484	MVZ	Shasta-West	Shasta	CA	USA	0.88
168485	MVZ	Shasta-West	Shasta	CA	USA	0.86
168486	MVZ	Shasta-West	Shasta	CA	USA	0.72
168487	MVZ	Shasta-West	Shasta	CA	USA	0.94
168488	MVZ	Shasta-West	Shasta	CA	USA	0.85
ACR307	ACR	Shasta-West	Shasta	CA	USA	0.74
ACR308	ACR	Shasta-West	Shasta	CA	USA	0.92
ACR309	ACR	Shasta-West	Shasta	CA	USA	0.87
ACR310	ACR	Shasta-West	Shasta	CA	USA	0.78
ACR311	ACR	Shasta-West	Shasta	CA	USA	0.84
ACR312	ACR	Shasta-West	Shasta	CA	USA	0.90
ACR313	ACR	Shasta-West	Shasta	CA	USA	0.89
ACR314	ACR	Shasta-West	Shasta	CA	USA	0.89
CC3417	MVZ	Red Bluff	Tehama	CA	USA	0.81
EAW57	MVZ	Red Bluff	Tehama	CA	USA	0.92
JAC272	MVZ	Red Bluff	Tehama	CA	USA	0.32
KEL82	MVZ	Red Bluff	Tehama	CA	USA	0.91
169262	MVZ	Rogue	Jackson	OR	USA	0.79
169263	MVZ	Rogue	Jackson	OR	USA	0.73
169266	MVZ	Rogue	Jackson	OR	USA	0.92
169267	MVZ	Rogue	Jackson	OR	USA	0.84
169268	MVZ	Rogue	Jackson	OR	USA	0.89
169285	MVZ	Rogue	Jackson	OR	USA	0.54
169286	MVZ	Rogue	Jackson	OR	USA	0.89
169287	MVZ	Rogue	Jackson	OR	USA	0.68
ACR116	ACR	Rogue	Jackson	OR	USA	0.80
ACR117	ACR	Rogue	Jackson	OR	USA	0.92
ACR118	ACR	Rogue	Jackson	OR	USA	0.72
ACR119	ACR	Rogue	Jackson	OR	USA	0.95
ACR120	ACR	Rogue	Jackson	OR	USA	0.92
RB132	JK	Central Oregon Cst	Lincoln	OR	USA	0.90
RB133	JK	Lincoln	Lincoln	OR	USA	0.95
RB134	JK	Lincoln	Lincoln	OR	USA	0.90
RB135	JK	Lincoln	Lincoln	OR	USA	0.92
RB136	JK	Lincoln	Lincoln	OR	USA	0.90
RB137	JK	Lincoln	Lincoln	OR	USA	0.96
RB138	JK	Lincoln	Lincoln	OR	USA	0.89
RB139	JK	Lincoln	Lincoln	OR	USA	0.74
JK10009	JK	Olympic Pen	Clallum	WA	USA	0.90
JK10010	JK	Olympic Pen	Clallum	WA	USA	0.93
JK10011	JK	Olympic Pen	Clallum	WA	USA	0.96
JK10012	JK	Olympic Pen	Clallum	WA	USA	0.81
JK10013	JK	Olympic Pen	Clallum	WA	USA	0.94

JK10014	JK	Olympic Pen	Clallum	WA	USA	0.85
JK10015	JK	Olympic Pen	Clallum	WA	USA	0.95
JK10017	JK	Olympic Pen	Clallum	WA	USA	0.90
JK10018	JK	Olympic Pen	Clallum	WA	USA	0.87
JK10019	JK	Olympic Pen	Clallum	WA	USA	0.93
ACR367	ACR	Skagit	Skagit, Whatcom	WA	USA	0.93
ACR368	ACR	Skagit	Skagit, Whatcom	WA	USA	0.87
ACR369	ACR	Skagit	Skagit, Whatcom	WA	USA	0.91
ACR370	ACR	Skagit	Skagit, Whatcom	WA	USA	0.91
ACR371	ACR	Skagit	Skagit, Whatcom	WA	USA	0.95
ACR372	ACR	Skagit	Skagit, Whatcom	WA	USA	0.94
ACR373	ACR	Skagit	Skagit, Whatcom	WA	USA	0.93
ACR374	ACR	Skagit	Skagit, Whatcom	WA	USA	0.81
ACR375	ACR	Skagit	Skagit, Whatcom	WA	USA	0.94
ACR376	ACR	Skagit	Skagit, Whatcom	WA	USA	0.83
ACR026	ACR	Chetwynd	-	BC	CA	0.45
ACR027	ACR	Chetwynd	-	BC	CA	0.57
ACR022	ACR	Christina Lake	-	BC	CA	0.32
ACR074	ACR	Christina Lake	-	BC	CA	0.72
ACR075	ACR	Christina Lake	-	BC	CA	0.37
ACR076	ACR	Christina Lake	-	BC	CA	0.30
ACR101	ACR	Christina Lake	-	BC	CA	0.47
ACR103	ACR	Christina Lake	-	BC	CA	0.78
ACR104	ACR	Christina Lake	-	BC	CA	0.57
ACR105	ACR	Christina Lake	-	BC	CA	0.49
ACR106	ACR	Christina Lake	-	BC	CA	0.43
ACR107	ACR	Christina Lake	-	BC	CA	0.58
ACR037	ACR	Kootenay Lake	-	BC	CA	0.40
ACR083	ACR	Kootenay Lake	-	BC	CA	0.59
ACR085	ACR	Kootenay Lake	-	BC	CA	0.37
ACR086	ACR	Kootenay Lake	-	BC	CA	0.26
ACR093	ACR	Lillooet	-	BC	CA	0.44
ACR018	ACR	OK-BC	-	BC	CA	0.33
ACR019	ACR	OK-BC	-	BC	CA	0.44
ACR061	ACR	OK-BC	-	BC	CA	0.57
ACR062	ACR	OK-BC	-	BC	CA	0.81
ACR065	ACR	OK-BC	-	BC	CA	0.55
ACR066	ACR	OK-BC	-	BC	CA	0.74
ACR100	ACR	OK-BC	-	BC	CA	0.44
ACR014	ACR	Princeton	-	BC	CA	0.74
ACR017	ACR	Princeton	-	BC	CA	0.77
ACR071	ACR	Princeton	-	BC	CA	0.60
ACR072	ACR	Princeton	-	BC	CA	0.56
ACR073	ACR	Princeton	-	BC	CA	0.53
ACR098	ACR	Princeton	-	BC	CA	0.53
ACR099	ACR	Princeton	-	BC	CA	0.79
ACR087	ACR	Williams Lake	-	BC	CA	0.38
ACR088	ACR	Williams Lake	-	BC	CA	0.77
ACR089	ACR	Williams Lake	-	BC	CA	0.88
ACR090	ACR	Williams Lake	-	BC	CA	0.58
ACR091	ACR	Williams Lake	-	BC	CA	0.60
ACR108	ACR	Williams Lake	-	BC	CA	0.66
ACR109	ACR	Williams Lake	-	BC	CA	0.63
ACR029	ACR	Kananaskis	-	AB	USA	0.52
ACR030	ACR	Kananaskis	-	AB	USA	0.62
ACR031	ACR	Kananaskis	-	AB	USA	0.64
ACR032	ACR	Kananaskis	-	AB	USA	0.50
ACR033	ACR	Kananaskis	-	AB	USA	0.56

ACR034	ACR	Kananaskis	-	AB	USA	0.36
ACR036	ACR	Kananaskis	-	AB	USA	0.63
ACR077	ACR	Kananaskis	-	AB	USA	0.35
ACR078	ACR	Kananaskis	-	AB	USA	0.72
ACR079	ACR	Kananaskis	-	AB	USA	0.58
ACR080	ACR	Kananaskis	-	AB	USA	0.63
ACR081	ACR	Kananaskis	-	AB	USA	0.60
ACR082	ACR	Kananaskis	-	AB	USA	0.59
RAM33097	RAM	Kananaskis	-	AB	USA	0.34
RAM33098	RAM	Kananaskis	-	AB	USA	0.41
RAM33099	RAM	Kananaskis	-	AB	USA	0.55
RAM33183	RAM	Kananaskis	-	AB	USA	0.84
RAM33808	RAM	Kananaskis	-	AB	USA	0.83
RAM33809	RAM	Kananaskis	-	AB	USA	0.63
166510	MVZ	Modoc	Modoc	CA	USA	0.14
ACR329	ACR	Modoc	Modoc	CA	USA	0.19
ACR330	ACR	Modoc	Modoc	CA	USA	0.33
ACR331	ACR	Modoc	Modoc	CA	USA	0.35
ACR333	ACR	Modoc	Modoc	CA	USA	0.52
ACR334	ACR	Modoc	Modoc	CA	USA	0.42
ACR335	ACR	Modoc	Modoc	CA	USA	0.28
ACR336	ACR	Modoc	Modoc	CA	USA	0.25
ACR337	ACR	Modoc	Modoc	CA	USA	0.17
ACR338	ACR	Modoc	Modoc	CA	USA	0.49
ACR339	ACR	Modoc	Modoc	CA	USA	0.09
ACR340	ACR	Modoc	Modoc	CA	USA	0.26
ACR341	ACR	Modoc	Modoc	CA	USA	0.39
ACR342	ACR	Modoc	Modoc	CA	USA	0.54
ACR343	ACR	Modoc	Modoc	CA	USA	0.30
JK10020	JK	Modoc	Modoc	CA	USA	0.20
JK10021	JK	Modoc	Modoc	CA	USA	0.32
JK10022	JK	Modoc	Modoc	CA	USA	0.12
JK10023	JK	Modoc	Modoc	CA	USA	0.12
JK10024	JK	Modoc	Modoc	CA	USA	0.21
JK10025	JK	Modoc	Modoc	CA	USA	0.41
JK10026	JK	Modoc	Modoc	CA	USA	0.43
JK10027	JK	Modoc	Modoc	CA	USA	0.28
JK10028	JK	Modoc	Modoc	CA	USA	0.10
JK10029	JK	Modoc	Modoc	CA	USA	0.21
132285	MVZ	Mono	Mono	CA	USA	0.92
168512	MVZ	Siskiyou	Siskiyou	CA	USA	0.49
168513	MVZ	Siskiyou	Siskiyou	CA	USA	0.79
168514	MVZ	Siskiyou	Siskiyou	CA	USA	0.58
168515	MVZ	Siskiyou	Siskiyou	CA	USA	0.47
168516	MVZ	Siskiyou	Siskiyou	CA	USA	0.90
168517	MVZ	Siskiyou	Siskiyou	CA	USA	0.44
168518	MVZ	Siskiyou	Siskiyou	CA	USA	0.78
168519	MVZ	Siskiyou	Siskiyou	CA	USA	0.49
168520	MVZ	Siskiyou	Siskiyou	CA	USA	0.85
168521	MVZ	Siskiyou	Siskiyou	CA	USA	0.82
168522	MVZ	Siskiyou	Siskiyou	CA	USA	0.79
168523	MVZ	Siskiyou	Siskiyou	CA	USA	0.54
168524	MVZ	Siskiyou	Siskiyou	CA	USA	0.35
168525	MVZ	Siskiyou	Siskiyou	CA	USA	0.83
168526	MVZ	Siskiyou	Siskiyou	CA	USA	0.94
168527	MVZ	Siskiyou	Siskiyou	CA	USA	0.45
168528	MVZ	Siskiyou	Siskiyou	CA	USA	0.90
178175	MVZ	Siskiyou	Siskiyou	CA	USA	0.86

178176	MVZ	Siskiyou	Siskiyou	CA	USA	0.54
178177	MVZ	Siskiyou	Siskiyou	CA	USA	0.80
ACR315	ACR	Siskiyou	Siskiyou	CA	USA	0.66
ACR316	ACR	Siskiyou	Siskiyou	CA	USA	0.64
ACR317	ACR	Siskiyou	Siskiyou	CA	USA	0.36
ACR318	ACR	Siskiyou	Siskiyou	CA	USA	0.89
ACR319	ACR	Siskiyou	Siskiyou	CA	USA	0.85
ACR320	ACR	Siskiyou	Siskiyou	CA	USA	0.72
ACR321	ACR	Siskiyou	Siskiyou	CA	USA	0.95
ACR322	ACR	Siskiyou	Siskiyou	CA	USA	0.45
ACR323	ACR	Siskiyou	Siskiyou	CA	USA	0.67
ACR325	ACR	Siskiyou	Siskiyou	CA	USA	0.64
ACR326	ACR	Siskiyou	Siskiyou	CA	USA	0.81
ACR327	ACR	Siskiyou	Siskiyou	CA	USA	0.66
ACR328	ACR	Siskiyou	Siskiyou	CA	USA	0.33
ACR296	ACR	Pocatello	Bannock, Power	ID	USA	0.18
ACR297	ACR	Pocatello	Bannock, Power	ID	USA	0.14
ACR298	ACR	Pocatello	Bannock, Power	ID	USA	0.05
ACR299	ACR	Pocatello	Bannock, Power	ID	USA	0.08
ACR300	ACR	Pocatello	Bannock, Power	ID	USA	0.42
ACR301	ACR	Pocatello	Bannock, Power	ID	USA	0.31
ACR344	ACR	Pocatello	Bannock, Power	ID	USA	0.39
ACR345	ACR	Pocatello	Bannock, Power	ID	USA	0.18
ACR346	ACR	Pocatello	Bannock, Power	ID	USA	0.20
ACR347	ACR	Pocatello	Bannock, Power	ID	USA	0.28
ACR348	ACR	Pocatello	Bannock, Power	ID	USA	0.36
ACR349	ACR	Pocatello	Bannock, Power	ID	USA	0.15
ACR146	ACR	Lk Pend Oreille	Bonner	ID	USA	0.30
ACR147	ACR	Lk Pend Oreille	Bonner	ID	USA	0.44
ACR148	ACR	Lk Pend Oreille	Bonner	ID	USA	0.72
ACR149	ACR	Lk Pend Oreille	Bonner	ID	USA	0.35
ACR150	ACR	Lk Pend Oreille	Bonner	ID	USA	0.44
ACR152	ACR	Lk Pend Oreille	Bonner	ID	USA	0.48
ACR153	ACR	Lk Pend Oreille	Bonner	ID	USA	0.43
ACR154	ACR	Lk Pend Oreille	Bonner	ID	USA	0.23
ACR155	ACR	Lk Pend Oreille	Bonner	ID	USA	0.31
ACR253	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.41
ACR254	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.19
ACR255	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.46
ACR256	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.13
ACR257	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.34
ACR258	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.32
ACR260	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.36
ACR261	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.30
ACR262	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.41
ACR268	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.27
ACR269	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.25
ACR270	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.27
ACR271	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.37
ACR272	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.31
ACR350	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.43
ACR351	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.54
ACR352	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.64
ACR353	ACR	Clearwater	Idaho, Nez Perce	ID	USA	0.38
ACR156	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.42
ACR157	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.27
ACR158	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.51
ACR159	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.57

