8-2018

Geographic Variation in Rock Wren (Salpinctes Obsoletus) Song Complexity

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Recommended Citation
Najar, Nadje Amal, "Geographic Variation in Rock Wren (Salpinctes Obsoletus) Song Complexity" (2018). *Dissertations*. 514.
https://digscholarship.unco.edu/dissertations/514

UNIVERSITY OF NORTHERN COLORADO

Greeley, Colorado

The Graduate School

GEOGRAPHIC VARIATION IN ROCK WREN (SALPINCTES OBSOLETUS) SONG COMPLEXITY

A Dissertation Submitted in Partial Fulfillment
Of the Requirements for the Degree of
Doctor of Philosophy

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College of Natural and Health Sciences
School of Biological Sciences
Biological Education

August 2018

This Dissertation by: Nadje Amal Najar

Entitled: *Geographic variation in rock wren (Salpinctes obsoletus) song complexity*

has been approved as meeting the requirement for the Degree of Doctor of Philosophy in the College of Natural and Health Sciences in the School of Biological Sciences, Program of Biological Education.

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ABSTRACT

Najar, Nadje Amal. *Geographic variation in rock wren (Salpinctes obsoletus) song complexity*. Published Doctor of Education dissertation, University of Northern Colorado, 2018.

Birds sing to advertise for mates and repel rivals, but there is enormous variety in how they do this. One of the best-studied and most intriguing questions in the field is how song varies in complexity from one bird to the next, at all taxonomic levels. Several studies have found associations between migratory behavior or latitudinal gradients and song complexity, but it remains unclear how universal this pattern is or what factors may be driving it. This small body of literature suffers from several problems, perhaps the most glaring of which is the lack of systematic, population-level studies. The main goals of this dissertation were to determine what evidence there is for the hypothesis that song complexity is influenced by latitude and/or migratory behavior and whether such a pattern can be detected in a single species, the rock wren (*Salpinctes obsoletus*). I recorded rock wren song at 11 sites in a latitudinal transect with both migratory and sedentary populations, and used morphological measurements and genome-level SNP scans to test my classification scheme of migratory versus sedentary populations. Song repertoire size was larger in sedentary rock wrens but did not vary with latitude, while migratory wrens had smaller mean repertoire sizes which increased with increasing latitude. Morphological measurements differed between migratory and sedentary populations, suggesting life history differences between these two groups. Population genetic structure was only apparent using outlier loci, but the resulting structure was not concordant with migratory behavior or site membership. Taken together, these results suggest migration does not pose a barrier to gene flow between migratory and sedentary populations, and that migratory and sedentary behavior is associated with differences in song complexity and morphology, although in a way inconsistent with any previously published hypotheses.

ACKNOWLEDGEMENTS

It's been a long time coming, but we're finally here. While it's my name on the front page of this document, and I've certainly spent blood, sweat, and tears (both literal and metaphorical) assembling it, this dissertation exists as the result of the efforts of a lot of people whose contributions I am happy to acknowledge.

I couldn't have done this project without the aid of several people who helped me with the nuts and bolts of data collection and analysis. I'd like to thank the people who assisted me in the field: Charmaine Holloway, Carissa King, and Sami Piper. Dr. Mit McGlaughlin taught me all the basics of genetics lab work, and Jenna McCullough gave me a crash course in target sequence capture. Sami Naibauer helped me get started learning Linux. Dr. Garth Spellman was absolutely essential to the genetics portion of this project, and served as a sort of ghost member of my committee. He persuaded us to go with targeted sequence capture, paid for the reagents, and was my lifeline in the analysis of the genetics data, and I can't thank him enough for volunteering to jump on board the rock wren project.

Most of the people who helped me through this project did so just by being there when I needed them. To talk to, bounce ideas off of, philosophize with, rant at, or just to be shoulders to lean on, I think I've had interesting, existential, sanity-rescuing conversations with most of the people in the department. In particular, I'd like to thank Tom McCabe, Stephanie Pitt, Karina Sanchez, and Cara Smith, all of whom probably know as much about my project as my committee does. My discussions with them were

just as therapeutic as they were intellectual, and I’m grateful for their patience and interest. I’d also like to thank the other members of the Benedict lab, my committee, my longtime (and long suffering) partner, Jordan Rose, and my bestest birdy friends Ilse, Baudelaire, and the dozen other birds that keep me company.

Finally, I’d like to thank my research advisor, Dr. Lauryn Benedict. She was the best mentor I could have had. She patiently helped me navigate the perils of graduate school, professional societies, acquiring funding, and my dissertation project. Dr. Benedict was always there to (very tactfully) poke, prod, and encourage me to go on. I’m a better student, writer, scientist, and human being as a result of her time spent mentoring me.

Thank you all so, so much,

Nadje

TABLE OF CONTENTS

CHAPTER

LIST OF TABLES

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER V

LIST OF FIGURES

CHAPTER I

CHAPTER III

CHAPTER IV

CHAPTER I

INTRODUCTION TO ANIMAL SIGNALING, SEXUAL SELECTION, BIRD SONG, AND BIRD MIGRATION

Introduction

Bird song has long been used to study how various selection pressures affect signal evolution, with particular emphasis on the role of sexual selection in generating signal complexity. Complexity in bird song, while widely studied (Catchpole and Slater 2008), is poorly-defined, but arguably the two best-accepted indicators of more complex songs are having larger syllable or song repertoires (Searcy and Nowicki 2005). However, “complexity” in bird song has a myriad of associations. Examples of variables associated with increased song complexity include higher habitat complexity (i.e. more vegetation structure, Hill et al. 2017), female fertility (Zhang et al. 2015), early male developmental experience (Schmidt et al. 2014), male breeding experience (Motes-Rodrigo et al. 2016), female investment in egg components (Krištofík et al. 2014), and ecological generalism (Gomes et al. 2017).

Many studies of bird song complexity literature explore the effects complexity has on features related directly to reproduction (Soma and Garamszegi 2011), such as age and experience (Nemeth et al. 2012) and aggression levels (Poot et al. 2012). However, a small corner of the literature has slowly been exploring the possible effects migratory behavior and high latitudes may have on complexity (Read and Weary 1992, Weir and

Wheatcroft 2011). The effect of migratory status on song complexity in birds has been the subject of some debate in the literature and is currently unresolved. Studies have alternatively found that migratory status is correlated with increased song complexity (Mountjoy and Leger 2001, Kroodsma et al. 2001, Collins et al. 2009), decreased song complexity (Ewert and Kroodsma 1994, Tietze et al. 2015), or not correlated with song complexity at all (Kroodsma and Verner 1987, Xing et al. 2017, Medina and Francis 2012), possibly reflecting taxon-specific patterns. Additionally, song complexity has been found to both increase with latitude (Cardoso et al. 2012, Irwin 2000, Read and Weary 1992, Weir and Wheatcroft 2011), and decrease with latitude (de Oliveira Gordinho et al. 2015, Ödeen and Björklund 2003, Pieplow and Francis 2011). Why song complexity changes with latitude is a fascinating question that requires closer examination. Most of these studies cite differing sexual selection pressures in migrants versus non-migrants and high-latitude vs low-latitude breeders, and make general predictions about what patterns should result. They posit there is some relationship between migration, latitude, sexual selection, and song complexity (although see Byers 2015), but few studies have attempted to test multiple hypotheses (Mountjoy and Leger 2001, Irwin 2000, Singh and Price 2015). It is important to try and resolve this issue to better understand how signals evolve in response to ecology and sexual selection.

My project's goal was to measure the differences in song repertoire size between migratory and non-migratory populations of a single passerine species across a latitudinal transect. I used recordings, morphology, genetics, and GIS to relate repertoire size to ecologically- and sexually-selected traits. Overall, I was interested in the posited relationship between migratory status, latitude, and repertoire size. My specific objectives were to 1) thoroughly investigate the literature to assess the nature of the evidence for and against the hypothesis that song complexity is associated with latitude and migratory status (Chapter II), 2) determine the song repertoires of migratory and sedentary populations (Chapter III), 3) determine population structure and gene flow among migratory and sedentary birds (Chapter IV), and to 4) use morphological measurements as proxy measures of the strength of selection for a migratory phenotype (Chapter IV), to assess whether song repertoire size in a single species is associated with migratory status, latitude, morphology, and/or genetic population membership. These topics are broad and not often discussed together. In this chapter I will provide background information on subjects related to signals and breeding ecology since this project is focused on how these forces interact.

Animal Communication

Animals are constantly making decisions (McFarland 1977). They choose where and how much time to spend foraging, hiding, and sleeping, whether to flee at any given instance of the threat of a predator, if it’s worth it to engage in territorial disputes, which individuals to pursue and/or mate with, how many young to rear and which should be prioritized or abandoned. The cost of making bad decisions can be very high, and evolution should favor the optimization of decision-making. Nearly all decisions animals make are, to some extent, mediated by both con- and heterospecifics. Animals communicate to influence the decision-making of or to gain some inference about the state of other individuals to make their own decisions (Endler 1993, Bradbury and Vehrencamp 1998).

Animals use a multitude of signals to communicate. From the rhythmic croaking of a frog and the drumming of a woodpecker, to the colorful flash of a lizard's dewlap and the deposition of a trail of pheromones by ants, these disparate traits are all linked by their similar functions as signals. The diversity and particulars of their modes of communication are the product of many selective pressures. Communication occurs when one individual (the sender or signaler) generates a cue or signal that is perceived by and influences the behavior of another individual (the receiver) (Endler 1993). This is an idea similar (but not identical) to modern conceptions of information transfer (Shannon and Weaver 1949, Owren et al. 2010). Information transfer occurs when data originating in one place (e.g. the photo you took of the peacock at the zoo) are faithfully recreated in another place (e.g. my computer screen). Barring digital applications however, this is not how 'information' goes from one individual to another in the real world. What happens is more akin to you describing the peacock while I form a mental picture of what it might have looked like. This would, by no definition, be a 'faithful' reproduction of the bird's features, and could result in me incorrectly identifying a difference species as a peacock. This distinction is not a semantic one. Given our reliance on modern computing it is easy to conflate the attempt to portray an idea with 'information transfer,' and it is important to remember that, for animals (including humans!) communication is not the latter. Despite this, discussing 'information transfer' is a useful metaphor and shorthand for what transpires during animal communication. It is important to remember that the transfer is imperfect and subject to outside forces and individual interpretation. So, when a frog croaks, the sound does not carry some information or meaning inherent to it. Rather, any sense of meaning comes from the interpretation of the signal by a receiver's nervous system (Ruxton and Schaefer 2011). This could be a rival frog, who interprets the croak as signaling his neighbor’s claim to a spot on the pond, it could be a female frog who finds the croak appealing or another female who finds it unappealing, and it could even be a predatory bat who interprets the croak to mean a meal is nearby.

Thus, it is apparent that signals are highly constrained by a myriad of problems, the least of which is the inability of the signaler to transfer information directly to some intended target (Endler 1993). Animals have evolved to produce signals detectable by the sense organs of other animals – visual, olfactory, auditory, etc. Many animals possess multiple sensory organs, so the particular modality of the signal is very important and depends on several factors. Is the signaler in water or on land? Are potential recipients nearby or far away and how often does the signaler encounter them? What are the possible obstructions to the signal in the environment? How important is it to avoid detection by eavesdroppers? Each potential signaling modality has both pros and cons. For example, a visual signal (e.g. a hand wave, a courtship dance, the color of a wattle) is transmitted essentially instantly, but it requires ambient light and line of sight to be perceived. Depending on the type of visual signal, it may or may not be able to be ‘turned off’ – a bright orange cock-of-the-rock cannot disguise his bright plumage but, a lizard can stop doing push-ups. Auditory signals, on the other hand, do not require light or line of sight to be detected, and they only persist for as long as they are actively produced. This means the signal’s effective radius is larger, both for intended and unintended recipients. Auditory signals may have to compete with other sources of noise, resulting in distortion, or travel through obstructions, resulting in degradation or attenuation, and by their very nature serve to help you locate the signaler. Chemical signals are emitted from the signaler, hanging about the emitter or being deposited directly onto a surface. A chemical signal can persist in the environment, allowing the animal to communicate without physically being present, and the variety of chemicals allows for highly specific signals. However, it may take some time for the signal to be received, and chemical signals may be difficult to track (both a pro and con depending on the recipient) (Endler 1993).

Signals are generated by the signaler for the purpose of communication. This is in contrast to cues, which are not deliberately generated as signals but are rather inherent to the animal's physiology or are byproducts of some other activity. For example, a pig digging up roots in the forest makes noise as it walks about and moves leaves and dirt. The sound being made can be heard by others and may be used to locate or avoid the pig, thereby influencing the behavior of another individual as if it were a signal. The pig, however, is not kicking up leaves specifically to attract attention but rather to forage, and the rustling is an unavoidable byproduct of this behavior, making it a cue. Cues benefit the receivers only, whereas signals benefit both signalers and receivers (Bradbury and Vehrencamp 1998).