ACR160	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.37
ACR161	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.51
ACR162	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.76
ACR163	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.36
ACR164	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.33
ACR165	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.16
ACR166	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.41
ACR167	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.66
ACR168	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.43
ACR230	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.45
ACR231	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.47
ACR232	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.72
ACR233	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.27
ACR234	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.80
ACR235	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.24
ACR236	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.52
ACR237	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.42
ACR238	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.56
ACR239	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.36
ACR240	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.60
ACR241	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.51
ACR242	ACR	Kootenai/Shoshone	Kootenai, Shoshone	ID	USA	0.42
ACR402	ACR	Pattee Crk	Lemhi	ID	USA	0.14
ACR403	ACR	Pattee Crk	Lemhi	ID	USA	0.25
ACR404	ACR	Pattee Crk	Lemhi	ID	USA	0.43
ACR405	ACR	Pattee Crk	Lemhi	ID	USA	0.21
ACR406	ACR	Pattee Crk	Lemhi	ID	USA	0.26
ACR407	ACR	Pattee Crk	Lemhi	ID	USA	0.55
ACR273	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.48
ACR274	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.19
ACR275	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.26
ACR276	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.22
ACR277	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.34
ACR278	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.35
ACR293	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.23
ACR294	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.39
ACR295	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.45
ACR379	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.25
ACR380	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.28
ACR381	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.38
ACR382	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.41
ACR383	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.19
ACR384	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.47
ACR385	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.15
ACR388	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.20
ACR389	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.15
ACR390	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.23
ACR391	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.30
ACR392	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.26
ACR393	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.43
ACR394	ACR	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA	0.11
ACR285	ACR	Pryor Mtns	Carbon	MT	USA	0.37
ACR286	ACR	Pryor Mtns	Carbon	MT	USA	0.22
ACR287	ACR	Pryor Mtns	Carbon	MT	USA	0.08
ACR288	ACR	Pryor Mtns	Carbon	MT	USA	0.36
ACR289	ACR	Pryor Mtns	Carbon	MT	USA	0.27
ACR292	ACR	Sweet Grass	Carbon	MT	USA	0.15
ACR182	ACR	Hungry Horse	Flathead	MT	USA	0.31

ACR183	ACR	Sawtooth Range	Lewis & Clark, Teton	MT	USA	0.59
ACR184	ACR	Sawtooth Range	Lewis & Clark, Teton	MT	USA	0.40
ACR185	ACR	Sawtooth Range	Lewis & Clark, Teton	MT	USA	0.40
ACR186	ACR	Sawtooth Range	Lewis & Clark, Teton	MT	USA	0.25
ACR187	ACR	Sawtooth Range	Lewis & Clark, Teton	MT	USA	0.68
ACR169	ACR	Bitterroot Mtns	Mineral	MT	USA	0.60
ACR172	ACR	Bitterroot Mtns	Mineral	MT	USA	0.19
ACR243	ACR	Bitterroot Mtns	Mineral	MT	USA	0.56
ACR173	ACR	Lolo Valley	Ravalli	MT	USA	0.60
ACR174	ACR	Lolo Valley	Ravalli	MT	USA	0.16
ACR175	ACR	Lolo Valley	Ravalli	MT	USA	0.41
ACR176	ACR	Lolo Valley	Ravalli	MT	USA	0.50
ACR177	ACR	Lolo Valley	Ravalli	MT	USA	0.44
ACR249	ACR	Lolo Valley	Ravalli	MT	USA	0.23
ACR250	ACR	Lolo Valley	Ravalli	MT	USA	0.43
ACR251	ACR	Lolo Valley	Ravalli	MT	USA	0.72
ACR252	ACR	Lolo Valley	Ravalli	MT	USA	0.35
ACR377	ACR	Lolo Valley	Ravalli	MT	USA	0.14
ACR378	ACR	Lolo Valley	Ravalli	MT	USA	0.31
ACR178	ACR	Thomposn River	Sanders	MT	USA	0.53
ACR179	ACR	Thomposn River	Sanders	MT	USA	0.56
ACR180	ACR	Thomposn River	Sanders	MT	USA	0.19
ACR181	ACR	Thomposn River	Sanders	MT	USA	0.61
ACR244	ACR	Thomposn River	Sanders	MT	USA	0.56
ACR245	ACR	Thomposn River	Sanders	MT	USA	0.58
ACR246	ACR	Thomposn River	Sanders	MT	USA	0.20
ACR247	ACR	Thomposn River	Sanders	MT	USA	0.35
ACR248	ACR	Thomposn River	Sanders	MT	USA	0.46
ACR395	ACR	Thomposn River	Sanders	MT	USA	0.57
ACR396	ACR	Thomposn River	Sanders	MT	USA	0.59
ACR398	ACR	Thomposn River	Sanders	MT	USA	0.47
ACR399	ACR	Thomposn River	Sanders	MT	USA	0.43
ACR400	ACR	Thomposn River	Sanders	MT	USA	0.39
ACR401	ACR	Thomposn River	Sanders	MT	USA	0.30
JK01286	JK	Spring Mtns	Clark	NV	USA	0.11
JK01287	JK	Spring Mtns	Clark	NV	USA	0.18
JK01408	JK	Spring Mtns	Clark	NV	USA	0.09
JK04512	JK	Spring Mtns	Clark	NV	USA	0.86
JK10032	JK	Spring Mtns	Clark	NV	USA	0.10
JK10033	JK	Spring Mtns	Clark	NV	USA	0.11
RB315	JK	Spring Mtns	Clark	NV	USA	0.49
JK05299	JK	Jarbidge Mtns	Elko	NV	USA	0.10
JK10185	JK	Jarbidge Mtns	Elko	NV	USA	0.10
JK10186	JK	Jarbidge Mtns	Elko	NV	USA	0.15
JK10187	JK	Jarbidge Mtns	Elko	NV	USA	0.37
JK10188	JK	Jarbidge Mtns	Elko	NV	USA	0.28
JK10189	JK	Jarbidge Mtns	Elko	NV	USA	0.31
JK10190	JK	Jarbidge Mtns	Elko	NV	USA	0.07
JK10191	JK	Jarbidge Mtns	Elko	NV	USA	0.47
JK10192	JK	Jarbidge Mtns	Elko	NV	USA	0.15
JK10193	JK	Jarbidge Mtns	Elko	NV	USA	0.20
ACR408	ACR	Ochoco Mtns	Crook	OR	USA	0.31
ACR409	ACR	Ochoco Mtns	Crook	OR	USA	0.36
ACR410	ACR	Ochoco Mtns	Crook	OR	USA	0.28
ACR411	ACR	Ochoco Mtns	Crook	OR	USA	0.30
ACR412	ACR	Ochoco Mtns	Crook	OR	USA	0.36
ACR413	ACR	Ochoco Mtns	Crook	OR	USA	0.64
ACR426	ACR	Ochoco Mtns	Crook	OR	USA	0.52

ACR122	ACR	Deschutes	Deschutes	OR	USA	0.40
ACR123	ACR	Deschutes	Deschutes	OR	USA	0.56
ACR418	ACR	Deschutes	Deschutes	OR	USA	0.65
ACR419	ACR	Deschutes	Deschutes	OR	USA	0.54
ACR420	ACR	Deschutes	Deschutes	OR	USA	0.35
ACR421	ACR	Deschutes	Deschutes	OR	USA	0.52
ACR422	ACR	Deschutes	Deschutes	OR	USA	0.17
ACR423	ACR	Deschutes	Deschutes	OR	USA	0.39
ACR424	ACR	Deschutes	Deschutes	OR	USA	0.70
ACR425	ACR	Deschutes	Deschutes	OR	USA	0.54
ACR414	ACR	PaulinaLk	Deschutes	OR	USA	0.19
ACR415	ACR	PaulinaLk	Deschutes	OR	USA	0.38
ACR416	ACR	PaulinaLk	Deschutes	OR	USA	0.20
ACR417	ACR	PaulinaLk	Deschutes	OR	USA	0.26
ACR204	ACR	Blue Mtns S	Grant, Umatilla, Union, Wallowa	OR	USA	0.33
ACR205	ACR	Blue Mtns S	Grant, Umatilla, Union, Wallowa	OR	USA	0.20
ACR206	ACR	Blue Mtns S	Grant, Umatilla, Union, Wallowa	OR	USA	0.38
ACR207	ACR	Blue Mtns S	Grant, Umatilla, Union, Wallowa	OR	USA	0.58
ACR208	ACR	Blue Mtns S	Grant, Umatilla, Union, Wallowa	OR	USA	0.43
ACR209	ACR	Blue Mtns S	Grant, Umatilla, Union, Wallowa	OR	USA	0.76
ACR210	ACR	Blue Mtns S	Grant, Umatilla, Union, Wallowa	OR	USA	0.46
ACR211	ACR	Blue Mtns S	Grant, Umatilla, Union, Wallowa	OR	USA	0.37
ACR212	ACR	Blue Mtns S	Grant, Umatilla, Union, Wallowa	OR	USA	0.40
ACR213	ACR	Blue Mtns S	Grant, Umatilla, Union, Wallowa	OR	USA	0.25
ACR121	ACR	Ft. Klamath	Klamath	OR	USA	0.38
ACR203	ACR	Ft. Klamath	Klamath	OR	USA	0.90
166895	MVZ	Warner Mtns N	Lake	OR	USA	0.12
166896	MVZ	Warner Mtns N	Lake	OR	USA	0.32
166897	MVZ	Warner Mtns N	Lake	OR	USA	0.25
166898	MVZ	Warner Mtns N	Lake	OR	USA	0.42
166899	MVZ	Warner Mtns N	Lake	OR	USA	0.12
166900	MVZ	Warner Mtns N	Lake	OR	USA	0.47
166901	MVZ	Warner Mtns N	Lake	OR	USA	0.53
166902	MVZ	Warner Mtns N	Lake	OR	USA	0.19
166903	MVZ	Warner Mtns N	Lake	OR	USA	0.23
166904	MVZ	Warner Mtns N	Lake	OR	USA	0.44
166905	MVZ	Warner Mtns N	Lake	OR	USA	0.61
166906	MVZ	Warner Mtns N	Lake	OR	USA	0.22
166907	MVZ	Warner Mtns N	Lake	OR	USA	0.62
169288	MVZ	Warner Mtns N	Lake	OR	USA	0.43
169289	MVZ	Warner Mtns N	Lake	OR	USA	0.51
169290	MVZ	Warner Mtns N	Lake	OR	USA	0.32
169291	MVZ	Warner Mtns N	Lake	OR	USA	0.25
169292	MVZ	Warner Mtns N	Lake	OR	USA	0.69
169293	MVZ	Warner Mtns N	Lake	OR	USA	0.53
169294	MVZ	Warner Mtns N	Lake	OR	USA	0.22
178151	MVZ	Warner Mtns N	Lake	OR	USA	0.56
178152	MVZ	Warner Mtns N	Lake	OR	USA	0.36

178153U	MVZ	Warner Mtns N	Lake	OR	USA	0.32
ACR112	ACR	Warner Mtns N	Lake	OR	USA	0.32
ACR113	ACR	Warner Mtns N	Lake	OR	USA	0.36
ACR114	ACR	Warner Mtns N	Lake	OR	USA	0.35
ACR115	ACR	Warner Mtns N	Lake	OR	USA	0.28
ACR192	ACR	Warner Mtns N	Lake	OR	USA	0.38
ACR193	ACR	Warner Mtns N	Lake	OR	USA	0.42
ACR194	ACR	Warner Mtns N	Lake	OR	USA	0.45
ACR195	ACR	Warner Mtns N	Lake	OR	USA	0.18
ACR196	ACR	Warner Mtns N	Lake	OR	USA	0.20
ACR197	ACR	Warner Mtns N	Lake	OR	USA	0.33
ACR198	ACR	Warner Mtns N	Lake	OR	USA	0.24
ACR199	ACR	Warner Mtns N	Lake	OR	USA	0.21
ACR200	ACR	Warner Mtns N	Lake	OR	USA	0.47
ACR201	ACR	Warner Mtns N	Lake	OR	USA	0.41
168602	MVZ	Black Hills	Lawrence	SD	USA	0.14
168603	MVZ	Black Hills	Lawrence	SD	USA	0.70
168604	MVZ	Black Hills	Lawrence	SD	USA	0.13
168605	MVZ	Black Hills	Lawrence	SD	USA	0.08
168606	MVZ	Black Hills	Lawrence	SD	USA	0.05
168607	MVZ	Black Hills	Lawrence	SD	USA	0.49
168608	MVZ	Black Hills	Lawrence	SD	USA	0.26
168610	MVZ	Black Hills	Lawrence	SD	USA	0.13
168612	MVZ	Black Hills	Lawrence	SD	USA	0.11
168614	MVZ	Black Hills	Lawrence	SD	USA	0.25
168615	MVZ	Black Hills	Lawrence	SD	USA	0.09
168620	MVZ	Black Hills	Lawrence	SD	USA	0.12
168621	MVZ	Black Hills	Lawrence	SD	USA	0.19
178162	MVZ	Black Hills	Lawrence	SD	USA	0.34
178172	MVZ	Black Hills	Lawrence	SD	USA	0.26
178173	MVZ	Black Hills	Lawrence	SD	USA	0.25
JK10218	JK	Black Hills	Lawrence	SD	USA	0.45
JK10220	JK	Black Hills	Lawrence	SD	USA	0.22
JK10221	JK	Black Hills	Lawrence	SD	USA	0.22
JK10222	JK	Black Hills	Lawrence	SD	USA	0.09
ACR191	ACR	Blue Mtns N	Asotin, Columbia, Walla Walla	WA	USA	0.29
ACR263	ACR	N Blue Mtns	Asotin, Columbia, Walla Walla	WA	USA	0.24
ACR264	ACR	N Blue Mtns	Asotin, Columbia, Walla Walla	WA	USA	0.49
ACR124	ACR	Kittitas	Kittitas	WA	USA	0.86
ACR125	ACR	Kittitas	Kittitas	WA	USA	0.50
ACR126	ACR	Kittitas	Kittitas	WA	USA	0.77
ACR127	ACR	Kittitas	Kittitas	WA	USA	0.79
ACR128	ACR	Kittitas	Kittitas	WA	USA	0.66
ACR214	ACR	Kittitas	Kittitas	WA	USA	0.72
ACR215	ACR	Kittitas	Kittitas	WA	USA	0.80
ACR216	ACR	Kittitas	Kittitas	WA	USA	0.76
ACR217	ACR	Kittitas	Kittitas	WA	USA	0.47
ACR218	ACR	Kittitas	Kittitas	WA	USA	0.62
ACR136	ACR	Okanogan E	Okanogan	WA	USA	0.61
ACR137	ACR	Okanogan E	Okanogan	WA	USA	0.65
ACR138	ACR	Okanogan E	Okanogan	WA	USA	0.65
ACR139	ACR	Okanogan E	Okanogan	WA	USA	0.58
ACR219	ACR	Okanogan E	Okanogan	WA	USA	0.36
ACR220	ACR	Okanogan E	Okanogan	WA	USA	0.56
ACR221	ACR	Okanogan E	Okanogan	WA	USA	0.59

ACR222	ACR	Okanogan E	Okanogan	WA	USA	0.72
ACR223	ACR	Okanogan E	Okanogan	WA	USA	0.53
ACR224	ACR	Okanogan E	Okanogan	WA	USA	0.42
ACR354	ACR	Okanogan E	Okanogan	WA	USA	0.44
ACR355	ACR	Okanogan E	Okanogan	WA	USA	0.69
ACR356	ACR	Okanogan E	Okanogan	WA	USA	0.40
ACR357	ACR	Okanogan E	Okanogan	WA	USA	0.81
ACR358	ACR	Okanogan E	Okanogan	WA	USA	0.62
ACR359	ACR	Okanogan E	Okanogan	WA	USA	0.70
ACR364	ACR	Okanogan E	Okanogan	WA	USA	0.73
ACR365	ACR	Okanogan E	Okanogan	WA	USA	0.61
ACR366	ACR	Okanogan E	Okanogan	WA	USA	0.47
ACR129	ACR	Okanogan W	Okanogan	WA	USA	0.53
ACR130	ACR	Okanogan W	Okanogan	WA	USA	0.62
ACR131	ACR	Okanogan W	Okanogan	WA	USA	0.70
ACR132	ACR	Okanogan W	Okanogan	WA	USA	0.57
ACR133	ACR	Okanogan W	Okanogan	WA	USA	0.67
ACR134	ACR	Okanogan W	Okanogan	WA	USA	0.33
ACR135	ACR	Okanogan W	Okanogan	WA	USA	0.56
ACR225	ACR	Okanogan W	Okanogan	WA	USA	0.69
ACR226	ACR	Okanogan W	Okanogan	WA	USA	0.44
ACR227	ACR	Okanogan W	Okanogan	WA	USA	0.44
ACR228	ACR	Okanogan W	Okanogan	WA	USA	0.46
ACR229	ACR	Okanogan W	Okanogan	WA	USA	0.46
ACR360	ACR	Okanogan W	Okanogan	WA	USA	0.61
ACR361	ACR	Okanogan W	Okanogan	WA	USA	0.55
ACR362	ACR	Okanogan W	Okanogan	WA	USA	0.50
ACR363	ACR	Okanogan W	Okanogan	WA	USA	0.62
ACR140	ACR	SullivanLk	Pend Oreille	WA	USA	0.59
ACR141	ACR	SullivanLk	Pend Oreille	WA	USA	0.78
ACR142	ACR	SullivanLk	Pend Oreille	WA	USA	0.27
ACR143	ACR	SullivanLk	Pend Oreille	WA	USA	0.53
ACR144	ACR	SullivanLk	Pend Oreille	WA	USA	0.28
ACR145	ACR	SullivanLk	Pend Oreille	WA	USA	0.35

Table A4. List of sound recordings used in Chapter 2. Source abbreviations: ACR = Andrew C. Rush, BL = Ohio State University Borror Laboratory, DAM = D. Archibald McCallum personal collection, DEI = Darren E. Irwin personal collection, KBB = Kelly B. Bryan personal collection, ML = Cornell Laboratory of Ornithology Macaulay Library, MVZ = University of California Museum of Vertebrate Zoology, NDP = Nathan D. Pieplow personal collection. Taxon abbreviations: PS = Pacific-slope Flycatcher, CO = Cordilleran Flycatcher, AD = “admixed”; a contact zone site of uncertain taxonomic affinity.

Catalogue	Source	Taxon	Location	County	State/Prov	Country
BL18572	BL	PS	Haida Gwaii	-	BC	CA
BL18573	BL	PS	Haida Gwaii	-	BC	CA
BL18574	BL	PS	Haida Gwaii	-	BC	CA
BL18575	BL	PS	Haida Gwaii	-	BC	CA
BL18577	BL	PS	Haida Gwaii	-	BC	CA
2006RUSH016	ACR	PS	Hope	-	BC	CA
2006RUSH017	ACR	PS	Hope	-	BC	CA
2006RUSH018	ACR	PS	Hope	-	BC	CA
2007RUSH010	ACR	PS	Hope	-	BC	CA
2007RUSH011	ACR	PS	Hope	-	BC	CA
2006RUSH003	ACR	PS	Vancouver	-	BC	CA
2006RUSH004	ACR	PS	Vancouver	-	BC	CA
DEI2006_001	DEI	PS	Vancouver	-	BC	CA
DEI2006_002	DEI	PS	Vancouver	-	BC	CA
DEI2006_003	DEI	PS	Vancouver	-	BC	CA
DEI2006_004	DEI	PS	Vancouver	-	BC	CA
DEI2006_005	DEI	PS	Vancouver	-	BC	CA
MVZ1446	MVZ	PS	Vancouver	-	BC	CA
BL19950427	BL	PS	Elk Cr	Glenn	CA	USA
2012RUSH779	ACR	PS	N California Cst	Humboldt, Del Norte	CA	USA
2012RUSH780	ACR	PS	N California Cst	Humboldt, Del Norte	CA	USA
2012RUSH781	ACR	PS	N California Cst	Humboldt, Del Norte	CA	USA
2012RUSH782	ACR	PS	N California Cst	Humboldt, Del Norte	CA	USA
2012RUSH783	ACR	PS	N California Cst	Humboldt, Del Norte	CA	USA
DAM2005061138	DAM	PS	N California Cst	Humboldt, Del Norte	CA	USA
DAM20050612	DAM	PS	N California Cst	Humboldt, Del Norte	CA	USA
DAM20050612	DAM	PS	N California Cst	Humboldt, Del Norte	CA	USA
DAM20050612	DAM	PS	N California Cst	Humboldt, Del Norte	CA	USA
2010RUSH001	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2010RUSH003	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2010RUSH005	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2010RUSH007	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2010RUSH008	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2010RUSH010	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2010RUSH011	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2010RUSH013	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2010RUSH015	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2010RUSH017	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2011RUSH131	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2011RUSH133	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2011RUSH135	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2011RUSH138	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2011RUSH139	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2011RUSH143	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2011RUSH146	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2011RUSH170	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA

2011RUSH171	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2011RUSH172	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2011RUSH173	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2011RUSH174	ACR	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
MAC111041	ML	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
MAC118835	ML	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
MAC126454	ML	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
MAC126470	ML	PS	San Francisco Bay N	Marin, Alameda, Sonoma	CA	USA
2012RUSH512	ACR	PS	Yosemite	Mariposa	CA	USA
2012RUSH513	ACR	PS	Yosemite	Mariposa	CA	USA
2012RUSH514	ACR	PS	Yosemite	Mariposa	CA	USA
2012RUSH515	ACR	PS	Yosemite	Mariposa	CA	USA
2012RUSH519	ACR	PS	Yosemite	Mariposa	CA	USA
2012RUSH522	ACR	PS	Yosemite	Mariposa	CA	USA
BL19950612	BL	PS	Yosemite	Mariposa	CA	USA
2010RUSH022	ACR	PS	Monterey	Monterey	CA	USA
2010RUSH022B	ACR	PS	Monterey	Monterey	CA	USA
2010RUSH024	ACR	PS	Monterey	Monterey	CA	USA
2010RUSH027	ACR	PS	Monterey	Monterey	CA	USA
DAM19930418	DAM	PS	Monterey	Monterey	CA	USA
MAC110916	ML	PS	Monterey	Monterey	CA	USA
MAC126424	ML	PS	Monterey	Monterey	CA	USA
MAC22985	ML	PS	Monterey	Monterey	CA	USA
MAC22986	ML	PS	Monterey	Monterey	CA	USA
MAC7600	ML	PS	Monterey	Monterey	CA	USA
DAM200704281	DAM	PS	San Diego	San Diego	CA	USA
DAM200704282b	DAM	PS	San Diego	San Diego	CA	USA
NDP20090321	NDP	PS	San Diego	San Diego	CA	USA
2011RUSH150	ACR	PS	San Francisco S	San Mateo	CA	USA
2011RUSH152	ACR	PS	San Francisco S	San Mateo	CA	USA
2011RUSH152B	ACR	PS	San Francisco S	San Mateo	CA	USA
2011RUSH155	ACR	PS	San Francisco S	San Mateo	CA	USA
2011RUSH156	ACR	PS	San Francisco S	San Mateo	CA	USA
2011RUSH158	ACR	PS	San Francisco S	San Mateo	CA	USA
2011RUSH159	ACR	PS	San Francisco S	San Mateo	CA	USA
2011RUSH160	ACR	PS	San Francisco S	San Mateo	CA	USA
2011RUSH161	ACR	PS	San Francisco S	San Mateo	CA	USA
2011RUSH162	ACR	PS	San Francisco S	San Mateo	CA	USA
2011RUSH163	ACR	PS	San Francisco S	San Mateo	CA	USA
2011RUSH164	ACR	PS	San Francisco S	San Mateo	CA	USA
2011RUSH165	ACR	PS	San Francisco S	San Mateo	CA	USA
2011RUSH167	ACR	PS	San Francisco S	San Mateo	CA	USA
2011RUSH168	ACR	PS	San Francisco S	San Mateo	CA	USA
2011RUSH169	ACR	PS	San Francisco S	San Mateo	CA	USA
2012RUSH151	ACR	PS	San Francisco S	San Mateo	CA	USA
2012RUSH154	ACR	PS	San Francisco S	San Mateo	CA	USA
2012RUSH156	ACR	PS	San Francisco S	San Mateo	CA	USA
2012RUSH158	ACR	PS	San Francisco S	San Mateo	CA	USA
2012RUSH166	ACR	PS	San Francisco S	San Mateo	CA	USA
2012RUSH167	ACR	PS	San Francisco S	San Mateo	CA	USA
2012RUSH168	ACR	PS	San Francisco S	San Mateo	CA	USA
2012RUSH169	ACR	PS	San Francisco S	San Mateo	CA	USA
2012RUSH170	ACR	PS	San Francisco S	San Mateo	CA	USA
2012RUSH171	ACR	PS	San Francisco S	San Mateo	CA	USA
2012RUSH180	ACR	PS	San Francisco S	San Mateo	CA	USA
2012RUSH181	ACR	PS	San Francisco S	San Mateo	CA	USA
2012RUSH182	ACR	PS	San Francisco S	San Mateo	CA	USA
MVZ1439	MVZ	PS	San Francisco N	Alameda	CA	USA

DAM200406041	DAM	PS	Central Oregon Cst	Coos, Lane, Lincoln	OR	USA
DAM200406043	DAM	PS	Central Oregon Cst	Coos, Lane, Lincoln	OR	USA
DAM200406044	DAM	PS	Central Oregon Cst	Coos, Lane, Lincoln	OR	USA
DAM200406046	DAM	PS	Central Oregon Cst	Coos, Lane, Lincoln	OR	USA
DAM20020607106072	DAM	PS	Central Oregon Cst	Coos, Lane, Lincoln	OR	USA
MAC40669	ML	PS	Central Oregon Cst	Coos, Lane, Lincoln	OR	USA
MAC44956	ML	PS	Central Oregon Cst	Coos, Lane, Lincoln	OR	USA
MAC44957	ML	PS	Central Oregon Cst	Coos, Lane, Lincoln	OR	USA
MAC50130	ML	PS	Central Oregon Cst	Coos, Lane, Lincoln	OR	USA
MAC50339	ML	PS	Central Oregon Cst	Coos, Lane, Lincoln	OR	USA
DAM200605291	DAM	PS	Willamette Vly	Lane, Douglas	OR	USA
DAM200605292	DAM	PS	Willamette Vly	Lane, Douglas	OR	USA
DAM20070613	DAM	PS	Willamette Vly	Lane, Douglas	OR	USA
DAM2003061510615200	DAM	PS	Willamette Vly	Lane, Douglas	OR	USA
DAM2005051910519200	DAM	PS	Willamette Vly	Lane, Douglas	OR	USA
DAM20070530	DAM	PS	Willamette Vly	Lane, Douglas	OR	USA
MAC106661	ML	PS	Willamette Vly	Lane, Douglas	OR	USA
MAC57623	ML	PS	Willamette Vly	Lane, Douglas	OR	USA
MAC19950529	ML	PS	Willamette Vly	Lane, Douglas	OR	USA
BL19960620	BL	PS	Olympic Pen	Clallum	WA	USA
BL19960619	BL	PS	Olympic Pen	Clallum	WA	USA
MAC59788	ML	PS	Olympic Pen	Clallum	WA	USA
2010RUSH156	ACR	PS	Skagit	Skagit, Whatcom	WA	USA
2010RUSH159	ACR	PS	Skagit	Skagit, Whatcom	WA	USA
2010RUSH160	ACR	PS	Skagit	Skagit, Whatcom	WA	USA
2010RUSH161	ACR	PS	Skagit	Skagit, Whatcom	WA	USA
2010RUSH162	ACR	PS	Skagit	Skagit, Whatcom	WA	USA
2010RUSH164	ACR	PS	Skagit	Skagit, Whatcom	WA	USA
2010RUSH166	ACR	PS	Skagit	Skagit, Whatcom	WA	USA
2010RUSH171	ACR	PS	Skagit	Skagit, Whatcom	WA	USA
2010RUSH172	ACR	PS	Skagit	Skagit, Whatcom	WA	USA
2010RUSH174	ACR	PS	Skagit	Skagit, Whatcom	WA	USA
DAM20080629	DAM	PS	Skagit	Skagit, Whatcom	WA	USA
MAC45279	ML	PS	Skagit	Skagit, Whatcom	WA	USA
MAC7601	ML	PS	Skagit	Skagit, Whatcom	WA	USA
MAC7602	ML	PS	Skagit	Skagit, Whatcom	WA	USA
MAC7611	ML	PS	Skagit	Skagit, Whatcom	WA	USA
2012RUSH578	ACR	CO	White Mtns	Apache	AZ	USA
2012RUSH579	ACR	CO	White Mtns	Apache	AZ	USA
2012RUSH590	ACR	CO	White Mtns	Apache	AZ	USA
2012RUSH591	ACR	CO	White Mtns	Apache	AZ	USA
2012RUSH592	ACR	CO	White Mtns	Apache	AZ	USA
2012RUSH593	ACR	CO	White Mtns	Apache	AZ	USA
2012RUSH577	ACR	CO	Chiricahua Mtns	Cochise	AZ	USA
2012RUSH646	ACR	CO	Chiricahua Mtns	Cochise	AZ	USA
2012RUSH647	ACR	CO	Chiricahua Mtns	Cochise	AZ	USA
2012RUSH648	ACR	CO	Chiricahua Mtns	Cochise	AZ	USA
2012RUSH661	ACR	CO	Chiricahua Mtns	Cochise	AZ	USA
2012RUSH663	ACR	CO	Chiricahua Mtns	Cochise	AZ	USA
2012RUSH664	ACR	CO	Chiricahua Mtns	Cochise	AZ	USA
DAM199506191	DAM	CO	Chiricahua Mtns	Cochise	AZ	USA
MAC21436	ML	CO	Chiricahua Mtns	Cochise	AZ	USA
MVZ1555	MVZ	CO	Chiricahua Mtns	Cochise	AZ	USA
MVZ1556	MVZ	CO	Chiricahua Mtns	Cochise	AZ	USA
2012RUSH549	ACR	CO	San Francisco Peaks	Coconino	AZ	USA
2012RUSH550	ACR	CO	San Francisco Peaks	Coconino	AZ	USA
2012RUSH551	ACR	CO	San Francisco Peaks	Coconino	AZ	USA
2012RUSH553	ACR	CO	San Francisco Peaks	Coconino	AZ	USA