Signals must be produced such that the signaler optimizes the energy spent on the signal versus its ability to transmit through a medium and be detected by intended recipients. Signals can be costly to produce, either in the production of the signal itself, the time spent signaling that could be used to do other things, or the risk of a predatory eavesdropper detecting it (Zahavi 1975). However, a signal needs to travel far or persist long enough and with enough intensity as to be detectable for communication to be effective. There are many sources of interference in a receiver's environment. Sound is attenuated and degraded by objects in the environment and masked by other sources of noise, visual displays require the receiver’s direct attention, electrical and tactile signals require very close contact, and chemicals can fade over time or be masked with other chemicals. Thus, selection against energy expenditure conflicts with selection for signal detectability. The cost of a signal versus the potential benefit of signaling will influence the frequency and intensity with which it is given.

Signals and Sexual Selection

Many animal signals do not appear to be optimal, despite strong selection for efficient generation, propagation, and reception of signals. Gazelles will frequently jump very high in the presence of predators, termed stotting or pronking (FitzGibbon and Fanshawe 1988), male stalk-eyed flies have massive eye spans placing their eyes in a precarious position (Wilkinson and Reillo 1994), and túngara frogs call for mates with a chuck call that is especially audible to their main predator, bats (Ryan 1985). Perhaps *the* classic example of an incredibly exaggerated and seemingly paradoxical signal is the peacock’s train, which consists of ~200 elongated back covert feathers, each ending in a round eyespot. Peacocks slowly molt their trains from September to February, although adult males are never completely without some sort of train on their backs. The train can be as long as 1.5m, up to 60% of the body length of the peacock (Ragupathy and James 1998). Males display by facing another individual and shaking out and erecting the train, using their tails to stridulate the feathers, causing the train to vibrate (Dakin et al. 2016). Clearly the train is used to communicate, but it would seem to come at a huge cost to personal survival. Males spend around six months growing their elaborate train, estimated to cost them ~10% of their basal metabolic rate (Lasiewski and Dawson 1967) and ~3% of total metabolic rate (Nagy et al. 1999) each day. It would appear that such a large trailing appendage would make them more conspicuous to predators and make flight more difficult. Peahens display their back coverts in much the same manner as males, vibrating the feathers, yet they are drab brown and do not bear the exaggerated trains of the males (Dakin et al. 2016). Charles Darwin, in a letter to Asa Gray responding to Gray's review of *On the Origin of Species* (Darwin 1859), wrote "The sight of a feather in a peacock's tail, whenever I gaze at it, makes me sick!" (Darwin Correspondence Project 2018), acknowledging that his newly published theory of natural selection alone could not adequately explain this trait.

It was, in part, this problematic bird that led to the publication of *The Descent of Man, and Selection in Relation to Sex* (Darwin 1871). From his theory of natural selection, in which the best adapted individuals leave the most offspring, Darwin postulated a subsidiary force: sexual selection. He wrote that "… the advantages which favoured males derive from conquering other males in battle or courtship, and thus leaving a numerous progeny, are in the long run greater than those derived from rather more perfect adaptation to their conditions of life." (Darwin 1871, p. 227). If females prefer trains during courtship, then no matter how much better-adapted the train-less males are for surviving in their environment, they will leave fewer offspring. Thus, the reproductive benefits imposed by female choice will start to overcome the survival benefits imposed by natural selection. 'Fitness' was coined by Herbert Spencer (1864 p. 444) as a way to describe this intersection of the forces of natural and sexual selection. In this view, the "favoured race," as Darwin put it, is not the one that can survive the longest in an absolute sense but is the one that leaves the most descendants. Today, fitness is usually thought of as a probability or propensity of a group rather than an absolute property of an individual (Maynard-Smith 1989). Thus, selection will, in general, favor higher fitness over superior survival.

Darwin's theory of sexual selection went a long way towards explaining the existence of seemingly paradoxical signals and traits, but there were many details which remained unclear and not satisfactorily explained. The most glaring problem lay in the existence of the preference itself. Darwin suggested that it is obvious that females should prefer ornamentation to plainness out of some inherent appreciation for beauty, which is none more apparent than in the birds (Darwin 1871, p. 359). The anthropomorphic, 'just so' nature of this rationale does not really offer an explanation for what maintains a preference for signals that render the signaler more vulnerable to predation. The concept of mate choice was not widely accepted by biologists, in no small part because of an active attempt by Alfred Russell Wallace to denounce it, until after R.A. Fisher championed the idea in his seminal work, *The Genetical Theory of Natural Selection* (Gayon 2010). Fisher proposed a new mechanism, termed 'runaway' selection, to explain features like the peacock's train. The preference for a trait, and the trait itself, are linked, such that successive generations will both have and prefer the trait more and more; this process only stops when natural selection imposes a wall against which the trait can no longer be exaggerated (Fisher 1930). Fisher's runaway model suggests a mechanism, namely that random mutations in sexual organisms provide the variation in physical traits, but, like Darwin, maintains that the 'aesthetic faculty' in females may confer nothing more than the arbitrary advantage of being more attractive to their sons (Fisher 1930, p. 145).

Today, the notion that females prefer beautiful males ‘just because’ has been overtaken by the theory that these traits are actually functional signals. Zahavi (1975) proposed a new idea, termed the ‘handicap principle,’ that traits which seem to lower the survival of the animal are actually a sort of quality test. Those individuals that can survive with the greatest handicap must be somehow better than their peers. Crucially, the handicap must honestly convey their quality – they must not be able to bluff the signal or it will not be useful to the selecting sex. In this view, the peacock’s train evolved not because peahens love long iridescent feathers, but because the train signals something about the peacock as a potential mate: he invested all this energy into growing and displaying a ridiculous appendage and survived in spite of it. The train cannot be bluffed; when a male erects and displays his feathers he is signaling to the female that what she sees is what she gets. Since peafowl are a lekking species with precocial chicks (i.e. they hatch well-developed and able to feed themselves) the male does not provide any parental care. What she ‘gets’ is the father’s genes for her offspring. Grafen’s (1990) model of how costly signals can exist as an evolutionarily stable strategy went a long way to convincing the scientific community of the validity of the handicap principle.

Not everyone agreed with Zahavi’s (1975) key assumption that *all* signals (not just courtship displays) must be costly to *produce* to maintain their honesty. Work by Számadó (Számadó 1999, Számadó 2003, Számadó 2011), particularly with reference to threat displays, demonstrated that, at least theoretically, the potential cost of being caught at cheating is sufficient to maintain honesty at no cost to the signaler, and if signalers spend less energy to produce the same signal as another individual, they are not cheating, they are efficient. A good example might be elk sizing each other up for combat by lowering their heads and pointing their racks at each other. The outcome of the contest should be the same regardless of whether the signaling took place – the same elk will lose, possibly badly. In this case, the signal would have been more valuable to the loser since that elk would not have had to incur the cost of losing the fight. If the losing elk had been able to bluff he would stand to gain quite a lot, but the risk of his bluff being called is enough to prevent bluffing in the first place. Not to say that bluffing does not exist; but most cases of animal bluffing seem to be when the animal is very weak (e.g. molting mantis shrimp, Steger and Caldwell 1983) so the bluff is more of a defensive strategy than an offensive one.

What about courtship displays? Males are not displaying to each other, they display to females. If a male is 'cheating,' the honest male *and* the female lose out on potential fitness gains, but there is no potential cost to the cheater. Indeed, he has everything to gain since he would not otherwise get to mate. This sets up a major conflict between male signalers and female choosers: males always want to seem better than they are and females always want the (truly) best individual. It behooves the females particularly to select males who accurately convey their quality in a way that cannot be bluffed, and high quality males benefit from the extra matings they would get, making cheating an unstable strategy. This situation would seem to support Zahavi's (1975), and not Számadó's (1999), position on the necessity of handicaps to explain elaborate ornaments like the peacock's train.

Handicaps are not the end-all, however. As Grafen (1990) was defending Zahavi's (1975) hypothesis, Endler and McLellan (1988) were developing a new one. While the adaptive value of signaling in mate choice is well-accepted, it is not obvious why those signals should be so ostentatious. If the peacock’s train is so long because he needs to demonstrate how much energy he put into it and how skilled he is at vibrating it, why does it need to be iridescent and brightly colored? Could he not accomplish the same signaling feat with a drab gray or brown train, like a peacock pheasant does? Is his bright, shimmery train a signal to predators, or to the females he is displaying to? Endler and McLellan (1988) and Endler (1992) proposed it is the latter, with the bright, shiny, shimmery colors tuned to the sensory systems of the females the male is trying to attract. He dazzles her and holds her attention by presenting her nervous system with a set of colors and patterns and movements it is particularly biased at perceiving, and making diverting attention difficult. The evolution of displays along these lines is termed ‘sensory drive’ (Endler and McLellan 1988). Sensory drive is, perhaps ironically, not that different from Darwin’s (1871) original suggestion that ‘aesthetic appeal’ is what leads females to choose ornamented males, and on the surface the net effect is just that. Unlike Darwin, the sensory drive hypothesis makes testable predictions about the mechanisms generating and maintaining signal ornamentation. Examples of sensory drive mediating mate choice and ornamentation include Lake Malawi cichlids (Seehausen et al. 2008), *Anolis* lizards (Ng et al. 2012), and great bowerbirds (Kelley and Endler 2012).

It is becoming clearer that no one mechanism can be held entirely responsible for the incredible diversity of animal signals (Bradbury and Vehrencamp 1998). Runaway selection, while difficult to definitively document, is theoretically possible (Bailey and Moore 2012). Conspicuous sexual signals may have evolved to be attuned to the receiver’s sensory systems, but predators are under selection to detect these conspicuous signals, so it is difficult to discount a handicapping effect (Zahavi 1975, Számadó 1999).

Many species do not acquire their conspicuous signals until they are sexually mature, highlighting the apparent disadvantage, in terms of pure survivorship, these signals can confer.

Bird Song as a Signal

While the peacock's train is perhaps the most famous of elaborate signals, it is not the best-studied. That honor almost certainly goes to the song of songbirds (Searcy and Nowicki 2005). The extremely elaborate and varied acoustic displays of some birds have been described as the 'acoustic equivalent of the peacock's tail' (Catchpole 1996). Song is used in communicating species identity and individual identity and is regarded today as a trait that functions mainly for attracting mates and repelling conspecific invaders. As such, the major hypotheses attempting to explain why species may have complex songs and/or large repertoires generally invoke sexual selection (Bradbury and Vehrencamp 1998, Searcy and Nowicki 2005, Catchpole and Slater 2008). An individual's song repertoire is the essential unit I studied for my project, below I define exactly how I used songs and song repertoires.

In general, birds are considered to produce two classes of vocalizations, songs and calls (Catchpole and Slater 2008). Calls are relatively short and simple, while songs are longer and more elaborate (Catchpole and Slater 2008). Songs are made up of notes and syllables (Catchpole and Slater 2008). A note is a continuous trace on a spectrogram, and a syllable is a grouping of notes. A song type is a stereotyped grouping of notes and syllables, such that each time it is produced it can be recognized as the same song type (Borror 1961). For example, a typical song sparrow (*Melospiza melodia*) sings one song made up of many notes arranged into syllables (Fig. 1.1). Some notes commonly appear grouped together, either as a trill like notes c and i, or not trilled, like notes d and e. This particular arrangement of notes is a song type. Other song types from the same bird may or may not include these notes or syllables. (Searcy and Nowicki 2005).

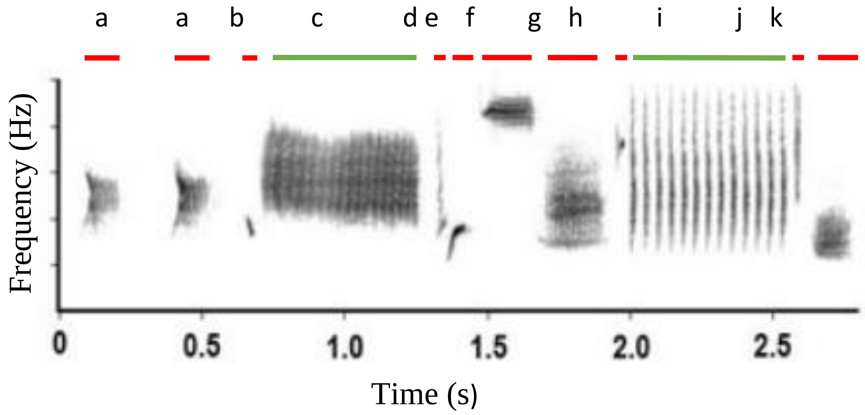

Figure 1.1. Typical song of a song sparrow (*Melospiza melodia*). Each letter represents a note type. Two syllables, c and i, are made up of repeated notes. Spectrogram from Wilson and Vehrencamp (2001).

A song repertoire is all of the unique song types an individual can produce. Song repertoires are commonly distinguished in the literature from syllable repertoires (all of the unique syllable types an individual can produce), and most authors choose to measure one or the other, but not typically both (Catchpole and Slater 2008). Many species of birds sing songs that seem to be virtually infinitely variable but are constructed from a limited repertoire of syllable types. Yet others sing songs according to a basic pattern, but with no two songs ever exactly alike (Catchpole and Slater 2008). It is unclear just how song and syllable repertoires relate to each other in terms of apparent complexity (and this division may or may not be biologically relevant) but is a way for us as observers to categorize song complexity. My focal species (rock wren) sings discrete song types and thus has a song repertoire, so song repertoires will be the focus of my project.

The size of a bird's song repertoire is considered by some to represent the quality of that individual, with larger repertoires indicating birds with higher fitness (Catchpole 1982, Peters et al. 2000). Since song is controlled by special centers in the brain, repertoire size may be the downstream result of an individual's brain development in infancy (Buchanan et al. 2004) as well as an indicator of current health (age/experience – Howard 1974, parasite resistance – Spencer et al. 2005). Song repertoire size has been shown to be positively correlated with brain nucleus HVC (formerly an abbreviation for Higher Vocal Center, now used as a proper noun) and the Robust nucleus of the Acropallium (RA) volume (two nuclei involved in song learning and song production) (Pfaff et al. 2007) (Fig. 1.2). Nutritionally stressed birds have smaller HVCs than non-stressed birds (Schmidt et al. 2013). Thus, there appears to be a cost associated with investing in song control nuclei. Territory size and nest provisioning rate are both positively correlated with repertoire size in male sedge warblers (Buchanan and Catchpole 1997), so females may be directly benefiting by pairing with such males. Additionally, isolation experiments on sedge warblers have shown that HVC size and song structure are under genetic control (Leitner et al. 2002). Hasselquist et al. (1996) found that female *Acrocephalus* warblers preferentially seek out extra-pair copulations with males that have larger repertoires, so females may use repertoire size to assess some indirect benefit they could gain for their offspring.

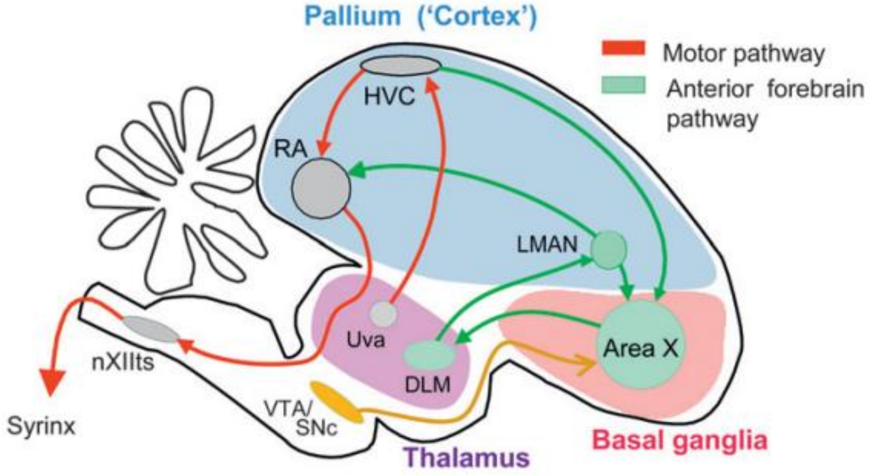

Figure 1.2. Regions and nuclei of a songbird's brain important in song learning and production. All regions are present in both hemispheres. Nucleus HVC (abbreviation used as a proper noun) directs both the learning and production pathways. Lesioning the HVC renders the bird mute. Nucleus Uva (uvaeformis) projects bilaterally across the hemispheres and has a role in interhemispheric coordination. HVC projects directly to RA (robust nucleus of the arcopallium) and indirectly via Area X (similar to mammal basal ganglia), DLM (dorsolateral anterior thalamic nucleus in the thalamus) and LMAN (lateral magnocellular nucleus of the nidopallium). RA projects to nXIIts (tracheosyringeal half of the hypoglossal nucleus), which projects to muscles in the syrinx controlling vocalizations. From Nottebohm (2005).

As such, song repertoire size and sexual selection are (theoretically) inextricably linked. One review of over 40 field and lab studies looking for evidence of female choice for males with larger repertoires found mixed results: lab studies almost always find evidence of female preference for larger repertoires while field studies almost never find such evidence (Byers and Kroodsma 2009). However, Soma and Garamszegi (2011) note several problems with Byers and Kroodsma (2009), including the omission of many relevant field studies and their method of simple paper-counting to determine whether there is an effect. Soma and Garamszegi (2011) take a model-based meta-analytic approach and conclude that while there is a positive association with song complexity and reproductive success, the effect is weak and is likely species-specific and modulated by many factors. There are many ways to interpret this sort of finding (too many variables in field studies, poor choice of proxies for female preference, preference is an artefact of lab studies, there are taxon-specific preferences, song is more important in male-male interactions, etc.), but most importantly it illustrates that we still do not completely understand what song repertoires or complex songs are useful for, or how they get large and elaborate. However, the prevailing idea is that while song features can be selected for as non-sexual signals (like species identity or audibility in a particular habitat), songs as a whole (and thus song repertoires) are frequently under sexual selection as they primarily function in conspecific communication (Catchpole and Slater 2008). This supposition is supported by the facts that songs are known to be produced more 1) in the presence of rivals, 2) in the presence of potential mates, 3) at the nest, 4) while feeding chicks, 5) before mating, and 6) during other breeding season-associated behaviors (Searcy and Nowicki 2005, Catchpole and Slater 2008).

Alternatively, it is possible that repertoire size is the result of cultural evolution via drift and is not necessarily under direct selection. Most oscine passerines learn their songs as chicks from adult tutors, resulting in the formation of local dialects that may span only a few dozens to a few hundred kilometers before reaching another dialect. Without direct selection for or against certain song types or repertoire sizes, local song characteristics can fluctuate in time. A species with populations that lack large geographic divisions may have dialects that blend into one another, while geographic barriers can produce distinct ‘song lineages’ as characteristics drift over time (Searcy and Nowicki 2005).

Migration

The seasonal disappearance and reappearance of migratory birds has fascinated people for millennia. Perhaps the earliest written account of bird migration was by Aristotle, who said in his *History of Animals, Vol. VIII*, “Of birds, the following are migratory – the crane, the swan, the pelican, and the lesser goose.” The rest he thought “go into hiding” to escape the winter, such as the swallow, which hides in holes “…quite denuded of its feathers…” For most of human history we have not been nearly as mobile as birds, so what happens to birds when they disappear for part of the year remained a mystery until relatively recently. People in Europe had known that large birds, like falcons and herons, could make long journeys for centuries. Indeed, the pigeon has been kept for millennia, at least as far back as the ancient Romans, for the purpose of long-distance communication. An early record of a metal band identifying an individual bird was from 1595 – a peregrine falcon belonging to Henry IV took off from England and showed up the next day in Malta, Spain, 1350 miles away (Wood 1945). Thomas Bewick’s *A History of British Birds* (1797) supported the idea that birds, in general, migrate using accounts of people seeing the birds in faraway lands and debunked the long-standing myth that swallows hibernate in wetlands. In 1803, John James Audubon made the first attempt to deliberately study migratory birds by tying strings around the legs of eastern phoebe chicks and recovering two of them in his neighborhood the following year (Craves 2010).

Modern study of bird migration has begun to reveal how even some of the smallest birds make incredible, continent-crossing journeys twice a year. The advent of the banding or ringing station has been the main source of information on the destinations and longevity of migratory birds. Paul Bartsch pioneered the use of numbered bands when he banded black-crowned night herons from 1902-03 in Washington, DC (Tautin 2005). Bartsch's work inspired many other groups to start banding birds with serial numbers and instructions (e.g. "return to …"), and led to the founding of the American Bird Banding Association which oversaw banding activities until the federal government took over in 1920 (Tautin 2005). There, the modern banding system was developed by Frederick Lincoln and purpose-built banding stations began to pop up all over the country (Wood 1945).

Banding recoveries rapidly revealed the elusive wintering locations of many small temperate-breeding migratory birds. A press release published in 1944 documented the discovery of the wintering grounds of chimney swifts in Peru, the last North American bird for whom no wintering grounds had been known (Lincoln 1944). By this time, it was well-acknowledged that even tiny birds fly hundreds to thousands of miles each year. However, the reality of these massive flights in small birds raised many more questions: how is it possible to fuel such a long journey? How do they know where to go? What causes this behavior in the first place? Answering these questions has been the focus of modern migration studies.

While we commonly describe the mass migrations of birds as if it were a singular behavior where species are either migrants or non-migrants, this is not really accurate (Zink 2002). Migratory behavior is the cumulative effect of several adaptations, each of which seems necessary for successful migrations. First and foremost, migratory birds need to *want* to migrate. This desire to migrate, termed migratory restlessness, or *zugunruhe*, has been observed in caged individuals when it is the appropriate time of year to migrate (Kramer 1949). During *zugunruhe*, caged birds show a sudden and pronounced desire to get out of their cage that coincides with the time they would have spent migrating. The 'appropriate' time is usually determined by photoperiod or ambient temperature (Farner 1950). This is also associated with the onset of hyperphagy and the deposition of fat reserves in many birds (Wolfson 1945). Once they begin their migration, they must know how to get to their destination. Methods for navigating include using the path of the sun (Kramer 1957, Alerstam et al. 2001), stars and constellations (Emlen 1967, Wiltschko et al. 1987), polarized light (Moore 1986, Horváth et al. 2009), landmarks like mountain ranges and rivers (Bingman et al. 1982, Williams et al. 2001), and the earth's magnetic field (Keeton 1971, Walcott et al. 1979, Beason and Nichols 1984, Wu and Dickman 2012), and many birds use multiple compasses (Mehlhorn and Rehkämper 2009). Many species alter the sizes of their organs, with species that stop frequently investing more in their digestive tracts, facilitating rapid acquisition of new fat reserves (Lindström et al. 1999, Guglielmo and Williams 2003), and species with few or no stopovers exhibiting atrophy of digestive organs and hypertrophy of muscle mass (Jehl 1997, Piersma and Gill 1998). It is this combination of restlessness, hyperphagy, internal map, navigational compass and metabolic changes, that results in the migratory phenotype.

This description of migratory behavior is typical for obligate migrants, birds for whom migration seems 'programmed' and is highly consistent and predictable in its timing and path (e.g. Wilson's warblers (Clegg et al. 2003), Swainson's thrushes (Ruegg and Smith 2002), and black swifts (Beason et al. 2012)). However, migration is not all-or-nothing. There are many species where some populations always migrate but others never do, such as the yellow-rumped warbler (*Setophaga coronata*) (Hunt and Flaspohler 1998). In other species individuals do not always migrate or migrate to the same place and are better described as facultative migrants. For example, American robins (*Turdus migratorius*) breed throughout North America and only retreat from Canada and Alaska in the winter and leave central Mexico to breed in the summer, with robins present in most of the United States year-round. Some robins overwinter at their breeding grounds, while others travel up to 1200km to reach wintering grounds. This behavior is not fixed – an individual who overwinters in place one year may migrate the next year (Vanderhoff et al. 2016). The most extreme manifestation of facultative behavior is irruption, where poor local conditions force masses of birds to move to find food (Koenig and Knops 2001). Commonly irrupting species in the United States include the pine siskin (*Spinus pinus*), common redpoll (*Carduelis flammea*), and short-eared owl (*Asio flammeus*) (Newton 2012), and whether and which species will irrupt in a given year can usually be predicted based on projections of winter weather and food conditions (Koenig and Knops 2001). Another term for species where not every individual migrates is 'partial' migration, which was originally defined in two ways: 1) migration is facultative in all individuals (like the robin) or 2) migration is obligate in some individuals but not others (like the yellow-rumped warbler) (Berthold 2001), although the term has since been most commonly used to refer only to the first definition (Pulido 2011).

Migratory behavior is thought to be endogenously controlled and highly heritable (Berthold 1996). In a seminal experiment on blackcaps (*Sylvia atricapilla*), Berthold and Querner (1982) crossed migratory with sedentary blackcaps and were able to selectively breed for entirely migratory or entirely sedentary behavior in successive generations. More recently Pulido and Berthold (2010) were able to generate entirely sedentary individuals from completely migratory ancestors, indicating that no cross-breeding is necessary and that migration and residency is highly evolutionarily labile. These results suggest that there is not one gene or allele that governs migratory behavior but rather a suite of genes, the particular combination of which determines whether the threshold for migratoriness is exceeded (Pulido 2011). Thus, facultative or partial migrants are those individuals who sit in an intermediate sweet spot between 'always migrate' and 'never migrate.'