2012RUSH567	ACR	CO	San Francisco Peaks	Coconino	AZ	USA
2012RUSH568	ACR	CO	San Francisco Peaks	Coconino	AZ	USA
2012RUSH569	ACR	CO	San Francisco Peaks	Coconino	AZ	USA
2012RUSH570	ACR	CO	San Francisco Peaks	Coconino	AZ	USA
2012RUSH604	ACR	CO	Pinaleno Mtns	Graham	AZ	USA
2012RUSH619	ACR	CO	Pinaleno Mtns	Graham	AZ	USA
2012RUSH620	ACR	CO	Pinaleno Mtns	Graham	AZ	USA
2012RUSH621	ACR	CO	Pinaleno Mtns	Graham	AZ	USA
2012RUSH622	ACR	CO	Pinaleno Mtns	Graham	AZ	USA
2012RUSH631	ACR	CO	Pinaleno Mtns	Graham	AZ	USA
2012RUSH632	ACR	CO	Pinaleno Mtns	Graham	AZ	USA
2012RUSH633	ACR	CO	Pinaleno Mtns	Graham	AZ	USA
2012RUSH634	ACR	CO	Pinaleno Mtns	Graham	AZ	USA
2012RUSH640	ACR	CO	Pinaleno Mtns	Graham	AZ	USA
BL10769	BL	CO	Rocky Mtn NP	Boulder	CO	USA
MAC105295	ML	CO	Rocky Mtn NP	Boulder	CO	USA
MAC105315	ML	CO	Rocky Mtn NP	Boulder	CO	USA
NDP20070601	NDP	CO	Rocky Mtn NP	Boulder	CO	USA
NDP20080525	NDP	CO	Rocky Mtn NP	Boulder	CO	USA
NDP20080618	NDP	CO	Rocky Mtn NP	Boulder	CO	USA
NDP20080708	NDP	CO	Rocky Mtn NP	Boulder	CO	USA
2011RUSH417	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH417	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH424	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH426	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH427	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH432	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH434	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH436	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH441	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH444	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH445	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH447	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH456	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH457	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH458	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH459	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH460	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH461	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH462	ACR	CO	Wet Mtns	Custer	CO	USA
2011RUSH474	ACR	CO	Zuni Mtns	Cibola, McKinley	NM	USA
DAMNMcibMfTaylor	DAM	CO	Zuni Mtns	Cibola, McKinley	NM	USA
DAM200607102	DAM	CO	Zuni Mtns	Cibola, McKinley	NM	USA
DAM200607103	DAM	CO	Zuni Mtns	Cibola, McKinley	NM	USA
DAM20060710710-1	DAM	CO	Zuni Mtns	Cibola, McKinley	NM	USA
DAM20060710710-3	DAM	CO	Zuni Mtns	Cibola, McKinley	NM	USA
DAM20060710710-4	DAM	CO	Zuni Mtns	Cibola, McKinley	NM	USA
DAM20060710710-5	DAM	CO	Zuni Mtns	Cibola, McKinley	NM	USA
DAM20060630M	DAM	CO	Zuni Mtns	Cibola, McKinley	NM	USA
DAM198106211	DAM	CO	Zuni Mtns	Cibola, McKinley	NM	USA
DAM198106212	DAM	CO	Zuni Mtns	Cibola, McKinley	NM	USA
DAM198106161	DAM	CO	Zuni Mtns	Cibola, McKinley	NM	USA
DAM200607081	DAM	CO	Zuni Mtns	Cibola, McKinley	NM	USA
DAM200607082	DAM	CO	Zuni Mtns	Cibola, McKinley	NM	USA
2011RUSH508	ACR	CO	Black Range	Grant	NM	USA
2011RUSH509	ACR	CO	Black Range	Grant	NM	USA
2011RUSH516	ACR	CO	Black Range	Grant	NM	USA
2011RUSH517	ACR	CO	Black Range	Grant	NM	USA

2011RUSH518	ACR	CO	Black Range	Grant		NM	USA
2011RUSH519	ACR	CO	Black Range	Grant		NM	USA
2011RUSH520	ACR	CO	Black Range	Grant		NM	USA
2011RUSH521	ACR	CO	Black Range	Grant		NM	USA
2011RUSH527	ACR	CO	Black Range	Grant		NM	USA
2011RUSH528	ACR	CO	Black Range	Grant		NM	USA
2011RUSH529	ACR	CO	Black Range	Grant		NM	USA
2011RUSH530	ACR	CO	Black Range	Grant		NM	USA
2011RUSH532	ACR	CO	Black Range	Grant		NM	USA
2011RUSH534B	ACR	CO	Black Range	Grant		NM	USA
2011RUSH535B	ACR	CO	Black Range	Grant		NM	USA
2011RUSH536	ACR	CO	Black Range	Grant		NM	USA
2012RUSH672	ACR	CO	Black Range	Grant		NM	USA
2012RUSH686	ACR	CO	Black Range	Grant		NM	USA
2012RUSH699	ACR	CO	Black Range	Grant		NM	USA
2012RUSH700	ACR	CO	Black Range	Grant		NM	USA
2012RUSH701	ACR	CO	Black Range	Grant		NM	USA
2011RUSH481	ACR	CO	Sacramento Mtns	Otero		NM	USA
2011RUSH491	ACR	CO	Sacramento Mtns	Otero		NM	USA
2011RUSH492	ACR	CO	Sacramento Mtns	Otero		NM	USA
2011RUSH493	ACR	CO	Sacramento Mtns	Otero		NM	USA
2011RUSH494	ACR	CO	Sacramento Mtns	Otero		NM	USA
2011RUSH495	ACR	CO	Sacramento Mtns	Otero		NM	USA
2011RUSH496	ACR	CO	Sacramento Mtns	Otero		NM	USA
2011RUSH497	ACR	CO	Sacramento Mtns	Otero		NM	USA
2011RUSH498	ACR	CO	Sacramento Mtns	Otero		NM	USA
2011RUSH500	ACR	CO	Sacramento Mtns	Otero		NM	USA
2011RUSH501	ACR	CO	Sacramento Mtns	Otero		NM	USA
2011RUSH502	ACR	CO	Sacramento Mtns	Otero		NM	USA
2012RUSH711	ACR	CO	Sacramento Mtns	Otero		NM	USA
2012RUSH712	ACR	CO	Sacramento Mtns	Otero		NM	USA
2012RUSH714	ACR	CO	Sacramento Mtns	Otero		NM	USA
MVZ1599	MVZ	CO	Sacramento Mtns	Otero		NM	USA
MVZ1603	MVZ	CO	Sacramento Mtns	Otero		NM	USA
MVZ1604	MVZ	CO	Sacramento Mtns	Otero		NM	USA
DAM20100601^1m	DAM	CO	Chisos, Davis, & Guadalupe Mtns	Brewster, Culbertson, Jeff Davis		TX	USA
DAM20100601^2m	DAM	CO	Chisos, Davis, & Guadalupe Mtns	Brewster, Culbertson, Jeff Davis		TX	USA
DAM20100601^4U	DAM	CO	Chisos, Davis, & Guadalupe Mtns	Brewster, Culbertson, Jeff Davis		TX	USA
DAM20100531^1mca	DAM	CO	Chisos, Davis, & Guadalupe Mtns	Brewster, Culbertson, Jeff Davis		TX	USA
DAM20100531^m2Li	DAM	CO	Chisos, Davis, & Guadalupe Mtns	Brewster, Culbertson, Jeff Davis		TX	USA
DAM20100531^m3As	DAM	CO	Chisos, Davis, & Guadalupe Mtns	Brewster, Culbertson, Jeff Davis		TX	USA
KBB20030611_U	KBB	CO	Chisos, Davis, & Guadalupe Mtns	Brewster, Culbertson, Jeff Davis		TX	USA
KBB20030712_U	KBB	CO	Chisos, Davis, & Guadalupe Mtns	Brewster, Culbertson, Jeff Davis		TX	USA
KBB20010519_U	KBB	CO	Chisos, Davis, & Guadalupe Mtns	Brewster, Culbertson, Jeff Davis		TX	USA
2006RUSH032B	ACR	AD	Kananaskis	-		AB	CA
2006RUSH035	ACR	AD	Kananaskis	-		AB	CA
2006RUSH038	ACR	AD	Kananaskis	-		AB	CA
2007RUSH099	ACR	AD	Kananaskis	-		AB	CA
2007RUSH100	ACR	AD	Kananaskis	-		AB	CA

2007RUSH102	ACR	AD	Kananaskis	-	AB	CA
2007RUSH105	ACR	AD	Kananaskis	-	AB	CA
2007RUSH106	ACR	AD	Kananaskis	-	AB	CA
2007RUSH107	ACR	AD	Kananaskis	-	AB	CA
2010RUSH132B	ACR	AD	Kananaskis	-	AB	CA
2010RUSH133	ACR	AD	Kananaskis	-	AB	CA
2010RUSH134B	ACR	AD	Kananaskis	-	AB	CA
2010RUSH136B	ACR	AD	Kananaskis	-	AB	CA
2012RUSH528	ACR	AD	Hualapai Mtns	Mohave	AZ	CA
2007RUSH049	ACR	AD	Christina Lk	-	BC	CA
2007RUSH051	ACR	AD	Christina Lk	-	BC	CA
2007RUSH052	ACR	AD	Christina Lk	-	BC	CA
2007RUSH053	ACR	AD	Christina Lk	-	BC	CA
2007RUSH060	ACR	AD	Christina Lk	-	BC	CA
2007RUSH061	ACR	AD	Christina Lk	-	BC	CA
2007RUSH062B	ACR	AD	Christina Lk	-	BC	CA
2007RUSH064B	ACR	AD	Christina Lk	-	BC	CA
2007RUSH065	ACR	AD	Christina Lk	-	BC	CA
2007RUSH066	ACR	AD	Christina Lk	-	BC	CA
2007RUSH067	ACR	AD	Christina Lk	-	BC	CA
2007RUSH069	ACR	AD	Christina Lk	-	BC	CA
2007RUSH070	ACR	AD	Christina Lk	-	BC	CA
DEI2006_007	DEI	AD	Christina Lk	-	BC	CA
DEI2006_008	DEI	AD	Christina Lk	-	BC	CA
DEI2006_009	DEI	AD	Christina Lk	-	BC	CA
2006RUSH044	ACR	AD	Kootenay Lk	-	BC	CA
2006RUSH042	ACR	AD	Kootneay Lk	-	BC	CA
2006RUSH048	ACR	AD	Kootneay Lk	-	BC	CA
2007RUSH109	ACR	AD	Kootneay Lk	-	BC	CA
2007RUSH084	ACR	AD	Peace Rv	-	BC	CA
2007RUSH086	ACR	AD	Peace Rv	-	BC	CA
2007RUSH087	ACR	AD	Peace Rv	-	BC	CA
2007RUSH093	ACR	AD	Peace Rv	-	BC	CA
2007RUSH095C	ACR	AD	Peace Rv	-	BC	CA
2006RUSH006	ACR	AD	Penticton	-	BC	CA
2006RUSH012	ACR	AD	Penticton	-	BC	CA
2006RUSH013	ACR	AD	Penticton	-	BC	CA
2007RUSH032	ACR	AD	Penticton	-	BC	CA
2007RUSH034	ACR	AD	Penticton	-	BC	CA
2007RUSH035	ACR	AD	Penticton	-	BC	CA
2007RUSH036	ACR	AD	Penticton	-	BC	CA
2007RUSH037	ACR	AD	Penticton	-	BC	CA
2007RUSH041	ACR	AD	Penticton	-	BC	CA
2007RUSH043	ACR	AD	Penticton	-	BC	CA
2006RUSH019	ACR	AD	Princeton	-	BC	CA
2006RUSH020	ACR	AD	Princeton	-	BC	CA
2006RUSH021	ACR	AD	Princeton	-	BC	CA
2006RUSH023	ACR	AD	Princeton	-	BC	CA
2007RUSH020	ACR	AD	Princeton	-	BC	CA
2007RUSH021	ACR	AD	Princeton	-	BC	CA
2007RUSH025	ACR	AD	Princeton	-	BC	CA
2007RUSH026B	ACR	AD	Princeton	-	BC	CA
2007RUSH029	ACR	AD	Princeton	-	BC	CA
2007RUSH078	ACR	AD	Williams Lk	-	BC	CA
2007RUSH079	ACR	AD	Williams Lk	-	BC	CA
2007RUSH080	ACR	AD	Williams Lk	-	BC	CA
2007RUSH081	ACR	AD	Williams Lk	-	BC	CA
2007RUSH083	ACR	AD	Williams Lk	-	BC	CA