It is a mischaracterization of migrant birds to suggest the adaptations that enable them to embark on their journeys must have evolved in birds for that purpose (Zink 2002). Zugunruhe has been reported in at least one sedentary species (*Saxicola torquata*, Helm and Gwinner 2006) and hyperphagy is common in sedentary birds preparing for the winter (Farner 1950). Photoperiod as a cue regulating annual rhythms probably evolved very early on and is vitally important to virtually all life (Hut and Beersma 2011). Internal compasses are not the sole purview of the birds: alligators orient using solar, stellar, and lunar cues (Murphy 1981), sea turtles use solar, stellar, and magnetic cues (Lohmann et al. 2004), monarch butterflies use solar and magnetic cues (Guerra et al. 2014), and newts can use the polarization of the earth's magnetic field (Wiltschko and Wiltschko 1995) to orient and navigate. Migration does not require massive physiological changes for all birds; for example, species that can forage on the wing (swallows, swifts, nightjars) or species that don't exert much effort in flying (hawks, vultures, falcons) do not need to reorganize their organs or spend huge amounts of extra energy to get to their destination (Newton 2010)

That being said, birds hold the record for the longest migrations, both in absolute distance and number of body lengths travelled (Alerstam et al. 2003), and long-distance migration in birds is one of the most extreme physiological challenges undertaken by any animals (Weber 2008). Migration poses a huge risk, not only because it is energetically taxing, but because the mass movement of birds attracts many predators (Ydenberg et al. 2004). Annual mortality in migrating birds has been measured to be up to 15x higher than during breeding or overwintering, with 85% of all mortality occurring on migration (Sillett and Holmes 2002). Any given individual is not likely to return the next year (particularly the case for small passerines), showcasing the strong pressure to maximize reproduction in the first breeding season since they are not likely to get another chance (Sillett and Holmes 2002, Alerstaam 2011, Klaassen et al. 2014).

Study Species - Rock Wrens

The rock wren (*Salpinctes obsoletus*) is a small, enigmatic passerine native to western North America, ranging from British Columbia and Saskatchewan to Nicaragua and Costa Rica (Fig 1.3). (Lowther et al. 2000). Males and females look almost exactly alike, and although females are slightly smaller than males, there is wide overlap in their morphological measurements (Fig. 1) (Pyle 1997). Males are only reliably distinguished from females in the field by their song (females are not known to sing). These birds are aptly named and are generally found on rocky, talus slopes and cliffs, although they are not obligate rock-dwellers and may be found anywhere where crevices and cavities exist (such as a sand bank) (Lowther et al. 2000). Rock wrens are fairly common in suitable habitat.

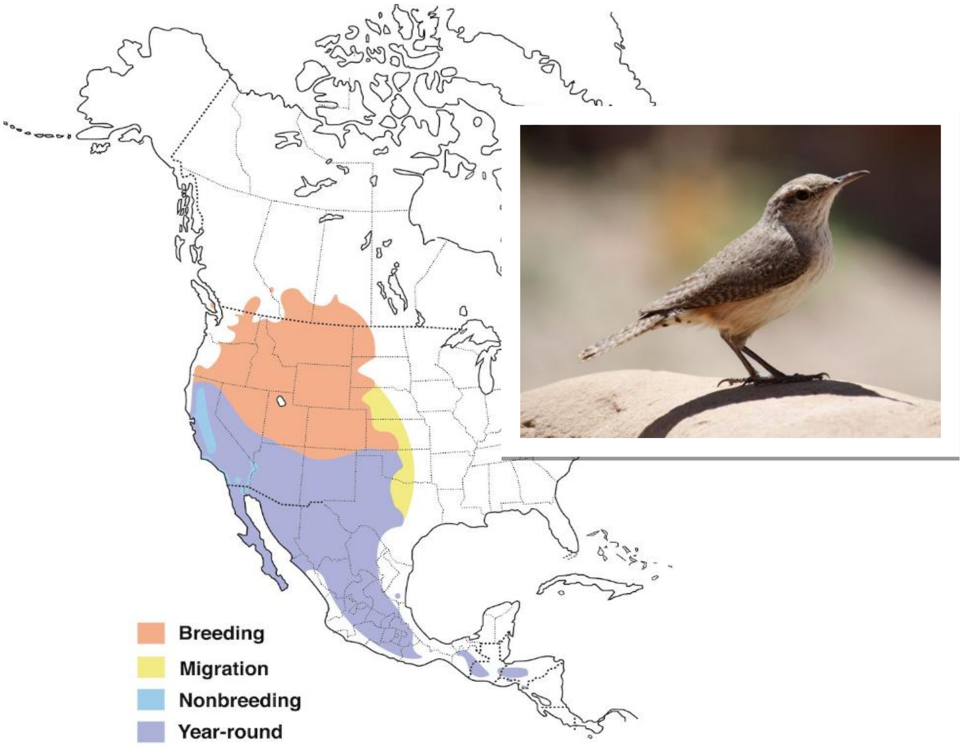

Figure 1.3. Rock wren distribution map. Map from Lowther et al. (2000), citing Christmas Bird Count data from 1992 and focusing on rock wren presence in California (National Audubon Society 2010). Adult rock wren photographed by me at Fort Davis National Historic Site, Jefferson County, Texas.

Despite this, rock wrens are one of the least-studied North American birds and are generally poorly known (Lowther et al. 2000). The best-studied aspect of rock wren life history is their nesting habits. Rock wrens build cup nests in rock cavities and pave the opening and front entrance with small flat stones. The function of this stone ‘patio’ has been the subject of several studies (Bailey 1904, Ray 1904, Peabody 1907, Merola 1995, Oppenheimer 1995, Warning and Benedict 2014, Warning and Benedict 2016). Otherwise there is very little known about this species’ biology, and much of our understanding is anecdotal. Before 2017 only one study explicitly examined rock wren song features (Kroodsma 1975, Benedict and Warning 2017), leaving open what could potentially be an extremely fruitful avenue of research of behavioral ecology and animal communication.

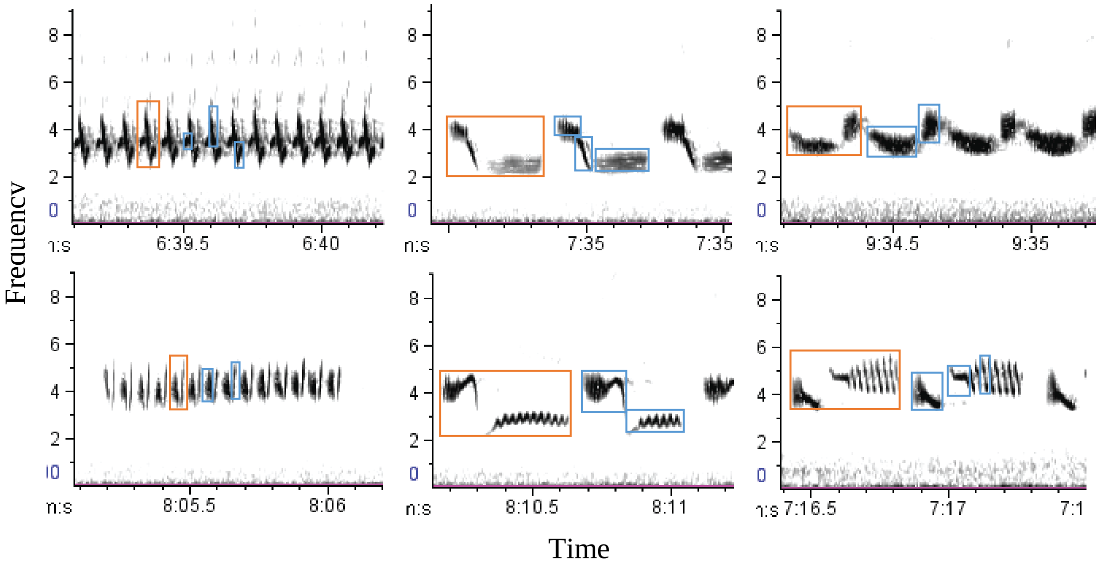

Figure 1.4. Six example spectrograms (visualization of sound) of typical song recorded from one rock wren at Devils Tower National Monument. The x-axis is time (minutes:seconds), the y-axis is frequency (kHz). The amplitude is visually represented by how dark the trace is – the darker the trace, the louder the sound. Rock wrens sing songs consisting of one syllable repeated several times. Each of the six spectrograms is one song, syllables are indicated by red boxes, and the notes making up each syllable is indicated by blue boxes.

Male rock wrens can be commonly found singing from exposed perches on the tops of cliffs, rocks, and trees. Most singing is done just before dawn and in the dawn twilight, although song bouts are given sporadically throughout the day (pers. obs.). Rock wren song is highly stereotyped, and individuals perform faithful renditions of the same song types. Individual song is generally constructed of a single syllable repeated 2-6 times, or a short trill, making each song type fairly simple to identify (Fig. 1.4), and resulting in the added bonus that rock wren song and syllable repertoires are almost equivalent. Individual males can have repertoires from 50 to 120 different song types (Benedict and Warning 2017). Rock wrens sing discrete song types with 'intermediate variety' a rare (and possibly unique) pattern of singing where a few song types are repeated several times before a new set of song types are switched to, as opposed to singing with 'immediate variety' where song types are switched after one repetition, or eventual variety, where one song type is repeated several times before switching to a second song type (Kroodsma 1975). This means rock wrens do not present their repertoire in a predictable way, therefore, making statistical inference of their repertoire size from a small song sample difficult.

Rock wrens are insectivores, using their long bills to probe for arthropods in cracks and crevices. They are not limited to foraging in rocks – rock wrens will climb up tree trunks, walk through short grass and into burrows, and flycatch to forage for insects (Lowther et al. 2000). Rock wrens mostly eat terrestrial arthropods like grasshoppers, crickets, spiders, ants, and beetles (Lowther et al. 2000). The annual cycle of the rock wren is similar to many North American passerines. The breeding season begins in April and continues until August, with high latitude birds starting and ending their breeding season later than low latitude birds (Lowther et al. 2000). Males advertise for mates and compete with neighboring males. Once paired, males and females choose a nest site together, almost always a cavity in a rock, and build a cup nest. Females may carry small stones to the cavity entrance, piling them up into a 'patio' or 'pavement' occluding the entrance and cascading down the hillside (Warning and Benedict 2014). Rocks are sometimes included in the nest cup as well (pers. obs.). Females brood the eggs (Oppenheimer 1995), and both males and females assist in feeding chicks until they fledge, after which the female will often build a new nest elsewhere and lay a new clutch of eggs, while the male continues to feed the fledglings (Merola 1995). Pairs will often produce two, sometimes three, clutches in a given breeding season (Merola 1995). Migrants depart in September and October for their wintering grounds, where they remain until March (Lowther et al. 2000).

Rock wrens are highly territorial during the breeding season, defending areas approximately 200m across (mean territory area 0.53±0.21 ha - 50% fixed kernel, 4.1±1.2 ha - 95% fixed kernel, Warning and Benedict 2015). Both males (Merola 1995) and females (pers. obs.) will vigorously defend territories from intrusion by members of the same sex (i.e. females will not chase away males but will chase and fight other females). Rock wrens in western Kansas do not appear to do defend territories during the winter and instead range more widely (Lowther et al. 2000). As insectivores, their wintering range and roaming behavior is likely driven by the distribution and relatively low abundance of insects during the winter. It should be noted that almost nothing is known of rock wrens in the winter, and Kansas is on the edge of their range and has very low numbers of rock wrens.

Unlike most wrens, which do not migrate, northern populations of rock wrens are migratory (Fig. 1, Lowther et al. 2000). Rock wrens do not migrate south of central Utah and southern Colorado. Whether rock wrens south of this divide are also migratory is unknown – it may be that these populations are sedentary and northern birds ‘leapfrog’ over them and reside further south, or it may be that these populations migrate south and wintering birds are actually northern migrants.

As a monomorphic partial migrant with large, discrete song repertoires, rock wrens are an excellent model with which to study the possible effects of latitude and migration on song complexity. The literature on this subject is populated by fairly diverse taxa and methodologies, despite being relatively sparse. Few studies examine the effects of both latitude and migration in birds, and no study has assessed this in a single species (Chapter II). Using this species as a launching point, I will start to address the question of whether and how latitude and migration influence the evolution of complex songs.

CHAPTER II

THE RELATIONSHIP BETWEEN LATITUDE, MIGRATION, AND THE EVOLUTION OF BIRD SONG COMPLEXITY

This chapter has been previously published or accepted for publication in *Ibis*.

Contribution of Authors and Co-Authors

Manuscript in Chapter II

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Contributions: Conceived the study topic, searched the literature, wrote first draft of the manuscript.

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Contributions: Helped conceive the study topic, helped search the literature, edited drafts of the manuscript.