2010RUSH080	ACR	AD	Modoc	Modoc	CA	USA
2010RUSH081	ACR	AD	Modoc	Modoc	CA	USA
2010RUSH089	ACR	AD	Modoc	Modoc	CA	USA
2010RUSH089B	ACR	AD	Modoc	Modoc	CA	USA
2010RUSH093B	ACR	AD	Modoc	Modoc	CA	USA
2010RUSH098	ACR	AD	Modoc	Modoc	CA	USA
2010RUSH098B	ACR	AD	Modoc	Modoc	CA	USA
2010RUSH100	ACR	AD	Modoc	Modoc	CA	USA
2011RUSH263	ACR	AD	Modoc	Modoc	CA	USA
2011RUSH264	ACR	AD	Modoc	Modoc	CA	USA
2011RUSH265	ACR	AD	Modoc	Modoc	CA	USA
2011RUSH268	ACR	AD	Modoc	Modoc	CA	USA
2011RUSH269	ACR	AD	Modoc	Modoc	CA	USA
2011RUSH279	ACR	AD	Modoc	Modoc	CA	USA
DAM200806151	DAM	AD	Mono	Mono	CA	USA
DAM200806152	DAM	AD	Mono	Mono	CA	USA
DAM200806153	DAM	AD	Mono	Mono	CA	USA
DAM200806155	DAM	AD	Mono	Mono	CA	USA
DAM200806161	DAM	AD	Mono	Mono	CA	USA
DAM200806163	DAM	AD	Mono	Mono	CA	USA
DAM200806164	DAM	AD	Mono	Mono	CA	USA
2010RUSH032B	ACR	AD	Shasta	Shasta	CA	USA
2010RUSH033	ACR	AD	Shasta	Shasta	CA	USA
2010RUSH035B	ACR	AD	Shasta	Shasta	CA	USA
2010RUSH039B	ACR	AD	Shasta	Shasta	CA	USA
2010RUSH043	ACR	AD	Shasta	Shasta	CA	USA
2010RUSH044B	ACR	AD	Shasta	Shasta	CA	USA
2010RUSH045	ACR	AD	Shasta	Shasta	CA	USA
2011RUSH191	ACR	AD	Shasta	Shasta	CA	USA
2011RUSH192	ACR	AD	Shasta	Shasta	CA	USA
2011RUSH196	ACR	AD	Shasta	Shasta	CA	USA
2011RUSH205	ACR	AD	Shasta	Shasta	CA	USA
2011RUSH213	ACR	AD	Shasta	Shasta	CA	USA
2011RUSH214	ACR	AD	Shasta	Shasta	CA	USA
2011RUSH215	ACR	AD	Shasta	Shasta	CA	USA
2011RUSH216	ACR	AD	Shasta	Shasta	CA	USA
2011RUSH221	ACR	AD	Shasta	Shasta	CA	USA
2011RUSH223	ACR	AD	Shasta	Shasta	CA	USA
DAM200806141	DAM	AD	Shasta	Shasta	CA	USA
2010RUSH047B	ACR	AD	Siskiyou	Siskiyou	CA	USA
2010RUSH058	ACR	AD	Siskiyou	Siskiyou	CA	USA
2010RUSH059	ACR	AD	Siskiyou	Siskiyou	CA	USA
2010RUSH064B	ACR	AD	Siskiyou	Siskiyou	CA	USA
2010RUSH064C	ACR	AD	Siskiyou	Siskiyou	CA	USA
2010RUSH065	ACR	AD	Siskiyou	Siskiyou	CA	USA
2010RUSH068	ACR	AD	Siskiyou	Siskiyou	CA	USA
2010RUSH074	ACR	AD	Siskiyou	Siskiyou	CA	USA
2010RUSH075	ACR	AD	Siskiyou	Siskiyou	CA	USA
2011RUSH224	ACR	AD	Siskiyou	Siskiyou	CA	USA
2011RUSH229	ACR	AD	Siskiyou	Siskiyou	CA	USA
2011RUSH2312	ACR	AD	Siskiyou	Siskiyou	CA	USA
2011RUSH233	ACR	AD	Siskiyou	Siskiyou	CA	USA
2011RUSH235	ACR	AD	Siskiyou	Siskiyou	CA	USA
2011RUSH250	ACR	AD	Siskiyou	Siskiyou	CA	USA
2011RUSH251	ACR	AD	Siskiyou	Siskiyou	CA	USA
2011RUSH253	ACR	AD	Siskiyou	Siskiyou	CA	USA
DAM200806141	DAM	AD	Siskiyou	Siskiyou	CA	USA
DAM200806142	DAM	AD	Siskiyou	Siskiyou	CA	USA

DAM200806145	DAM	AD	Siskiyou	Siskiyou	CA	USA
DAM200806146	DAM	AD	Siskiyou	Siskiyou	CA	USA
MAC87907	ML	AD	Siskiyou	Siskiyou	CA	USA
MAC87908	ML	AD	Siskiyou	Siskiyou	CA	USA
MVZ1506	MVZ	AD	Siskiyou	Siskiyou	CA	USA
MVZ1569	MVZ	AD	Siskiyou	Siskiyou	CA	USA
MVZ1573	MVZ	AD	Siskiyou	Siskiyou	CA	USA
2011RUSH410	ACR	AD	W Elk Mtns	Gunnison	CO	USA
2011RUSH412	ACR	AD	W Elk Mtns	Gunnison	CO	USA
2009RUSH038	ACR	AD	Payette	Boise	ID	USA
2008RUSH061	ACR	AD	Lk Pend Oreille	Bonner	ID	USA
2008RUSH063	ACR	AD	Lk Pend Oreille	Bonner	ID	USA
2008RUSH066	ACR	AD	Lk Pend Oreille	Bonner	ID	USA
2008RUSH067	ACR	AD	Lk Pend Oreille	Bonner	ID	USA
2008RUSH068	ACR	AD	Lk Pend Oreille	Bonner	ID	USA
2008RUSH069	ACR	AD	Lk Pend Oreille	Bonner	ID	USA
2008RUSH073	ACR	AD	Lk Pend Oreille	Bonner	ID	USA
2009RUSH032	ACR	AD	Clearwater Rv	Idaho	ID	USA
2009RUSH034	ACR	AD	Clearwater Rv	Idaho	ID	USA
2010RUSH112	ACR	AD	Clearwater Rv	Idaho	ID	USA
2010RUSH120	ACR	AD	Clearwater Rv	Idaho	ID	USA
2010RUSH122	ACR	AD	Clearwater Rv	Idaho	ID	USA
2010RUSH124	ACR	AD	Clearwater Rv	Idaho	ID	USA
2010RUSH125B	ACR	AD	Clearwater Rv	Idaho	ID	USA
2010RUSH127	ACR	AD	Clearwater Rv	Idaho	ID	USA
2008RUSH060	ACR	AD	Coeur d' Alene	Kootenai, Shoshone	ID	USA
2008RUSH079	ACR	AD	Coeur d' Alene	Kootenai, Shoshone	ID	USA
2009RUSH020	ACR	AD	Coeur d' Alene	Kootenai, Shoshone	ID	USA
2009RUSH025	ACR	AD	Coeur d' Alene	Kootenai, Shoshone	ID	USA
DAM200606101	DAM	AD	Coeur d' Alene	Kootenai, Shoshone	ID	USA
DAM200606106	DAM	AD	Coeur d' Alene	Kootenai, Shoshone	ID	USA
2010RUSH106	ACR	AD	Pocatello	Power	ID	USA
DAM200606084	DAM	AD	Palouse	Latah, Whitman	ID, WA	USA
DAM20060608	DAM	AD	Palouse	Latah, Whitman	ID, WA	USA
DAM20060608	DAM	AD	Palouse	Latah, Whitman	ID, WA	USA
DAM200706212	DAM	AD	Palouse	Latah, Whitman	ID, WA	USA
DAM200706221	DAM	AD	Palouse	Latah, Whitman	ID, WA	USA
MVZ1508	MVZ	AD	Palouse	Latah, Whitman	ID, WA	USA
2010RUSH189B	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH192B	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH196	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH197	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH198	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH199	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH200	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH205	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH206	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH207	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH208	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH209	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH210	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH211	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH214	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH215	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH216	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2010RUSH217	ACR	AD	Big Belt Mtns	Broadwater, Lewis & Clark	MT	USA
2008RUSH085	ACR	AD	Sawtooth Range	Lewis & Clark, Teton	MT	USA
2010RUSH224	ACR	AD	Sawtooth Range	Lewis & Clark, Teton	MT	USA

2010RUSH225	ACR	AD	Sawtooth Range	Lewis & Clark, Teton	MT	USA
2010RUSH226	ACR	AD	Sawtooth Range	Lewis & Clark, Teton	MT	USA
2010RUSH182	ACR	AD	Lolo Vly	Ravalli	MT	USA
2010RUSH184	ACR	AD	Lolo Vly	Ravalli	MT	USA
2010RUSH185	ACR	AD	Lolo Vly	Ravalli	MT	USA
2008RUSH083	ACR	AD	Thompson Rv	Sanders	MT	USA
2009RUSH028	ACR	AD	Thompson Rv	Sanders	MT	USA
2009RUSH029	ACR	AD	Thompson Rv	Sanders	MT	USA
2010RUSH234B	ACR	AD	Thompson Rv	Sanders	MT	USA
2010RUSH235	ACR	AD	Thompson Rv	Sanders	MT	USA
2010RUSH236	ACR	AD	Thompson Rv	Sanders	MT	USA
2010RUSH237	ACR	AD	Thompson Rv	Sanders	MT	USA
2010RUSH242	ACR	AD	Thompson Rv	Sanders	MT	USA
2011RUSH320	ACR	AD	Ruby Mtns	Elko	NV	USA
2011RUSH333	ACR	AD	Ruby Mtns	Elko	NV	USA
2011RUSH357	ACR	AD	Snake Range	White Pine	NV	USA
2011RUSH369	ACR	AD	Snake Range	White Pine	NV	USA
DAM200305141	DAM	AD	Mt. Hood	Clackamas	OR	USA
DAM200706231	DAM	AD	Mt. Hood	Clackamas	OR	USA
DAM200706232	DAM	AD	Mt. Hood	Clackamas	OR	USA
DAM200305141	DAM	AD	Mt. Hood	Clackamas	OR	USA
DAM20050507	DAM	AD	Mt. Hood	Clackamas	OR	USA
DAM200505072	DAM	AD	Mt. Hood	Clackamas	OR	USA
DAM20050507x	DAM	AD	Mt. Hood	Clackamas	OR	USA
DAM20050507y	DAM	AD	Mt. Hood	Clackamas	OR	USA
MVZ_NKJ19650618_	MVZ	AD	Mt. Hood	Clackamas	OR	USA
2008RUSH024	ACR	AD	Deschutes	Deschutes	OR	USA
2011RUSH311	ACR	AD	Deschutes	Deschutes	OR	USA
2011RUSH312	ACR	AD	Deschutes	Deschutes	OR	USA
DAMm306weflm3	DAM	AD	Deschutes	Deschutes	OR	USA
DAM2005061910619	DAM	AD	Deschutes	Deschutes	OR	USA
DAM20050619m1	DAM	AD	Deschutes	Deschutes	OR	USA
DAM20050619Mtwo	DAM	AD	Deschutes	Deschutes	OR	USA
DAM2005061850618200	DAM	AD	Deschutes	Deschutes	OR	USA
DAM2005061860618200	DAM	AD	Deschutes	Deschutes	OR	USA
DAM200506181061820	DAM	AD	Deschutes	Deschutes	OR	USA
DAM200506182061820	DAM	AD	Deschutes	Deschutes	OR	USA
DAM20050617106172	DAM	AD	Deschutes	Deschutes	OR	USA
DAM20050617306172	DAM	AD	Deschutes	Deschutes	OR	USA
2009RUSH011	ACR	AD	Blue Mtns S	Grant, Umatilla, Wallowa, Union	OR	USA
2009RUSH012	ACR	AD	Blue Mtns S	Grant, Umatilla, Wallowa, Union	OR	USA
2009RUSH013	ACR	AD	Blue Mtns S	Grant, Umatilla, Wallowa, Union	OR	USA
DAM19810607A	DAM	AD	Blue Mtns S	Grant, Umatilla, Wallowa, Union	OR	USA
DAM198106072	DAM	AD	Blue Mtns S	Grant, Umatilla, Wallowa, Union	OR	USA
DAM198106073	DAM	AD	Blue Mtns S	Grant, Umatilla, Wallowa, Union	OR	USA
2008RUSH010	ACR	AD	Rogue Rv	Jackson	OR	USA
2008RUSH011	ACR	AD	Rogue Rv	Jackson	OR	USA
2008RUSH012	ACR	AD	Rogue Rv	Jackson	OR	USA
2008RUSH013	ACR	AD	Rogue Rv	Jackson	OR	USA
MVZ1442	MVZ	AD	Rogue Rv	Jackson	OR	USA
2008RUSH018	ACR	AD	Ft. Klamath	Klamath	OR	USA
2008RUSH020	ACR	AD	Ft. Klamath	Klamath	OR	USA
2009RUSH004B	ACR	AD	Ft. Klamath	Klamath	OR	USA

2009RUSH005	ACR	AD	Ft. Klamath	Klamath	OR	USA
2009RUSH006	ACR	AD	Ft. Klamath	Klamath	OR	USA
2011RUSH289	ACR	AD	Ft. Klamath	Klamath	OR	USA
2011RUSH291	ACR	AD	Ft. Klamath	Klamath	OR	USA
2011RUSH296	ACR	AD	Ft. Klamath	Klamath	OR	USA
2011RUSH305	ACR	AD	Ft. Klamath	Klamath	OR	USA
DAM200906261	DAM	AD	Ft. Klamath	Klamath	OR	USA
DAM200906262	DAM	AD	Ft. Klamath	Klamath	OR	USA
DAM200906264	DAM	AD	Ft. Klamath	Klamath	OR	USA
DAM200906265	DAM	AD	Ft. Klamath	Klamath	OR	USA
DAM200906263	DAM	AD	Ft. Klamath	Klamath	OR	USA
DAM20090626_	DAM	AD	Ft. Klamath	Klamath	OR	USA
2008RUSH008	ACR	AD	Warner Mtns N	Lake	OR	USA
2009RUSH002	ACR	AD	Warner Mtns N	Lake	OR	USA
2009RUSH003	ACR	AD	Warner Mtns N	Lake	OR	USA
DAM2005062810	DAM	AD	Warner Mtns N	Lake	OR	USA
DAM200506281062820	DAM	AD	Warner Mtns N	Lake	OR	USA
DAM200506283062820	DAM	AD	Warner Mtns N	Lake	OR	USA
DAM200506285062820	DAM	AD	Warner Mtns N	Lake	OR	USA
DAM200506289062820	DAM	AD	Warner Mtns N	Lake	OR	USA
DAM506weflm5	DAM	AD	Warner Mtns N	Lake	OR	USA
DAM20070607	DAM	AD	Warner Mtns N	Lake	OR	USA
DAM200307041	DAM	AD	Willamette Vly	Lane, Douglas	OR	USA
2012RUSH734	ACR	AD	Black Hills	Lawrence	SD	USA
2012RUSH735	ACR	AD	Black Hills	Lawrence	SD	USA
2012RUSH736	ACR	AD	Black Hills	Lawrence	SD	USA
2012RUSH737	ACR	AD	Black Hills	Lawrence	SD	USA
2012RUSH738	ACR	AD	Black Hills	Lawrence	SD	USA
2012RUSH739	ACR	AD	Black Hills	Lawrence	SD	USA
2012RUSH760	ACR	AD	Black Hills	Lawrence	SD	USA
2012RUSH761	ACR	AD	Black Hills	Lawrence	SD	USA
2012RUSH762	ACR	AD	Black Hills	Lawrence	SD	USA
2012RUSH763	ACR	AD	Black Hills	Lawrence	SD	USA
MVZ_NKJ19650630_	MVZ	AD	Black Hills	Lawrence	SD	USA
2011RUSH374	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH376	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH376B	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH382B	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH383	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH384	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH385	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH386	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH387	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH388	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH389	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH3892	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH390	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH3912	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH392	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH393B	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH395B	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH396	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH400	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH401	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2011RUSH402	ACR	AD	Wasatch Mtns	Juab, Utah	UT	USA
2009RUSH036	ACR	AD	Blue Mtns N	Asotin, Columbia, Walla Walla	WA	USA
2009RUSH037	ACR	AD	Blue Mtns N	Asotin, Columbia, Walla Walla	WA	USA
DAM20060611cab3	DAM	AD	Blue Mtns N	Asotin, Columbia, Walla Walla	WA	USA

DAM20060611cab4	DAM	AD	Blue Mtns N	Asotin, Columbia, Walla Walla	WA	USA
DAM20060611cs	DAM	AD	Blue Mtns N	Asotin, Columbia, Walla Walla	WA	USA
DAM20060611cw5	DAM	AD	Blue Mtns N	Asotin, Columbia, Walla Walla	WA	USA
DAM20060611	DAM	AD	Blue Mtns N	Asotin, Columbia, Walla Walla	WA	USA
DAM200606111	DAM	AD	Blue Mtns N	Asotin, Columbia, Walla Walla	WA	USA
DAM20060611cw5a	DAM	AD	Blue Mtns N	Asotin, Columbia, Walla Walla	WA	USA
MAC45301	ML	AD	Blue Mtns N	Asotin, Columbia, Walla Walla	WA	USA
2008RUSH026	ACR	AD	Kittitas	Kittitas	WA	USA
2008RUSH027	ACR	AD	Kittitas	Kittitas	WA	USA
2008RUSH030	ACR	AD	Kittitas	Kittitas	WA	USA
2008RUSH032	ACR	AD	Kittitas	Kittitas	WA	USA
2008RUSH033	ACR	AD	Okanogan	Okanogan	WA	USA
2008RUSH035	ACR	AD	Okanogan	Okanogan	WA	USA
2008RUSH037	ACR	AD	Okanogan	Okanogan	WA	USA
2008RUSH038	ACR	AD	Okanogan	Okanogan	WA	USA
2008RUSH045	ACR	AD	Okanogan	Okanogan	WA	USA
2009RUSH015	ACR	AD	Okanogan	Okanogan	WA	USA
2009RUSH016	ACR	AD	Okanogan	Okanogan	WA	USA
2009RUSH018	ACR	AD	Okanogan	Okanogan	WA	USA
2009RUSH019	ACR	AD	Okanogan	Okanogan	WA	USA
2010RUSH144	ACR	AD	Okanogan	Okanogan	WA	USA
2010RUSH145	ACR	AD	Okanogan	Okanogan	WA	USA
2010RUSH146	ACR	AD	Okanogan	Okanogan	WA	USA
2010RUSH149	ACR	AD	Okanogan	Okanogan	WA	USA
2010RUSH152	ACR	AD	Okanogan	Okanogan	WA	USA
2010RUSH154	ACR	AD	Okanogan	Okanogan	WA	USA
2008RUSH053	ACR	AD	Sullivan Lk	Pend Oreille	WA	USA
2008RUSH054	ACR	AD	Sullivan Lk	Pend Oreille	WA	USA
2008RUSH055	ACR	AD	Sullivan Lk	Pend Oreille	WA	USA
2008RUSH056	ACR	AD	Sullivan Lk	Pend Oreille	WA	USA
DAM200606101	DAM	AD	Sullivan Lk	Pend Oreille	WA	USA
DAM200606103	DAM	AD	Sullivan Lk	Pend Oreille	WA	USA
DAM200606104	DAM	AD	Sullivan Lk	Pend Oreille	WA	USA
DAM200606106	DAM	AD	Sullivan Lk	Pend Oreille	WA	USA
DAM20060610Cab1	DAM	AD	Sullivan Lk	Pend Oreille	WA	USA
DAM20060610Cab2	DAM	AD	Sullivan Lk	Pend Oreille	WA	USA
2012RUSH766	ACR	AD	Sinks Canyon	Fremont	WY	USA

APPENDIX B – Chapter 2 Song Playback Response Model Results

Table B1. Table of AIC values for Generalized Linear Mixed Model (GLMM) of approach responses by the three focal taxa to different categories of song stimulus.

Model Terms	k	AIC_c	ΔAIC_c	likelihood	w_i
taxon + stimulus + (taxon x stimulus)	9	3422.84	0.00	1.00	1.00
taxon	3	3457.29	34.45	0.00	0.00
taxon + stimulus	5	3460.87	38.02	0.00	0.00
(.)	1	3470.23	47.39	0.00	0.00
stimulus	3	3473.78	50.93	0.00	0.00

Table B2. Table of AIC values for Generalized Additive Mixed Model (GAMM) of the effect of song distance on the approach responses of the three focal taxa.

Model Terms	k	AIC_c	ΔAIC_c	likelihood	w_i
taxon + song distance + (song distance X taxon)	6	3426.96	0.00	1.00	1.00
taxon + song distance	6.3	3438.62	11.66	0.00	0.00
song distance	4.3	3451.08	24.12	0.00	0.00
taxon	3	3457.29	30.33	0.00	0.00
(.)	1	3470.23	43.27	0.00	0.00

APPENDIX C – Sample lists for Chapter 3

Table C1. Complete sample list with locations for mtDNA analysis. Sequences of all samples provided by John Klicka, University of Washington Burke Museum of Natural History and Culture.

Taxon	Catalogue	Country	State	County	Latitude	Longitude
<i>E. d. difficilis</i>	MGL125	Mexico	Baja California	-	32.03	-115.93
<i>E. d. difficilis</i>	MGL128	Mexico	Baja California	-	32.03	-115.93
<i>E. d. difficilis</i>	MGL140	Mexico	Baja California	-	32.03	-115.93
<i>E. d. difficilis</i>	RB324	USA	California	Mendocino	39.82	-122.99
<i>E. d. difficilis</i>	RB343	USA	California	Monterey	36.24	-121.70
<i>E. d. difficilis</i>	RB344	USA	California	Monterey	36.24	-121.70
<i>E. f. flavescens</i>	CR158	Costa Rica	-	-	-	-
<i>E. f. flavescens</i>	GMS2057	Panama	Chiriqui	-	8.77	-82.66
<i>E. f. flavescens</i>	GMS2107	Panama	Chiriqui	-	8.77	-82.66
<i>E. f. salvinii</i>	JK02039	Guatemala	Quetzaltenango	-	14.66	-91.61
<i>E. f. salvinii</i>	BMM433	Mexico	Chiapas	-	16.50	-92.46
<i>E. f. salvinii</i>	BRB722	Mexico	Chiapas	-	16.50	-92.46
<i>E. flaviventris</i>	DHB4611	Guatemala	Quetzaltenango	-	14.66	-91.61
<i>E. flaviventris</i>	TUX252	Mexico	Veracruz	-	-	-
<i>E. o. hellmayri</i>	RB274	USA	Arizona	Cochise	31.85	-109.98
<i>E. o. hellmayri</i>	RB231	USA	Arizona	Coconino	35.27	-111.77
<i>E. o. hellmayri</i>	JK09551	USA	Arizona	Graham	32.71	-109.64
<i>E. o. occidentalis</i>	JK09376	Mexico	Chihuahua	-	29.65	-108.17
<i>E. o. occidentalis</i>	DHB5311	Mexico	Jalisco	-	21.88	-103.87
<i>E. o. occidentalis</i>	JK06455	Mexico	Michoacan	-	19.43	-102.26
<i>E. o. occidentalis</i>	JK11265	Mexico	Nuevo Leon	-	23.81	-99.85
<i>E. o. occidentalis</i> (Guerrero)	JK11161	Mexico	Guerrero	-	17.56	-99.69
<i>E. o. occidentalis</i> (Guerrero)	JK11162	Mexico	Guerrero	-	17.56	-99.69
<i>E. o. occidentalis</i> (Guerrero)	JK11192	Mexico	Guerrero	-	17.56	-99.69

Table C2. Complete sample list with locations for bioacoustics analysis. Catalog abbreviations: Rush = Andrew Rush, BL = Ohio State University Borror Laboratory, DAM = D. Archibald McCallum personal collection, DEI = Darren E. Irwin personal collection, GDS = Hector and Monica Gómez de Silva personal collection, KBB = Kelly B. Bryan personal collection, ML = Cornell Laboratory of Ornithology Macaulay Library, MVZ = University of California Museum of Vertebrate Zoology, NDP = Nathan D. Pieplow personal collection, SNGH = Steve N.G. Howell personal collection, XC = xeno-canto.org.

Taxon	Catalog	Location	Country	State/Prov	County
<i>E. d. difficilis</i>	BL18574	Haida Gwaii	CA	BC	-
<i>E. d. difficilis</i>	BL18578	Haida Gwaii	CA	BC	-
<i>E. d. difficilis</i>	2006DEI004	Vancouver	CA	BC	-
<i>E. d. difficilis</i>	2006DEI005	Vancouver	CA	BC	-
<i>E. d. difficilis</i>	DEI2006001	Vancouver	CA	BC	-
<i>E. d. difficilis</i>	MVZ1446	Vancouver	CA	BC	-
<i>E. d. difficilis</i>	MVZ1447	Vancouver	CA	BC	-
<i>E. d. difficilis</i>	ML59788	Vancouver Island	CA	BC	-
<i>E. d. difficilis</i>	2012Rush180	Berkeley	USA	CA	Alameda
<i>E. d. difficilis</i>	2012Rush181	Berkeley	USA	CA	Alameda
<i>E. d. difficilis</i>	2012Rush182	Berkeley	USA	CA	Alameda
<i>E. d. difficilis</i>	2010Rush022	Carmel Vly	USA	CA	Monterey
<i>E. d. difficilis</i>	2010Rush026	Carmel Vly	USA	CA	Monterey
<i>E. d. difficilis</i>	2010Rush027	Carmel Vly	USA	CA	Monterey
<i>E. d. difficilis</i>	ML110916	Carmel Vly	USA	CA	Monterey
<i>E. d. difficilis</i>	ML22983	Carmel Vly	USA	CA	Monterey
<i>E. d. difficilis</i>	ML22985	Carmel Vly	USA	CA	Monterey
<i>E. d. difficilis</i>	ML22986	Carmel Vly	USA	CA	Monterey
<i>E. d. difficilis</i>	ML7600	Carmel Vly	USA	CA	Monterey
<i>E. d. difficilis</i>	ML7610	Carmel Vly	USA	CA	Monterey
<i>E. d. difficilis</i>	BL28297	Elk Creek	USA	CA	Glenn
<i>E. d. difficilis</i>	BL28311	Elk Creek	USA	CA	Glenn
<i>E. d. difficilis</i>	2012Rush782	N Calif Coast	USA	CA	Humboldt
<i>E. d. difficilis</i>	2012Rush784	N Calif Coast	USA	CA	Humboldt
<i>E. d. difficilis</i>	2012Rush785	N Calif Coast	USA	CA	Humboldt
<i>E. d. difficilis</i>	2012Rush786	N Calif Coast	USA	CA	Humboldt
<i>E. d. difficilis</i>	2011Rush150	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2011Rush158	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2011Rush159	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2011Rush160	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2011Rush161	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2011Rush164	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2011Rush165	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2012Rush151	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2012Rush154	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2012Rush170	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2012Rush171	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2013Rush053	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2013Rush054	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2013Rush055	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2013Rush056	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2013Rush057	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2013Rush058	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	2013Rush059	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	BL24860	Pescadero	USA	CA	San Mateo
<i>E. d. difficilis</i>	ML126424	Pescadero	USA	CA	San Mateo