Abstract

For the last several decades it has been proposed that birds show latitudinal variation in song complexity. How universal this variation may be and what factors generate it, however, is still largely unknown. Furthermore, while migration is confounded with latitude, migratory behavior alone may also be associated with variation in song complexity. In this paper we review the literature to assess current ideas on how latitude and migratory behavior may drive large-scale geographic patterns of song complexity. At least seven distinct hypotheses have been proposed in 29 studies of the topic. Four of these hypotheses posit that sexual selection pressures co-vary with latitude and/or migration, resulting in concordant changes in song. Other hypotheses suggest that mechanisms other than sexual selection, such as large-scale changes in environmental sound transmission properties, may be at play. Sixteen studies found support for increased song complexity with increased latitude and/or migration, while 13 did not. Relatively few studies exist on this topic, and methodological differences between them and variable definitions of "complexity" make it difficult to determine whether results are comparable and concordant. At a minimum, it is possible to conclude there is no strong evidence that song complexity increases with latitude and/or migration in all birds. Future work should focus on examining multiple hypotheses at once to further advance our understanding of how latitude, migration, and song complexity may or may not be related.

Introduction

Bird song has served as an excellent model for understanding how acoustic signal complexity evolves. There is copious research on the subject of what promotes and constrains song complexity in birds (Andersson 1994, Irwin 2000, Nowicki and Searcy 2004, Catchpole and Slater 2008, Freeberg et al. 2012). Across these studies and others, song "complexity" is a variably defined concept which typically incorporates combinations of measurements of song repertoire size, acoustic frequency, timing, and structure (Nowicki and Searcy 2004).

Several authors have suggested that large-scale patterns of bird song variation evolve in high latitude temperate regions because the conditions there are conducive to increases in song complexity. The idea that high latitude breeding may relate to song complexity was first advanced when Catchpole (1982) noted that migratory *Acrocephalus* warblers have larger repertoires than sedentary congeners. He suggested that migrants have less time to pair and breed, increasing pre-breeding sexual selection pressures which promote vocal complexity. A subsequent comparative analysis by Read and Weary (1992) found the connection between song complexity and migratory behavior may be widespread in passerines and may represent common selective pressures acting on migrants. Of course, the propensity to migrate is confounded with breeding latitude and all its associated environmental variables, potentially complicating interpretation of Read and Weary's (1992) findings. Irwin (2000) found that song complexity increased with latitude in Greenish Warblers *Phylloscopus trochiloides,* a species with no sedentary populations, which suggested migration alone is not sufficient to explain an apparent propensity for signals to get more elaborate towards the poles. Since then, many studies have attempted to re-evaluate how latitude and/or migration may influence the evolution of song complexity. However, few studies explicitly test or consider more than a single hypothesis, and the field as a whole lacks cohesion.

Geographic variation in bird song has been well studied with regard to song learning, dialect formation and functions, variability in song form and frequencies, and patterns of song sharing among individuals (Podos and Warren 2007). Far fewer studies have compared song complexity among multiple latitudinally widespread conspecific populations or different species (Table 2.1). Nevertheless, this is an important topic if we wish to understand the processes that drive species-, family-, and higher-level variation. The few geographically large-scale studies of this topic illustrate some interesting patterns and propose a multitude of ideas to explain them (Table 2.2). However, aside from an oft-cited emphasis on sexual selection, those patterns and ideas have not been discussed within a single theoretical framework. These studies cover a wide range of passerine diversity and nearly every author has their own definition of “complexity”, a problem in itself that makes collective discussion and analysis difficult at best for any studies of bird song. This paper will review the available evidence to address an open question: does song complexity vary consistently across avian groups in relation to latitude and migratory behavior, and if it does, what processes drive that variation?

Table 2.1. Summary of studies examining variation in bird song complexity in relation to latitude and migratory behavior. The ‘measure of complexity’ is reported as the authors themselves report it in their respective studies. Unless otherwise noted, song measurements were made by the authors. Abbreviations used: M = migration comparison, L = latitudinal comparison, M & L = both migration and latitude considered, W = within species, B = between species, ind. = individuals, pop. = populations, subsp. = subspecies, sp. = species.

Study Design	Study System	Breeding Range	More Complexity: Migratory or Sedentary	More Complexity: Higher or Lower Latitude	Measure of Complexity	Reference
M & L: W	Common Yellowthroat *Geothlypis trichas* – 9 subsp.	North temperate	Sedentary[1]	No pattern	# notes/phrase, # elements/note	Bolus (2014)
M & L: W	Marsh Wren *Cistothorus palustris* – 18 ind.	North temperate	No pattern[1]	No pattern	song repertoire size	Kroodsma and Verner (1987)
M & L: W	Marsh Grassbird *Locustella pryeri* – 7 pop.	North temperate	No pattern	Lower[2]	# element types, element rate, song length, 3 frequency traits	Xing et al. (2017)
M & L: B	“Fringillidae”[3] – 65 pop.	Worldwide	Sedentary	Lower	song/syllable repertoire size (from Read & Weary 1992)	Handley and Nelson (2005)
M & L: B	Troglodytidae – 3 sp.	North temperate, neotropical	Migratory	Higher	song repertoire size	Kroodsma et al. (2001)[4]
M & L: B	Passeriformes – 44 sp.	North temperate	No pattern	No pattern	PCA: 3 element diversity traits and 1 frequency trait	Medina and Francis (2012)
M & L: B	*Vireo* – 18-28 sp.	North temperate, neotropical	Migratory	Higher	repertoire size (from the literature)	Mountjoy and Leger (2001)
M & L: B	*Phylloscopus* – 80 sp.	Worldwide	No pattern	Lower[5]	3 traits derived from element, frequency, and tempo measures	Tietze et al. (2015)
M & L: B	Cettidae – 30 sp.	Worldwide	No pattern	Higher	# notes, strophe duration, longest note duration, 4 frequency traits	Wei et al. (2017)
M: W	Blackcap *Sylvia atricapilla* – 4 pop.	North temperate	Migratory	Not tested	song length, # note types/# notes in song	Collins et al. (2009)[6]
M: W	Eastern Towhee *Pipilo erythrophthalmus* – 2 pop.	North temperate	Sedentary	Not tested	song repertoire size	Ewert and Kroodsma (1994)
M: W	Red-winged Blackbird *Agelaius phoeniceus* – 5 pop.	North temperate	Migratory	Not tested	song repertoire size (from the literature)	Morton (1986)[7]
M: W	White-crowned Sparrow *Zonotrichia leuchophrys* – 3 subsp.	North temperate	Migratory	Not tested	repertoire size, # complex syllables	Nelson et al. (1996)
M: W	Song Sparrow *Melospiza melodia* – 5 pop.	North temperate	Sedentary	Not tested	repertoire size, minimal units of production	Peters et al. (2000)
M: B	Mimidae – 29 sp.	North temperate, neotropical	Migratory	Not tested	song duration, syllable duration, syllable types/song	Botero et al. (2009)

Table 2.1, continued.

Study Design	Study System	Breeding Range	More Complexity: Migratory or Sedentary	More Complexity: Higher or Lower Latitude	Measure of Complexity	Reference
M: B	*Geothlypis* – 9 sp.	North temperate, neotropical	No pattern	Not tested	song duration, # notes/song, # elements/note, # note types/song	Byers (2015)
M: B	*Acrocephalus* – 6 sp.	North temperate	Migratory	Not tested	(full) syllable repertoire	Catchpole (1982)
M: B	Passeriformes – 165 sp.	Worldwide[*]	Migratory	Not tested	Song/syllable repertoire size, versatility (from the literature)	Read and Weary (1992)
L: W	Red-faced Cisticola, *Cisticola erythrops* - rangewide	Paleotropical	Not applicable[8]	No pattern	# unique syllables	Benedict and Bowie (2009)
L: W	Common Reed Bunting, *Emberiza schoeniclus* – 3 subsp.	North temperate	Not tested	Lower	# unique syllables/song	de Oliveria Gordinho et al. (2015)
L: W	Greenish Warbler, *Phylloscopus trochiloides* – 5 subsp.	North temperate	Not applicable	Higher	PCA: song length, # units/song, # unit types/song, bandwidth, # units/unit song length	Irwin (2000)
L: W	House Wrens *Troglodytes aedon* - rangewide	North temperate, neotropical	Not tested	Higher	5 note traits, 8 tempo traits, 3 frequency traits	Kaluthota et al. (2016)
L: W	Yellow Wagtail, *Motacilla flava* – 5 subsp.	North temperate	Not tested	Lower	# elements/syllable	Ödeen and Björklund (2003)
L: W	3 subspecies of Yellow-eyed Junco, *Junco phaeonotus* – 3 subsp.	North temperate, paleotropical	Not applicable	Lower	# unique syllables, #unique notes/trill	Pieplow and Francis (2011)
L: B	*Serinus* and *Carduelis* – 44 sp.	North temperate, neotropical, paleotropical	Not tested	Higher	PCA: frequency range, two-voiced syllables, buzzy syllables, song duration, # syllables/song	Cardoso et al. (2012)
L: B	Maluridae – 16 sp.	Paleotropical	Not applicable	Higher	song versatility, note variety	Greig et al. (2013)
L: B	*Phylloscopus* – 30 sp.	North temperate, paleotropical	Not tested	Higher	PCA: song duration, # song types, # syllable types, # element types, # elements/song, # syllables/song	Mahler and Gil (2009)
L: B	*Phylloscopus* – 2 sp.	North temperate	Not applicable	Higher	song repertoire, song rate, repetition rate	Singh and Price (2015)
L: B	Passeriformes – 232 sp.	North temperate, neotropical	Not tested	Higher[9]	# syllable types/song	Weir and Wheatcroft (2011)

[*] Heavily biased to temperate breeders

[1] Found pattern of complexity difference between eastern and western lineages.

[2] Xing et al. (2017) note that, counter to their expectations, song duration decreased with latitude.

[3] Major revisions have since been made to this group.

[4] Song length, however, does increase with latitude, and this trait is associated with increased extra-pair paternity (Gil et al., 2007) (and may therefore be more informative than song “complexity”).

[5] This paper does not explicitly compare these three wren species in terms of migration, latitude, and complexity, but it has been referred to in Byers (2015) as such an example.

[6] See Byers (2011) and Collins et al. (2011) for further discussion of the limitations of this study.

[7] Only one published study is referenced in this example (Yasukawa 1981), the rest are personal communications.

[8] Refers to either completely migratory or sedentary species so differences among these classes cannot be compared.

[9] Complexity increases for oscines but not for suboscines, which have no pattern.

Latitude, Migration, and Song Complexity

Latitudinal Effects

Several studies have found latitudinal variation in song complexity that is not necessarily related to migratory behavior (e.g. Irwin 2000, Mahler and Gil 2009, Weir and Wheatcroft 2011, Cardoso et al. 2012, Table 2.1). Hypotheses proposed to explain this variation invoke both ecological selection (Weir et al. 2012) and sexual selection (Catchpole 1982) as the causes of these patterns (Table 2.2).

Ecological hypotheses. Bird vocalizations are signals adapted to propagate through an environment (Brumm and Naguib 2009) and as more sound space becomes available over a latitudinal gradient, songs may become more complex (Weir et al. 2012). The availability of sound space is negatively influenced by the amount of background noise generated by other animals (especially insects), the closeness of habitat (i.e. forests versus grasslands), and the sound-attenuating properties of the vegetation (Morton 1975). Overall, habitat does become more open as one moves away from the equator, with larger frequency windows and less sound attenuation in the predominantly evergreen forests of high latitudes and elevations (as compared with the tropical forests of lower latitudes and elevations) (Weir et al. 2012). There are both fewer bird species (Botero et al. 2014, Weir and Lawson 2015) and less background noise from insects (Weir et al. 2012) at higher latitudes, potentially freeing bird song to evolve more complex forms. Irwin (2000), Singh and Price (2015), and Wei et al. (2017) suggest latitudinal variation in sound space may explain some or most of the variation in song form detected in their respective studies.

Table 2.2. Published hypotheses on how latitude and migration may drive song complexity.

Hypothesis	Reference	Synopsis	Predictions
Sound space	Weir et al. (2012)	Songs becomes more complex with more available frequency windows.	Background noise and habitat features vary with latitude. ↑Latitude → ↑Complexity
Rapid pairing	Catchpole (1982)	At high latitudes birds have less time to pair and breed. Increased sexual selection drives elaboration of signals.	Elaboration varies with latitude and/or migratory distance ↑Latitude/↑Migration → ↑Complexity
Temporal isolation	Bolus (2014)	Migration isolates metapopulations both temporally and spatially, decreasing genetic/cultural transmission between them.	Migrants have greater variability in song via isolation by distance ↑Migration → ↑Complexity
Panmictic migrants	Bolus (2014)	Migrants disperse farther than residents, leading to greater mixing.	Migrants have less variation than residents ↑Migration → ↓Complexity
Good migrations	Fitzpatrick (1994)	Migrants are under strong selection to find good wintering grounds. Females will choose males whose genes can guide offspring to these places.	Signal elaboration and genetic variation greater in migrants[2] Migration → ↑Complexity
Ranging	Morton (1986)	Dialects exist in sedentary birds to assess sound degradation and threat level, migrants have no dialects and larger repertoires to disrupt this process.	Sedentary birds form small dialects, migrants have larger repertoires with no dialects Migration → ↑Complexity
Territory lottery	Mountjoy and Leger (2001)	Sedentary birds acquire territories by chance; selection is lower in these populations.	Signal elaboration higher in migrants Migration → ↑Complexity

[1] Fitzpatrick (1994) is discussing plumage elaboration and moult as a mechanism for determining how well an individual did on their wintering grounds. Mountjoy and Leger (2001) argue this could also apply to song complexity.

Sexual selection hypotheses. Catchpole (1982) theorized that short breeding seasons offer birds less time to pair and breed, so there may be greater selection on traits that quickly allow a bird to choose the best mate available. This rapid pairing hypothesis was first suggested for migratory birds, but the effect is correlated with latitude and would hold true for non-migrants that do not retain their pair bond from year to year. Both migrant and non-migrant species breeding at high latitudes have shorter breeding seasons than most of their low latitude counterparts, with this effect being more pronounced at the highest latitudes (Wyndham 1950). On the other hand, it is possible that the need to pair rapidly would result in birds making worse choices, on average, than those birds breeding at lower latitudes. It is not well-known how long an individual bird spends assessing potential partners or what the consequences are of having more or less time to pair (but see Sullivan 1994).

Migration Effects

While both non-migrant and migrant birds may breed in the same habitats at the same time, migrants face unique challenges. For clarity, we refer to "migratory species" as any birds that make seasonal movements such that there is a distinct and different breeding and non-breeding location for a particular individual tens to thousands of kilometers apart, even if not all members of the species make such a movement (Newton 2010). The ability to migrate requires physiological and navigational adaptations that may be absent in non-migrants (Hedenström 2008). The potentially long distance travelled and the fact that migrants reside in at least two often dramatically different locations confers different selective pressures on them irrespective of breeding latitude. A number of hypotheses have been advanced to explain how these pressures might affect song complexity.

Ecological hypotheses. Bolus (2014) recently proposed two hypotheses to explain how migrant dispersal patterns may influence song evolution in migrants compared with non-migrants. Because they move away from their breeding grounds each year, migrants are likely to vary more in both the timing and location of their subsequent nesting than non-migrants. The temporal isolation hypothesis posits that if individuals return to a particular location on different schedules, the staggering of their arrivals could isolate sub-populations breeding in the same place (e.g. Bearhop et al. 2005). Migrants would exhibit greater song variation through a mechanism similar to the one that drives sedentary populations to form local dialects, and this variation would be generated mainly via drift rather than as a result of selection for variability per se. The panmictic migrants hypothesis suggests the opposite: if migrants disperse further than non-migrants, song variation in migrants is expected to be less than that of non-migrants. Bolus (2014) found support for this latter hypothesis in her study of Common Yellowthroats *Geothlypis trichas*.

Sexual selection hypotheses. Migrants must successfully navigate to their breeding and wintering grounds each year, a process thought to be largely under genetic control (Pulido 2007). The good migrations hypothesis posits that molt taking place on the wintering grounds indicates the condition of the bird, and individuals with the genetic propensity to find the best wintering grounds will have the highest quality plumage for the following breeding season (Fitzpatrick 1994). Mountjoy and Leger (2001) suggest this mechanism may extend to song complexity, although they do not propose a mechanism. Perhaps birds that migrate to the best wintering grounds have more time, energy, and resources to devote to crystallizing a large song repertoire (Brainard and Doupe 2002), possibly by devoting more resources to growth of the song nuclei in the brain during their first winter. Females that then choose to mate with those males acquire “good migration” genes for their offspring.

Sedentary species or populations are more likely to have dialects, which often include only a small number of song types (Podos and Warren 2007). The ranging hypothesis (Morton 1986) posits that dialects evolved in sedentary birds to more accurately convey the location of the singer to its neighbors. Because birds within a dialect zone are all familiar with the common song type(s), they can compare songs that they hear with their own song in order to assess the amount of degradation and thus how far away the song originates and whether it constitutes a threat. Morton (1986) proposed that migratory species evolved repertoires to disrupt this ranging function of song. Unfamiliar, unrangeable songs could lead a neighbor to waste time and energy searching for a far-away signal or ignore a song that was actually a threat. This benefit would drive the evolution of constantly changing or larger repertoires in all individuals. There is evidence that birds are better able to range songs that are in their own repertoire (McGregor et al. 1983, Morton et al. 2006) and respond to unfamiliar song types from outside their territory more strongly than familiar songs (Shy and Morton 1986). However, this hypothesis makes no inference as to whether the territory holder recognizes the song as coming from a new rival or a familiar neighbor, factors which are known to affect a bird’s singing response (e.g. Stoddard et al. 1991, Stoddard et al. 1992). It is not clear what benefit neighbors gain from this disruption or how they avoid it, and does not satisfactorily explain the propensity for sedentary species to form dialects as there is no reason why they should not also benefit from repertoire ‘disruption.’

Regardless of how far migrants travel, the fact that they do not overwinter in their breeding territory obliges them to reacquire a territory the following year. The authors of the territory lottery hypothesis suggest this should manifest as greater elaboration of traits in migrants irrespective of migration distance (Mountjoy and Leger 2001). This ignores reports of winter territoriality in some migratory species (Marra et al. 1993, Cuadrado 1994, Stutchbury 1994). Then again, it is just as plausible to predict elaboration in the opposite direction: if sedentary birds must continuously defend their territories from intruders (e.g. Salomonson and Balda 1977, Kraaijeveld and Dickinson 2001), while migrants do not, sedentary birds could evolve more elaborate traits to honestly advertise their ability to defend that territory. Winter singing or territorial behavior is poorly understood, while the relationship between either serial acquisition or continuous defense of a territory and song evolution is even less so. These subjects present an interesting avenue for future research.

Methods

We used Google Scholar, Semantic Scholar and Web of Science to search for combinations of variations of key terms: migration (e.g. migrate, migratory, migrating), latitude (latitudinal), geographic variation (variability), (bird) song complexity, repertoire, and song evolution (elaboration). At a minimum we read the first 200 hits for each combination of terms. We also searched through all the citations of the papers found studying this topic and papers that present relevant hypotheses. Our goal was not to conduct a meta-analytic review of all studies of the song characteristics of a species or group to search for geographic patterns, but to review studies where bird song complexity was explicitly considered in relation to latitude and/or migration. We did not consider studies featuring relatively short migratory distances (i.e. overall geographic extent < 500 km).

Results

Our search of the literature discovered 29 papers that report the presence or absence of a latitudinal trend or a difference between migrants and non-migrants in some measure of song complexity. Measures of complexity were defined by the authors in most studies. Several studies of repertoire size were included because, despite the authors of these studies not characterizing this metric as "complexity," repertoire size is often used as a complexity metric.

Of the 29 papers, 15 are comparisons between species and 14 are comparisons among subspecies or populations within one species. All of the studies are of passerines, only one of which considers suboscines. Nine studies looked for differences between migrants and non-migrants, 11 studies looked for latitudinal patterns, and nine studies considered both latitude and migratory strategy. Song complexity was assessed in many ways, mostly by measuring song repertoire size, counting and/or measuring the number and duration of elements, notes, syllables, or phrases per unit, estimating song versatility or variety, or consulting previously published metrics (summarized in Table 2.1).

Increased song complexity was associated with migratory behavior in eight studies, decreased song complexity in four studies, there was no pattern in six studies (Table 2.1). When considering potential effects of latitude, the outcomes are similarly variable: song complexity increased towards the poles in ten studies but decreased in six studies, and four studies found no pattern (Table 2.1). Overall, more studies found a relationship between migration and/or latitude and increased song complexity (16) than with decreased song complexity (9), but this is not significantly different from an expected proportion of 0.50 (two-tailed binomial test $p = 0.59$). Most studies do, however, find an effect of latitude and/or migration (25) rather than no pattern at all (4) (expected proportion 0.50, two-tailed binomial test $p < 0.001$). Because patterns may vary with taxonomic level, we also asked how studies comparing populations of a single species might differ from studies comparing multiple species. Of the within-species studies, five found support for complexity increasing with latitude (2) or migratory behavior (3), seven found support for complexity decreasing with latitude (4) or migratory behavior (3), and two studies found no pattern (Table 2.1). Of the between-species studies, 11 found support for complexity increasing with latitude (6) or migratory behavior (5), two found support for complexity decreasing with migratory behavior, and two studies reported no pattern (Table 2.1).

Discussion

Seven of the hypotheses discussed here predict and 16 studies found positive correlations between avian song complexity and latitude and/or migration, while only one hypothesis predicts and nine studies found negative correlations. Four studies reported no pattern. This may be an underestimate due to publication bias, or may signal that in most species latitude and migration influence song complexity, but do so variably.

With only 29 studies considering such a broad topic it is difficult to reach general conclusions. Nevertheless, it is clear that not all existing studies draw similar conclusions (Table 2.1). Additionally, the many hypotheses imply that the field is nowhere near consensus on how latitude and migratory behavior might affect song complexity. Six different hypotheses are similar in that they broadly associate complexity with increases in migration or movement towards the poles, but the existing research does not overwhelmingly support this predicted pattern. Moreover, the studies reviewed here suggest (and some support) five fairly distinct biological drivers of any proposed patterns: acoustic adaptation, rapid mate choice, cultural drift versus mixing, navigational abilities, and resource defense (Table 2.2). The authors of these hypotheses discuss these drivers as either ecological or sexual, but at least two (territory lottery and ranging) may be better characterized as socially selected since they describe competition for territories, not mates (West-Eberhard 1983). Considering the results of published studies, it seems highly unlikely that only one mechanism is at play or that all species would exhibit similar patterns in geographic variation in song complexity. Different selection pressures may result in similarly increased or decreased song complexity in different species, further complicating our understanding of causes. This should be evident simply by noting that not all species are suitable for studying all hypotheses (e.g. species that are entirely sedentary or migratory) but the effects of latitude or migration are still apparent. Additionally, different methodological approaches may be appropriate for different species, and comparisons between studies that use these different measures may not always be valid (see below).

Song Complexity

Thus far, in our discussions of song “complexity”, we have neglected to define the term. We are not alone in this: many authors do not precisely define (if at all) what constitutes complexity, or they may use statistical analysis to define complexity post-hoc.

It is generally agreed that increases in song and syllable repertoire size as well as increases in note variability represent complexity (Catchpole and Slater 2008), but each study may define complexity in different ways. For example, Irwin (2000) states "Females… prefer greater song complexity and repertoire size…" implying complexity and repertoire size are conceptually different (albeit both influenced by sexual selection), while Peters et al. (2000) note that "Song complexity is most often measured as song repertoire size…" This difference often stems from the different samples available to researchers and song differences between species. Studies that measure sound files obtained from sound libraries or using published estimates are limited in how many songs from a single individual they can acquire, and cannot measure repertoires or structural elements in the same way that studies which collect their own recordings can. Similarly, studies examining species that sing only a single song type might measure complexity in terms of the structure of the song, making those studies difficult to compare with studies of species whose complexity is largely characterized by having a song or syllable repertoire. Multi-species studies face additional challenges in choosing song complexity metrics. The more divergent the species are taxonomically, the fewer homologous measures are available for comparison. Studies that examine one species or genus often include many taxonomically-specific measurements (such as whether a syllable uses two voices or the length of an introductory phrase common to the study species).

Of the studies in Table 2.1, four do not offer any definition of complexity or describe their measurements as reflecting song elaboration. Instead they are focused on the functions of repertoire size specifically, irrespective of the identity or features of the song types. Thirteen publications do offer an explicit definition, ranging from general (e.g. “Complexity… generally includes a measure of note, syllable or song variety…” (Singh and Price 2015) to more specific (e.g. “… and complexity (note variety and song versatility)” (Greig et al. 2013). Twelve studies mention complexity but do not offer explicit definitions or are vague in their use of the term; complexity is instead defined implicitly using statistics (e.g. Cardoso et al. 2012) or in the text (e.g. “complex syllables” in Nelson et al. 1996). Despite this lack of consensus, there is a general pattern of agreement in what an author measures as “complexity”. All of the listed studies include some count of unique elements per unit song length or among different songs as their key metric for complexity, suggesting that diversity in element structure is fundamental to defining “complex” songs among song researchers.

If hypotheses that sexual selection pressure increases with latitude or migratory behavior are largely correct, then we might also expect performance (the ability to sing physically challenging songs) to vary with these factors (Gil and Gahr 2002). Complexity may even be traded off for performance; in cases where complexity does not seem to vary geographically it may be that performance does. Performance is not commonly discussed specifically in the reviewed studies, and the one study that refers to it explicitly lumps it together with complexity (Kaluthota et al. 2016). This suggests that researchers either do not generally consider performance to be directly related to complexity, or think it is perfectly correlated and complexity is simply easier to measure (although this is unlikely given the lack of supporting evidence). Performance in bird song is a comparatively new idea and there simply may not be enough research yet to draw conclusions one way or the other. It may be interesting to consider performance in future studies to help determine whether and how estimates of performance co-vary with song complexity and geography overall.