<i>E. d. difficilis</i>	2010Rush001	Pt Reyes	USA	CA	Marin
<i>E. d. difficilis</i>	2010Rush005	Pt Reyes	USA	CA	Marin
<i>E. d. difficilis</i>	2010Rush017	Pt Reyes	USA	CA	Marin
<i>E. d. difficilis</i>	2011Rush131	Pt Reyes	USA	CA	Marin
<i>E. d. difficilis</i>	2011Rush139	Pt Reyes	USA	CA	Marin
<i>E. d. difficilis</i>	2011Rush146	Pt Reyes	USA	CA	Marin
<i>E. d. difficilis</i>	2011Rush171	Pt Reyes	USA	CA	Marin
<i>E. d. difficilis</i>	ML111041	Pt Reyes	USA	CA	Marin
<i>E. d. difficilis</i>	ML126454	Pt Reyes	USA	CA	Marin
<i>E. d. difficilis</i>	ML126470	Pt Reyes	USA	CA	Marin
<i>E. d. difficilis</i>	NDP20090433	Santiago Oaks	USA	CA	Orange
<i>E. d. difficilis</i>	NDP20090437	Santiago Oaks	USA	CA	Orange
<i>E. d. difficilis</i>	ML118835	Healdsburg	USA	CA	Sonoma
<i>E. d. difficilis</i>	2012Rush516	Yosemite	USA	CA	Mariposa
<i>E. d. difficilis</i>	2012Rush517	Yosemite	USA	CA	Mariposa
<i>E. d. difficilis</i>	2012Rush522	Yosemite	USA	CA	Mariposa
<i>E. d. difficilis</i>	ML44956	Coos Bay	USA	OR	Coos
<i>E. d. difficilis</i>	ML44957	Coos Bay	USA	OR	Coos
<i>E. d. difficilis</i>	ML50334	Coos Bay	USA	OR	Coos
<i>E. d. difficilis</i>	BL18670	Olympic Pen	USA	WA	Clallum
<i>E. d. difficilis</i>	BL28972	Olympic Pen	USA	WA	Clallum
<i>E. d. difficilis</i>	BL28986	Olympic Pen	USA	WA	Clallum
<i>E. d. difficilis</i>	ML45289	Olympic Pen	USA	WA	Clallum
<i>E. d. difficilis</i>	2010Rush160	Skagit Rv	USA	WA	Glacier
<i>E. d. difficilis</i>	2010Rush162	Skagit Rv	USA	WA	Glacier
<i>E. d. difficilis</i>	ML7601	Skagit Rv	USA	WA	Glacier
<i>E. d. difficilis</i>	ML7602	Skagit Rv	USA	WA	Glacier
<i>E. d. difficilis</i>	ML7611	W Washington	USA	WA	Pierce
<i>E. f. flavescens</i>	DAM032420101	Talamanca Mtns	CR	SJ	-
<i>E. f. flavescens</i>	ML165380	Talamanca Mtns	CR	SJ	-
<i>E. f. flavescens</i>	ML165382	Talamanca Mtns	CR	SJ	-
<i>E. f. flavescens</i>	ML51728	Talamanca Mtns	CR	SJ	-
<i>E. f. flavescens</i>	MVZ161993	Talamanca Mtns	CR	SJ	-
<i>E. f. salvinii</i>	2012Rush420	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush429	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush430	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush431	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush432	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush433b	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush436	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush438	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush439	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush440	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush441	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush442	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush448	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush451	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush453	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush454	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush455	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush461	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush462	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush463	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush464	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush465	Antigua	GT	SA	-
<i>E. f. salvinii</i>	2012Rush383	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2012Rush384	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2012Rush385	San Cristobal Casas	MX	CP	-

<i>E. f. salvinii</i>	2012Rush386	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2012Rush387	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2012Rush388	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2012Rush406	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2012Rush407	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2012Rush472	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2012Rush473	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2012Rush475	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2012Rush478	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2012Rush483	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2012Rush486	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2012Rush490	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2013Rush155	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2013Rush156	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2013Rush161	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2013Rush162	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2013Rush164	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2013Rush165	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2013Rush168	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2013Rush175	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2013Rush178	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2013Rush180	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2013Rush186	San Cristobal Casas	MX	CP	-
<i>E. f. salvinii</i>	2013Rush188	San Cristobal Casas	MX	CP	-
<i>E. o. hellmayri</i>	2011Rush586	Chiricahua Mtns	USA	AZ	Cochise
<i>E. o. hellmayri</i>	2012Rush646	Chiricahua Mtns	USA	AZ	Cochise
<i>E. o. hellmayri</i>	2012Rush647	Chiricahua Mtns	USA	AZ	Cochise
<i>E. o. hellmayri</i>	2012Rush648	Chiricahua Mtns	USA	AZ	Cochise
<i>E. o. hellmayri</i>	2012Rush653	Chiricahua Mtns	USA	AZ	Cochise
<i>E. o. hellmayri</i>	2012Rush655	Chiricahua Mtns	USA	AZ	Cochise
<i>E. o. hellmayri</i>	2012Rush661	Chiricahua Mtns	USA	AZ	Cochise
<i>E. o. hellmayri</i>	2012Rush664	Chiricahua Mtns	USA	AZ	Cochise
<i>E. o. hellmayri</i>	ML21108	Chiricahua Mtns	USA	AZ	Cochise
<i>E. o. hellmayri</i>	ML21436	Chiricahua Mtns	USA	AZ	Cochise
<i>E. o. hellmayri</i>	ML21455	Chiricahua Mtns	USA	AZ	Cochise
<i>E. o. hellmayri</i>	ML59789	Chiricahua Mtns	USA	AZ	Cochise
<i>E. o. hellmayri</i>	ML87920	Chiricahua Mtns	USA	AZ	Cochise
<i>E. o. hellmayri</i>	2012Rush609	Pinaleno Mtns	USA	AZ	Graham
<i>E. o. hellmayri</i>	2012Rush611	Pinaleno Mtns	USA	AZ	Graham
<i>E. o. hellmayri</i>	2012Rush612	Pinaleno Mtns	USA	AZ	Graham
<i>E. o. hellmayri</i>	2012Rush613	Pinaleno Mtns	USA	AZ	Graham
<i>E. o. hellmayri</i>	2012Rush615	Pinaleno Mtns	USA	AZ	Graham
<i>E. o. hellmayri</i>	2012Rush617	Pinaleno Mtns	USA	AZ	Graham
<i>E. o. hellmayri</i>	2012Rush619	Pinaleno Mtns	USA	AZ	Graham
<i>E. o. hellmayri</i>	2012Rush620	Pinaleno Mtns	USA	AZ	Graham
<i>E. o. hellmayri</i>	2012Rush621	Pinaleno Mtns	USA	AZ	Graham
<i>E. o. hellmayri</i>	2012Rush622	Pinaleno Mtns	USA	AZ	Graham
<i>E. o. hellmayri</i>	2012Rush631	Pinaleno Mtns	USA	AZ	Graham
<i>E. o. hellmayri</i>	2012Rush632	Pinaleno Mtns	USA	AZ	Graham
<i>E. o. hellmayri</i>	2012Rush633	Pinaleno Mtns	USA	AZ	Graham
<i>E. o. hellmayri</i>	2012Rush634	Pinaleno Mtns	USA	AZ	Graham
<i>E. o. hellmayri</i>	2012Rush549	San Francisco Pks	USA	AZ	Coconino
<i>E. o. hellmayri</i>	2012Rush550	San Francisco Pks	USA	AZ	Coconino
<i>E. o. hellmayri</i>	2012Rush551	San Francisco Pks	USA	AZ	Coconino
<i>E. o. hellmayri</i>	2012Rush553	San Francisco Pks	USA	AZ	Coconino
<i>E. o. hellmayri</i>	2012Rush559	San Francisco Pks	USA	AZ	Coconino
<i>E. o. hellmayri</i>	2012Rush563	San Francisco Pks	USA	AZ	Coconino
<i>E. o. hellmayri</i>	2012Rush567	San Francisco Pks	USA	AZ	Coconino

<i>E. o. hellmayri</i>	2012Rush568	San Francisco Pks	USA	AZ	Coconino
<i>E. o. hellmayri</i>	2012Rush569	San Francisco Pks	USA	AZ	Coconino
<i>E. o. hellmayri</i>	2012Rush570	San Francisco Pks	USA	AZ	Coconino
<i>E. o. hellmayri</i>	2012Rush575	San Francisco Pks	USA	AZ	Coconino
<i>E. o. hellmayri</i>	MVZ1555	Sta Catalina Mtns	USA	AZ	Pima
<i>E. o. hellmayri</i>	MVZ1556	Sta Catalina Mtns	USA	AZ	Pima
<i>E. o. hellmayri</i>	ML7605	Sta Catalina Mtns	USA	AZ	Pima
<i>E. o. hellmayri</i>	2012Rush577	White Mtns	USA	AZ	Apache
<i>E. o. hellmayri</i>	2012Rush578	White Mtns	USA	AZ	Apache
<i>E. o. hellmayri</i>	2011Rush527	Black Range	USA	NM	Grant
<i>E. o. hellmayri</i>	2011Rush529	Black Range	USA	NM	Grant
<i>E. o. hellmayri</i>	2011Rush530	Black Range	USA	NM	Grant
<i>E. o. hellmayri</i>	2011Rush532	Black Range	USA	NM	Grant
<i>E. o. hellmayri</i>	2011Rush533	Black Range	USA	NM	Grant
<i>E. o. hellmayri</i>	2011Rush534b	Black Range	USA	NM	Grant
<i>E. o. hellmayri</i>	2011Rush535b	Black Range	USA	NM	Grant
<i>E. o. hellmayri</i>	2011Rush536	Black Range	USA	NM	Grant
<i>E. o. hellmayri</i>	2011Rush540	Black Range	USA	NM	Grant
<i>E. o. hellmayri</i>	2012Rush672	Black Range	USA	NM	Grant
<i>E. o. hellmayri</i>	2012Rush686	Black Range	USA	NM	Grant
<i>E. o. hellmayri</i>	2012Rush699	Black Range	USA	NM	Grant
<i>E. o. hellmayri</i>	2012Rush700	Black Range	USA	NM	Grant
<i>E. o. hellmayri</i>	ML112616	Black Range	USA	NM	Grant
<i>E. o. hellmayri</i>	KBB003	Davis Mtns	USA	TX	Jeff Davis
<i>E. o. hellmayri</i>	KBB006	Davis Mtns	USA	TX	Jeff Davis
<i>E. o. hellmayri</i>	KBB013b10	Davis Mtns	USA	TX	Jeff Davis
<i>E. o. hellmayri</i>	KBB020b10	Davis Mtns	USA	TX	Jeff Davis
<i>E. o. hellmayri</i>	KBB033b08	Davis Mtns	USA	TX	Jeff Davis
<i>E. o. hellmayri</i>	KBB027b08	Guadalupe Mtns	USA	TX	Culbertson
<i>E. o. hellmayri</i>	KBB028a03	Guadalupe Mtns	USA	TX	Culbertson
<i>E. o. hellmayri</i>	KBB028a06	Guadalupe Mtns	USA	TX	Culbertson
<i>E. o. hellmayri</i>	KBB030a04	Guadalupe Mtns	USA	TX	Culbertson
<i>E. o. occidentalis</i>	HGDS1	Creel	MX	CH	-
<i>E. o. occidentalis</i>	HGDS2	Creel	MX	CH	-
<i>E. o. occidentalis</i>	SNGH57	Tlanchinol	MX	HI	-
<i>E. o. occidentalis</i>	MVZ1415	Cuernevaca	MX	MR	-
<i>E. o. occidentalis</i>	MVZ1417	Cuernevaca	MX	MR	-
<i>E. o. occidentalis</i>	XC65757	Pollo Nino	MX	OA	-
<i>E. o. occidentalis</i>	MVZ1423	Xilitla	MX	QE	-
<i>E. o. occidentalis</i>	ML53151	Durango Hwy	MX	SI	-
<i>E. o. occidentalis</i>	2012Rush195	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush199	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush202	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush210	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush223	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush224	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush239	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush240	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush242	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush243	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush244	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush245	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush247	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush248	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush249	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush250	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush251	Xalapa	MX	VE	-
<i>E. o. occidentalis</i>	2012Rush252	Xalapa	MX	VE	-

<i>E. o. occidentalis</i>	Guerrero	2013Rush112	Omiltemi	MX	GR	-
<i>E. o. occidentalis</i>	Guerrero	2013Rush113	Omiltemi	MX	GR	-
<i>E. o. occidentalis</i>	Guerrero	2013Rush114	Omiltemi	MX	GR	-
<i>E. o. occidentalis</i>	Guerrero	2013Rush115	Omiltemi	MX	GR	-
<i>E. o. occidentalis</i>	Guerrero	2013Rush118	Omiltemi	MX	GR	-
<i>E. o. occidentalis</i>	Guerrero	2013Rush130	Omiltemi	MX	GR	-
<i>E. o. occidentalis</i>	Guerrero	2013Rush131	Omiltemi	MX	GR	-
<i>E. o. occidentalis</i>	Guerrero	2013Rush134	Omiltemi	MX	GR	-
<i>E. o. occidentalis</i>	Guerrero	2013Rush138	Omiltemi	MX	GR	-
<i>E. o. occidentalis</i>	Guerrero	2013Rush139	Omiltemi	MX	GR	-
<i>E. o. occidentalis</i>	Guerrero	2013Rush141	Omiltemi	MX	GR	-
<i>E. o. occidentalis</i>	Guerrero	2013Rush142	Omiltemi	MX	GR	-

APPENDIX D – Description of the vocal landmarks used in Chapter 3

The vocal repertoire of the *E. difficilis-occidentalis-flavescens* superspecies consists of a position note and the three song types that make up the typical three-part song phrase. I hypothesize that these four vocal types are homologous. This is based on the presence of common landmarks (LM) evident in the spectrograms of each vocalization type in each taxon. For this study, I marked 10 landmarks on each of the four vocalization types for each taxon in this complex (Figure D1). Landmarks were based largely on inflection points evident in the spectrogram. I marked a maximum of five homologous inflection points in each vocalization type, although in some simpler vocalizations I collapsed multiple landmarks into one point when inflection points were not detectable. In general, inflection points seem to create decreases in amplitude, evident in the waveform. There seem to be amplitude peaks immediately preceding or following inflection points, suggesting that the transition that occurs before and after a peak requires an input of sound energy. I assume that the orientation of the first part of each vocalization remains unchanged and that the apparent rotation of the spectrogram in certain vocal types is due to changes in second part of the vocalization. I also assume that the first part of the vocalization is preserved in all vocal types, but that the second part can become severely truncated. This is most important with respect to S1. I assume that the reduced S1 evident in most taxa has been formed by reduction of the vocalization pre-LM04 and post-LM05. These assumptions are based on careful analysis of hundreds of vocal samples from these taxa. Some of these trends are more evident in the vocalizations of genetically admixed individuals (analyzed in Chapter 2, but not included here).