Study System and Data

The studies referenced in this review considered their questions in many ways: they may have studied populations of one species or compared multiple species, they used variable numbers of populations or species, and they may or may not have corrected for phylogeny. Song complexity analyses varied from comparisons of one or a few song measurements using t-tests and correlations, to principal coordinate analysis and model-based analyses of many song measurements. All of these studies were designed and analyzed in different ways and are taxonomically diverse, making comparisons imperfect (Table 2.1). While there is no way to completely circumvent this comparability problem, it is important to note these methodological differences so they can be considered in comparisons.

Studies conducted between species seem to find support for increased complexity with latitude or migration more frequently than within-species studies, which are more equivocal in their results (Table 2.2). This could be a telling symptom of an ecological fallacy, where this apparent “effect of scale” could be due to one or several causal variables going unmeasured (Simpson 1951, Selvin 1958). It is also possible that this is an illustration of the comparability problem (Read and Weary 1992), whereby studies between distantly related species must necessarily use fewer metrics to compare them, although many within-species studies also use only one or a few metrics. Alternatively, multiple metrics may evolve along different trajectories, following different hypotheses. Of the 11 studies that examined repertoire size, seven find complexity to be correlated with latitude or migration, but four do not. Of the eight studies that use only song or syllable repertoire size, or both, as their metrics of complexity, four find increases, three find decreases, and one finds no pattern of song complexity related to latitude or migration. Collectively, these studies use ten different bird “groups” (e.g. Old World warblers, New World sparrows), making it difficult to assign differences in results to differences in life history. Thus, while most studies do find an effect of latitude or migration, either positive or negative, there is no clear indication that certain methods of comparison or measures of complexity show more or less tendency to vary with latitude.

Data sources for song features also varied widely for these studies; some gleaned song measurements from the literature, while others measured them from sound recordings. Those studies using sound recordings varied in the number and geographic distribution of recordings available to them depending on whether they used archived recordings from sound libraries or made their own field recordings. Some studies generated new measurement practices, and others relied on applying approaches or reanalyzing data previously described in the literature. While using previously published descriptions is not a bad practice, care must be taken in their use. This is particularly true when discussing variation in structural elements because different people may have different tolerances as to when to call something the same or not. A good example of this was discussed in Ewert and Kroodsma’s (1994) study of Eastern Towhee *Pipilo erythrophthalmus* song. They found that their method of classifying towhee song types differed significantly from a previous author’s classification scheme, and identified fewer song types. Clear description of methods for defining structural elements is vitally important to facilitate comparisons.

Future Directions

Comparative analyses by Read and Weary (1992), and later Weir and Wheatcroft (2011), seem largely responsible for popularizing the idea that migration and latitude may play a role in the evolution of complex bird song, specifically that song complexity increases with being migratory and with increasing latitude of breeding. However, not all studies agree with this assessment and even those that do are difficult to compare in order to determine the underlying mechanism(s) at play. While the idea that there is an “expected outcome” of increasing complexity towards the poles has become common, this review of the empirical literature finds that there is no broad consensus and no expectation to be violated. Rather, more research is needed to better demonstrate whether patterns exist and, if so, what the possible mechanisms generating them are.

Future studies of a variety of species that measure song complexity in conjunction with relevant ecological or sexual selection variables will go a long way towards advancing our understanding of the relationships between latitude, migration, and song elaboration. Table 2.1 is dominated by old world warblers, new world sparrows, and new world wrens. More diversity of study species would indicate whether these hypotheses hold up for other groups of birds. For example, broadly distributed non-passerines with vocalizations much like passerine song such as members of the Columbidae, Trochilidae, or Cuculidae may prove to be interesting groups for study. Noticeably missing from these studies are suboscines. Studying species that do not learn their songs would be informative both as controls for hypotheses where song learning is part of the proposed mechanism and as parallel comparisons for hypotheses where song learning is inconsequential.

Most hypotheses included here made (or implied) predictions about the breeding biology of tropical and temperate-zone species, but few quantified traits of tropical breeders. While theory predicts certain characteristics of tropical species, such as low rates of extra-pair paternity and long breeding seasons, there is not enough data to confidently conclude this (e.g. Macedo et al. 2008, Cramer et al. 2011, Ferretti et al. 2016). More study is needed to assess the ecological correlates of sexual selection in tropical regions, and more studies of tropical species are needed to characterize patterns of song complexity at all latitudes.

Both ecology and sexual selection are often cited as factors driving geographic patterns of song complexity, but it is rare for researchers to measure their effects in this context. While many studies do attempt to take the environment into account in some way (such as by classifying them as ‘boreal’ vs. ‘tropical’ forest or ‘open’ vs. ‘closed’ species), only two studies included here actually measured the habitat and acoustic features at their study sites (Irwin 2000, Singh and Price 2015), while another three used mean climate measures (Botero et al. 2009, Medina and Francis 2012, Xing et al. 2017). To fully test the ecological hypotheses included in this review the field needs more studies that explicitly quantify the sound space available at varying latitudes and whether more complex songs fill a wider swath of that space.

The underlying assumption of sexual selection hypotheses is that song complexity or song repertoire size is a good proxy for the strength of sexual selection and that given a choice, birds will choose to mate with individuals that have a more elaborate or complex song. However, this is often not explicitly tested and there is debate in the literature about how this should be done (Wilkins et al. 2013, Byers 2015, Price 2017).

None of the studies included here measured proxies of sexual selection, such as time to pairing, reproductive success, or level of parasite or disease infection, leaving room for improvement in future studies. Researchers positing connections between song complexity and female choice should test whether females actually prefer the ‘complex’ traits in question. Additionally, several hypotheses mention resource defense in the context of sexual selection, but which could be more accurately described as being mediated by social selection. None of the studies reviewed here invoke social selection (i.e. selection for competition for resources other than mates, such as nesting sites, food, or space, West-Eberhard 1983) as a force driving changes in song complexity. Future studies considering both social and sexual selection would be valuable.

Conclusions

The studies reviewed here attempted to document latitudinal patterns of avian song complexity. Despite a variety of hypotheses mostly rooted in sexual selection theory, it is still unclear whether and to what extent song complexity may be influenced by latitude and its correlate, migration. Certainly there is no overarching theory on the topic beyond the observation that vegetation is different at high latitudes or a weak appeal to ‘higher sexual selection pressures’ assumed to exist in these places. What we can clearly conclude is that latitude and migration do not universally affect song complexity in the same way among birds, and that increased latitude or migratory behavior is not always associated with increased complexity. This is perhaps an unsurprising result but an important one to acknowledge given the overwhelming bias in the theoretical literature towards predicting a universal directional trend. High latitudes and migration affect birds in a multitude of ways, and it is unrealistic to expect one hypothesis to explain all or even most of the variation observed in bird song features. Many of the studies discussed here are observational and correlational, an excellent approach for initial studies, but none go any further. That being said, many studies cited in this review do find an effect of latitude or migration in their study system and future studies should explicitly test the hypotheses that offer the most potential to explain these outcomes.

CHAPTER III

GEOGRAPHIC PATTERN OF ROCK WREN SONG REPERTOIRE SIZE

Contribution of Authors and Co-Authors

Manuscript in Chapter III

Author: Nadje A. Najar

Contributions: Conceived the study topic and design, collected song recordings and measured territories at 10 sites, analyzed the data, and wrote the manuscript.

Co-Author: Dr. Lauryn Benedict

Contributions: Collected song recordings at one site, assisted analyzing the data, and edited drafts of the manuscript.

Abstract

A small body of literature devoted to studying the possible effects of latitude, migration, and song complexity has resulted in a number of hypotheses despite inconsistent and inconclusive results (see Chapter II). A variety of problems remain unsolved, such as how to meaningfully compare the songs of different species or the fact that studies of single species tend to focus on only a few populations. The goal of this study was to examine the pattern of song repertoire size within a single, geographically widespread species consisting of both migratory and sedentary populations. Rock wren song repertoire size was measured and compared amongst five migratory and six sedentary populations along a latitudinal transect spanning northern Montana to west Texas. Repertoire size was significantly larger in sedentary (mean 102.6±20.8) versus migratory (mean 87±23) rock wrens, but latitude was only significantly correlated with song repertoire size in migratory (R^2=0.30, p=0.006, F_{23}=9.2) and not sedentary (R^2=0.013, p=0.63, F_{23}=0.23) populations. This is a pattern of song complexity that has not been previously predicted and suggests our understanding of the factors governing geographic patterns of song complexity is still in its infancy.

Introduction

Bird vocalizations continue to fascinate and intrigue, motivating the publication of hundreds of studies dedicated to unraveling the evolution, ontogeny, and functions of complex songs. Broadly speaking, the main factors proposed to drive the evolution of song complexity are ecological (Morton 1975), sexual (Andersson 1994), and social selection (Freeberg et al. 2012). Some patterns of evolution are thought to have resulted from the interaction of these three forces at large scales. Such broad scale interactions of evolutionary forces have likely driven latitudinal gradients and migratory divides in the elaboration of bird song.

At least 29 studies have explored the possible link between migration, latitude, and song complexity at both the species- and population-levels, considering wholly, partially, and non-migratory groups (Chapter II). Collectively, this small body of literature has given rise to at least eight hypotheses focused largely on sexual, and to a lesser extent, ecological selection. These hypotheses may be best summarized as predicting that song complexity will increase with latitude and that migrants will have more complexity than non-migrants (Read and Weary 1992, Irwin 2000, Peters et al. 2000, Kaluthota et al. 2016).

The plethora of hypotheses is not matched by an abundance of evidence, however, and these studies are plagued by a number of problems outlined in a previous chapter, which may be best summarized as issues with sampling and comparability. About half of the studies examining these relationships find no pattern or one not consistent with prior predictions (Chapter II). Considering the large-scale nature of the patterns being discussed and the varied nature of the studies addressing them, 29 publications is a meager number.

Support for the basic hypothesis that latitude and/or migration lead to increased song complexity remains mixed. The studies comparing the most species and/or covering the greatest geographic extent generally draw on (and are therefore limited by) archived recordings and must often reduce their song analyses to one or a few comparisons (e.g.

Read and Weary 1992, Mountjoy and Leger 2001, Weir and Wheatcroft 2011, Medina and Francis 2012). Studies focusing on a single species often have poor sampling, examining only a few individuals or populations (e.g. Ewert and Kroodsma 1994, Nelson et al. 1996, Morton 1986, Ödeen and Björklund 2003, Collins et al. 2009). Only eight studies attempted to examine the effects of both latitude and migration (Kroodsma and Verner 1987, Kroodsma et al. 2001, Mountjoy and Leger 2001, Handley and Nelson 2005, Medina and Francis 2012, Bolus 2014, Tietze et al. 2015, Wei et al. 2017, Xing et al. 2017). Finally, some authors have called into question the logic of continuing to cite a phenomenon that has not been convincingly demonstrated to exist (e.g. Byers 2011, Byers 2015).

I sought to add to this discussion by comprehensively studying the interaction of latitude, migration, and song complexity in a single species, the rock wren (*Salpinctes obsoletus*). By examining populations of a single species across a latitudinal gradient, I examined whether any pattern of song complexity exists, and depending on the pattern, what hypotheses might be supported. I predicted that I would see both a latitudinal gradient and a migratory divide in song repertoire size. That is, my southernmost population will have the smallest repertoire size, my northernmost population will have the largest repertoire size, and there will be a significant difference between the populations closest to the migratory divide.

Methods

Study Species

Rock wrens are drab, monomorphic passerines in the family Troglodytidae with a large range in western north and middle America. Males and females are nearly indistinguishable except that only males are known to sing. Rock wren song is characterized by the repetition of a single syllable 2-8 times followed by a silence of 6-10 seconds, making song types easy to identify. This also results in a strong correlation between an individual's song and syllable repertoire. Individual males have repertoires ranging from 50-130 song types. Rock wrens are unusual among wrens in that northern populations are migratory. This combination of large, variable, easily characterized song repertoires, a large geographic range, and migratory and sedentary populations make rock wrens almost uniquely suited to studying how latitude and migration influence the evolution of bird song complexity.

Site Selection

Rock wrens are resident throughout the United States, and migratory in the northern half of that range. I was able to choose multiple study sites that varied in latitude and migratory status. Initially, site selection was carried out remotely. I used recently surveyed high density (>25 rock wrens counted) Breeding Bird Survey tracks (Sauer et al. 2013) as candidate sites in Montana, Wyoming, Colorado, New Mexico, and Texas. Of these, I selected tracks occurring on or near public lands and narrowed these sites to those occurring approximately 300 kilometers apart in a longitudinal transect.

Table 3.1. Study site locations and abbreviations.