Each vocalization can be preceded by a ‘tick’, a short, relatively low frequency sound that is barely noticeable to the ear, but evident on many spectrograms. The tick most commonly precedes S2 and S3 (in some taxa). Because the tick is not always present, I did not use it in any analyses (although I marked it as LM01 if present).

Following is a detailed description of the vocal landmarks used in this study.

LM01. The end (or last low frequency point) of the tick.

LM02. The true beginning of the vocalization, used primarily to measure duration. This is often the same point as LM03, unless the spectrogram begins with a descending flourish. In this case, LM03 will differ, and LM02 may be the same as LM04.

LM03. This is the low point of the beginning of the song. It will differ from LM02 only if the vocalization begins with a descending flourish. This LM is necessary to measure the frequency change in the first part of the vocalization (i.e., LM05 minus LM03).

LM04. This is the first inflection point, between the beginning of the vocalization and the frequency peak of part 1 (LM5). This is most obvious in S2, where it appears in the spectrogram as a bulge that occurs soon after the end of the tick. The waveform typically shows an increase in amplitude at this point. Some *difficilis* S2s start with a downward flourish that then leads up to LM05. Careful inspection of multiple S2 examples shows that the peak of this flourish is LM04.

LM05. The peak frequency of the first part of the vocalization. This often coincides with a decrease in amplitude evident in the waveform. This is often the peak frequency of the entire vocalization.

LM06. The transition between the first and second parts of the vocalization. In continuous vocalizations, LM06 occurs as a change in slope that occurs after the descent from the peak frequency, and may be accompanied by an increase in amplitude. In (non-linear) vocalizations with an amplitude gap (e.g., the S2 of *difficilis*, or the MPNs of *hellmayri*, *occidentalis*, or “Guerrero”), this marks the beginning of the amplitude gap.

LM07. The beginning (and low point) of the second half of the vocalization, which often coincides with a slight decrease in amplitude in the waveform. In vocalizations with an amplitude gap, this marks the end of the amplitude gap.

LM08. The positive inflection point following LM07, which often coincides with a slight decrease in amplitude in the waveform.

LM09. The last high frequency point of the second half of the vocalization.

LM10. The true end of the vocalization. This differs from LM09 if the vocalization “trails off” after LM09 (usually to a lower frequency).

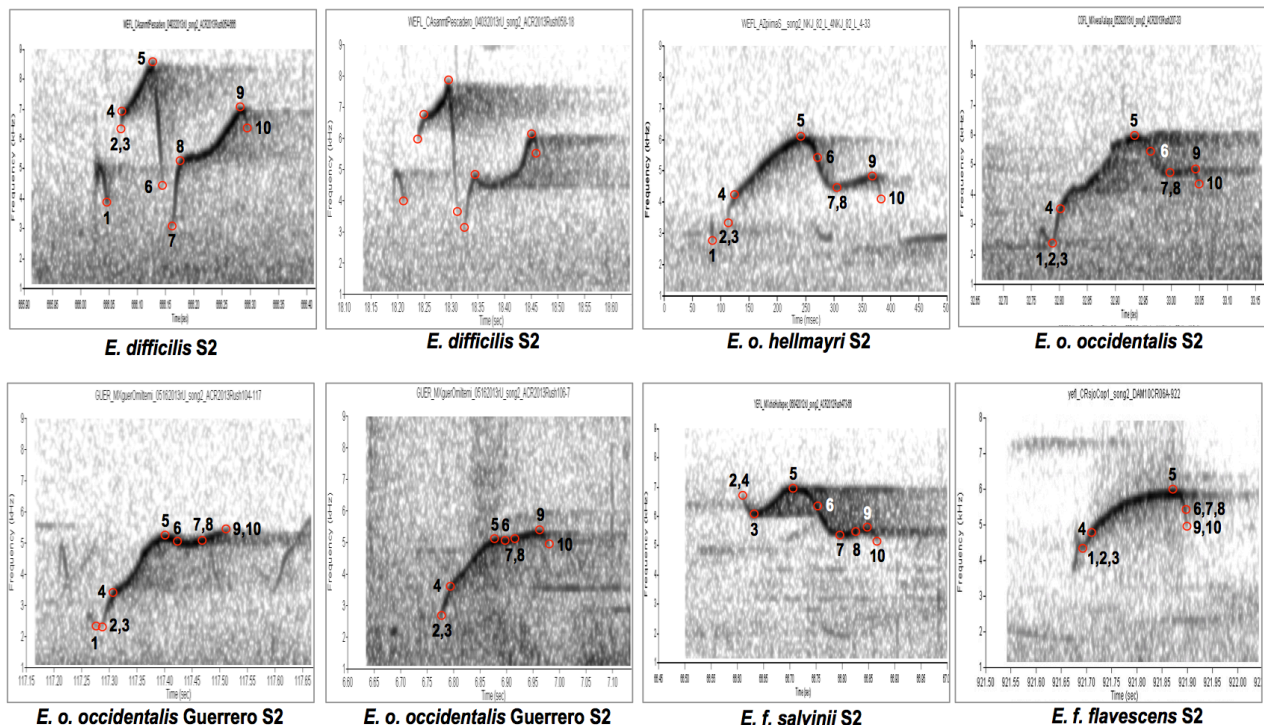


Figure D1. Song spectrograms of the six taxa illustrating the placement of the 10 landmarks used in this analysis. Here, Song 2 is used as an exemplar vocalization.

APPENDIX E – Results of Principal Components Analyses performed in Chapter 3

Table E1. Eigenvectors and eigenvalues for PCA of each of the four vocalization types. See Methods for explanation of the input variables.

SONG 1 Eigenvectors	PC1	PC2	PC3	PC4	PC	Eigenvalue	Percent	Cumulative Percent
LM05 <i>f</i>	0.315	0.137	-0.152	0.401	1	3.308	30.074	30.074
Δf LM05-LM02	0.372	-0.242	0.040	0.461	2	3.114	28.309	58.383
Δf LM06-LM05	0.371	-0.259	-0.016	-0.428	3	1.359	12.352	70.735
Δf LM05-LM07	-0.376	0.214	0.141	0.441	4	1.288	11.708	82.443
Δf LM09-LM07	0.288	0.269	0.074	0.046	5	0.719	6.536	88.979
Duration (Δt LM02-LM10)	0.372	-0.302	0.110	0.368	6	0.526	4.780	93.759
Reltv. Δt LM02-LM05	-0.244	-0.470	-0.169	0.134	7	0.322	2.928	96.687
Reltv. Δt LM05-LM06	-0.119	0.212	0.704	0.061	8	0.168	1.525	98.212
Reltv. Δt LM07-LM10	0.325	0.400	-0.130	-0.213	9	0.126	1.142	99.354
Slope LM05-LM02	0.192	0.424	-0.099	0.119	10	0.052	0.476	99.83
Slope LM05-LM06	0.215	-0.201	0.622	-0.172	11	0.019	0.170	100

SONG 2 Eigenvectors	PC1	PC2	PC3	PC4	PC	Eigenvalue	Percent	Cumulative Percent
LM05 <i>f</i>	0.333	-0.237	0.161	0.082	1	6.801	61.825	61.825
Δf LM05-LM02	-0.083	0.649	-0.307	0.154	2	1.899	17.260	79.084
Δf LM06-LM05	-0.373	-0.015	-0.080	-0.129	3	1.196	10.874	89.958
Δf LM05-LM07	0.370	0.033	0.133	0.110	4	0.586	5.325	95.284
Δf LM09-LM07	0.374	0.054	0.036	0.081	5	0.266	2.422	97.705
Duration (Δt LM02-LM10)	-0.071	0.445	0.687	-0.140	6	0.100	0.907	98.612
Reltv. Δt LM02-LM05	-0.335	0.239	0.159	-0.151	7	0.049	0.441	99.053
Reltv. Δt LM05-LM06	-0.243	-0.083	0.307	0.897	8	0.038	0.342	99.395
Reltv. Δt LM07-LM10	0.305	0.268	0.316	-0.125	9	0.030	0.269	99.664
Slope LM05-LM02	0.237	0.430	-0.404	0.269	10	0.023	0.205	99.869
Slope LM05-LM06	-0.376	-0.007	-0.013	0.006	11	0.014	0.131	100

SONG 3 Eigenvectors	PC1	PC2	PC3	PC4	PC	Eigenvalue	Percent	Cumulative Percent
LM05 <i>f</i>	0.425	0.121	-0.047	-0.082	1	3.862	35.112	35.112
Δf LM05-LM02	-0.447	0.058	-0.032	0.031	2	1.914	17.397	52.508
Δf LM06-LM05	-0.293	0.395	0.394	0.176	3	1.405	12.769	65.277
Δf LM05-LM07	0.404	0.314	-0.078	-0.048	4	1.177	10.700	75.977
Δf LM09-LM07	0.241	0.513	0.040	0.174	5	0.857	7.790	83.768
Duration (Δt LM02-LM10)	-0.184	-0.287	-0.432	0.361	6	0.797	7.247	91.015
Reltv. Δt LM02-LM05	-0.430	0.218	-0.168	-0.200	7	0.389	3.534	94.549
Reltv. Δt LM05-LM06	0.245	-0.424	0.193	-0.270	8	0.290	2.635	97.184
Reltv. Δt LM07-LM10	0.175	-0.075	-0.141	0.782	9	0.195	1.771	98.955
Slope LM05-LM02	0.016	-0.377	0.268	0.057	10	0.079	0.722	99.677
Slope LM05-LM06	-0.051	-0.084	0.700	0.267	11	0.036	0.323	100

MPN Eigenvectors	PC1	PC2	PC3	PC4	PC	Eigenvalue	Percent	Cumulative Percent
LM05 <i>f</i>	0.322	0.380	-0.048	-0.164	1	4.607	41.878	41.878
Δf LM05-LM02	0.330	0.181	-0.028	-0.319	2	1.995	18.140	60.019
Δf LM06-LM05	0.269	0.245	-0.074	0.661	3	1.307	11.878	71.896
Δf LM05-LM07	-0.336	0.230	0.134	-0.201	4	1.088	9.889	81.785
Δf LM09-LM07	-0.427	0.161	0.086	0.030	5	0.831	7.555	89.34
Duration (Δt LM02-LM10)	0.048	-0.171	0.783	0.033	6	0.434	3.947	93.287
Reltv. Δt LM02-LM05	0.412	-0.130	0.261	-0.106	7	0.247	2.247	95.534
Reltv. Δt LM05-LM06	-0.047	0.479	0.316	-0.378	8	0.200	1.821	97.355
Reltv. Δt LM07-LM10	-0.338	0.062	0.320	0.376	9	0.123	1.122	98.477
Slope LM05-LM02	-0.296	0.356	-0.244	-0.041	10	0.101	0.920	99.397
Slope LM05-LM06	0.208	0.527	0.152	0.309	11	0.066	0.603	100

Table E2. Eigenvectors and eigenvalues for PCA of taxon song. Input variables are the first two PCs from the PCA of each of the three song types (Table E1).

TAXON SONG Eigenvectors	PC1	PC2	PC3	PC4	PC	Eigenvalue	Percent	Cumulative Percent
Song 1 PC1	0.200	-0.590	-0.204	0.308	1	2.790	46.492	46.492
Song 1 PC2	-0.534	-0.232	0.311	0.281	2	2.437	40.623	87.115
Song 2 PC1	-0.437	0.419	0.199	-0.330	3	0.689	11.481	98.596
Song 2 PC2	0.374	0.383	0.574	0.604	4	0.072	1.202	99.798
Song 3 PC1	0.580	0.138	0.028	-0.420	5	0.012	0.202	100
Song 3 PC2	-0.090	0.507	-0.701	0.419	-	-	-	-

Table E3. Eigenvectors and eigenvalues for PCA of four vocalization types (top) and three vocalization types (bottom) for six taxa. See Methods for explanation of the input variables.

4 VOCAL TYPES Eigenvectors	PC1	PC2	PC3	PC4	PC	Eigenvalue	Percent	Cumulative Percent
LM05 <i>f</i>	-0.039	0.526	-0.484	-0.150	1	5.729	52.077	52.077
Δf LM05-LM02	-0.188	0.365	0.541	0.188	2	1.944	17.672	69.748
Δf LM06-LM05	-0.332	-0.338	0.152	0.002	3	1.479	13.441	83.19
Δf LM05-LM07	0.383	0.191	-0.123	-0.044	4	0.913	8.300	91.49
Δf LM09-LM07	0.374	0.065	0.171	-0.049	5	0.356	3.233	94.723
Duration (Δt LM02-LM10)	-0.186	0.511	0.295	0.318	6	0.228	2.075	96.798
Reltv. Δt LM02-LM05	-0.367	0.147	-0.139	-0.135	7	0.156	1.417	98.215
Reltv. Δt LM05-LM06	0.101	-0.093	-0.408	0.855	8	0.080	0.724	98.939
Reltv. Δt LM07-LM10	0.365	0.156	0.237	0.162	9	0.061	0.551	99.49
Slope LM05-LM02	0.332	-0.282	0.272	0.063	10	0.044	0.399	99.889
Slope LM05-LM06	-0.378	-0.198	0.039	0.242	11	0.012	0.111	100

3 VOCAL TYPES Eigenvectors	PC1	PC2	PC3	PC4	PC	Eigenvalue	Percent	Cumulative Percent
LM05 <i>f</i>	0.180	-0.464	0.339	-0.064	1	5.402	49.106	49.106
Δf LM05-LM02	0.074	0.483	0.444	-0.322	2	2.159	19.626	68.732
Δf LM06-LM05	-0.396	0.222	-0.073	0.123	3	1.585	14.405	83.137
Δf LM05-LM07	0.385	-0.246	0.125	-0.053	4	0.912	8.291	91.428
Δf LM09-LM07	0.393	0.097	-0.178	0.063	5	0.432	3.929	95.357
Duration (Δt LM02-LM10)	0.140	0.390	0.511	-0.086	6	0.281	2.558	97.915
Reltv. Δt LM02-LM05	-0.303	-0.203	0.324	-0.333	7	0.114	1.040	98.955
Reltv. Δt LM05-LM06	-0.076	-0.115	0.453	0.807	8	0.067	0.609	99.564
Reltv. Δt LM07-LM10	0.393	0.148	0.084	0.191	9	0.040	0.365	99.929
Slope LM05-LM02	0.285	0.374	-0.239	0.170	10	0.007	0.064	99.993
Slope LM05-LM06	-0.387	0.252	0.012	0.186	11	0.001	0.007	100