Abbreviation	Locality	County	State	GPS N	GPS W
CBL	Coal Banks Landing	Chouteau	MT	48.037616	-110.229292
MC	Milligan Canyon Road	Jefferson	MT	45.881958	-111.683048
DETO	Devils Tower National Monument	Crook	WY	44.588621	-104.714628
BSP	Boysen State Park	Fremont	WY	43.419939	-108.092617
FOCO	Horsetooth Reservoir	Larimer	CO	40.589961	-105.183689
CNG	Comanche National Grasslands	Baca	CO	37.012971	-102.746630
RGG	Rio Grande Gorge	Taos	NM	36.290282	-105.779375
BOX	The Box National Recreation Area	Socorro	NM	34.002822	-106.991115
OM	Desert Peaks National Monument	Dona Ana	NM	32.323647	-106.991115
FODA	Fort Davis National Historic Site	Jefferson Davis	TX	30.598757	-103.892149
BBR	Big Bend Ranch State Park	Presidio	TX	29.475857	-103.964853

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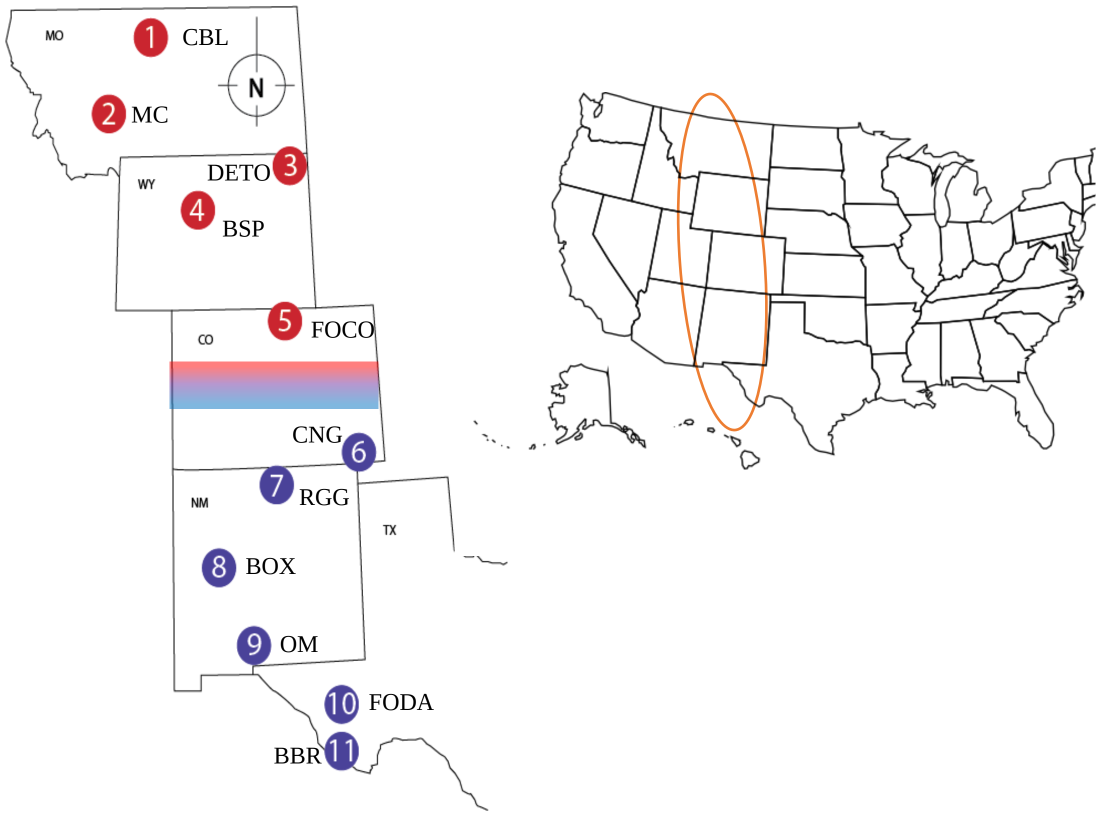


Figure 3.1. Map of study sites with approximate locations and population name abbreviations. Red populations are migratory, purple populations sedentary. The colored rectangle indicates the location of the migratory divide. Inset map indicates location of transect in the USA.

This method was sufficient for most sites. Upon arriving I found a few sites either to not be accessible (2 sites) or not to have rock wrens present (1 site); I used ebird.org to find a nearby site with rock wrens to replace them. The number of sites I chose were such that I could visit half of my sites for two weeks each in one field season. The final list of study site locations is outlined in Table 3.1 and graphically illustrated in Figure 3.1.

Populations were determined to be either migratory or sedentary by comparing presence during summer (as determined by Breeding Bird Survey reports) with presence or absence during the winter (using Christmas Bird Count reports) from 10 recent years (2003-2013) (National Audubon Society 2010). A site was considered sedentary if it (or nearby count locations) had at least 6 rock wrens detected in the area for at least 7 of the 10 count years. From these criteria, I determined that the point at which rock wrens cease to be migratory and overwinter was south of Denver and north of Colorado Springs in Colorado. The region between Boulder and Colorado Springs had many singular sightings of overwintering rock wrens, with some years or sites having many more rock wrens than others. This region of variable migrator behavior constitutes a fairly wide (~70 miles) cline in central Colorado, and my data may underestimate its true size given singular reports of overwintering rock wrens as far north as Lander, Wyoming. To avoid this region of migratory and sedentary overlap I chose a site in the far south (CNG) and in the far north (FOCO) of Colorado. To check the assumption that sites north of Denver were migratory I looked for reports of wintering northern rock wrens on ebird.org, and I returned to each of my northern populations in early December 2014 and surveyed for rock wrens. I conducted at least one playback survey at each of the locations where I had previously recorded rock wrens by playing conspecific song. No rock wrens were detected in the winter, either during my visit or reported online. Populations south of Denver were considered to be sedentary. A banded population of rock wrens observed over three years were seen to remain on their territories in successive breeding seasons in northern New Mexico (Merola 1995), suggesting those birds were sedentary. To test whether birds at my study sites were sedentary, I banded rock wrens at each site during the breeding season. I was unable to search for banded rock wrens in the winters following my field work, but locals at some sites were able to sight banded rock wrens and report their color combinations (FODA, OM). I returned to two field sites (FODA, BOX) in the summer the year after I banded there and was able to re-sight just over half of my banded rock wrens. This is a recovery rate consistent with these populations being completely sedentary. Only three rock wrens were re-sighted over the course of this study at northern sites, two at Horsetooth Reservoir and one at Devils Tower National Monument. The poor re-sight rate for northern rock wrens could be due to higher mortality and/or a lack of site fidelity, and is consistent with these birds being migrants.

Recording Protocol

Rock wrens at all sites were audio recorded from 30 minutes before civil sunrise until 14:00 each day until 2000 songs had been recorded or for three days, whichever came first. Repertoire curves previously generated for rock wrens (as in Kroodsma 1975) indicate that 1000 to 2000 songs reliably estimate a bird’s repertoire. Two recordists (myself and an assistant) followed and recorded one male rock wren each until it was time to move onto another one. Rock wrens were not banded during recording efforts, but each pair is highly territorial and males can be recognized by the perches they sing from. After audio recording an individual, we recorded GPS coordinates from each of the locations it was observed singing or foraging. We recorded up to 12 rock wrens for a total of two weeks at each site. Recordings were made using a Sennheiser short shotgun microphone (MKH 60 P48) with a windscreen (MZW 60-1) and Marantz handheld PMD solid state recorder (PMD661 MKII, mono input, 48KHz, 768 kbps). Memory and battery power in the field was limited; to maximize the number of songs recorded we did not record continuous song bouts but rather one song at a time using the record/pause function. This eliminated periods of silence and resulted in tracks with one song every three seconds. While recordings were made throughout four field seasons (2013 – 2016) of work, the majority were made during the summers of 2013 and 2014.

Song Analysis

For the purposes of this study, rock wren song complexity is measured as song repertoire size (Chapter I). Songs were visualized in RAVEN PRO (version 1.3, Bioacoustics Research Program 2008) where I identified song types by eye following Borror (1967) and Kroodsma (1975). Reference pictures and sound files of each song type were kept as libraries for each individual wren. Song repertoire size was determined visually by generating a repertoire curve for each individual. Repertoire size was used as the main data point for subsequent analyses. General linear mixed models were used to account for potentially confounding variables to see if there was any correlation between repertoire size (total song types identified, song types at 500th song), migratory status (migratory, sedentary), and latitude (site membership) among sampled rock wrens. For analyses using total number of song types identified, number of songs sampled was included as a random effect. I used a stepwise linear regression to identify the combination of model effects that best describe the data.

Measuring Territories

All points were collected on a Garmin GPSMAP 60 using the NAD83 datum then transformed to WGS84. I used the “aggregate points” function in ArcMap 10.5 to estimate rock wren territory sizes. I grouped points using an aggregation distance of 200m, then calculated the area of the resultant polygon in hectares. Territory size was significantly correlated with number of GPS points up to five points (5 or more points $p<0.001$, $R^2=0.44$, 6 or more points $p=0.12$, $R^2=0.04$) so I only used territories estimated with at least six points in subsequent analyses ($n=53$). I used a stepwise linear regression to identify the model effects that best fit the data (population membership or migratory strategy, with year and number of points included as random effects) as well as individual analyses using one-way analysis of variance. All analyses were conducted in JMP 9.0. Territory sizes were not directly correlated with repertoire size because very few individuals with repertoires included in the analysis also had territory sizes measured. Not all individuals recorded or captured had territories accessible to logging GPS points from. This was particularly true of individuals occupying highly vertical rock formations or whose territories crossed rivers or onto land I was not permitted to access.

Capture and Handling

Rock wrens were captured using mist nets in 2015 and 2016. Birds were lured into the net with playback of conspecific song and, on occasion, a taxidermied rock wren mount. Each wren was marked with a unique combination of plastic color bands and a USFWS metal band. Mass and the lengths of the culmen, tarsus, wing chord, first secondary, and tail were measured. Approximately 15µl of blood was collected by puncturing the brachial vein with a 26-gauge hypodermic needle and drawing blood with a capillary tube. Blood was stored in Longmire’s solution without refrigeration in a chest cooler until the end of the field season, after which samples were frozen at -20°C. Finally, a photograph of the outstretched wing was taken against a 1cmx1cm grid. The bird was then released.

I was able to capture 109 rock wrens. Of these, 107 were adults and 2 were juveniles. Of the 107 adults, 96 were males and 11 were females. The Horsetooth Reservoir site was managed by Dr. Benedict, who captured 15 individuals in 2015 and 2016. Capture locations and totals are outlined in Table 3.2.

Table 3.2. Capture localities and demographics.

Abbreviation	Locality	Total captures	Males	Females	Juveniles
CBL	Coal Banks Landing, MT	9	7	1	1
MC	Milligan Canyon, MT	12	12	0	0
DETO	Devils Tower, WY	12	9	3	0
BSP	Boysen State Park, WY	12	12	0	0
FOCO	Horsetooth Reservoir, CO	15	14	1	0
CNG	Comanche National Grasslands, CO	12	9	3	0
RGG	Rio Grande Gorge, NM	12	11	1	0
BOX	The Box National Recreation Area, NM	12	11	1	0
OM	Desert Peaks National Monument, NM	9	9	0	0
FODA	Fort Davis National Historic Site, TX	13	10	2	1
BBR	Big Bend Ranch State Park, TX	6	5	1	0

Results

My colleague and I assessed the repertoires of a total of 60 individuals from eleven sites. The mean number of songs recorded per individual was 909.6 ± 630.9 songs (range 17-2105) (Table 3.3). Of these, 42 individuals had over 500 songs recorded. All individuals with very low numbers of songs recorded (<500) were monitored for at least three consecutive days for 10 hours per day, indicating that some rock wrens sing very infrequently, even during the peak of the breeding season. Of the 18 individuals that sang fewer than 500 songs, five sang fewer than 200 songs and 13 individuals sang only 200-400 songs over three consecutive days totaling 28-30 recording hours. Observations of their behavior did not yield any obvious clues as to why they were not singing. These individuals (n=18) were excluded from all analyses. In the two weeks spent recording a given population, rock wren song production did not vary day-to-day. Individuals were fairly consistent in their singing rates from day to day, but varied in their singing rates relative to each other (e.g. one bird sings five minutes of every hour and his neighbor sings 25 minutes of every hour). However, individuals that were relatively quiet did not pick up their singing, even when stimulated with conspecific playback. Since nearly all birds recorded were clearly paired and engaged in breeding activities (e.g. nest building, incubation, feeding chicks) this difference cannot be broadly attributed to pairing or nesting status.

Table 3.3. Summary of recording effort and repertoire size for each population. Migrants in red and residents in blue.

pop	# inds	# songs	mean songs / ind	mean rep size	# inds over 500	mean rep size at 500th song	sd
CBL	4	2436	609.0	82.3	3	83.7	2.9
MC	4	2688	672.0	96.8	3	80.0	18.9
DETO	7	3169	452.7	61.3	3	78.0	11.3
BSP	5	2060	412.0	52.2	2	59.3	35.2
FOCO	12	19376	1614.7	76.8	12	59.0	14.5
subtotal	32	29729	929.0	87	23	68	18.8
CNG	7	6711	958.7	78.4	5	80.4	9.1
RGG	5	4550	910.0	52.0	3	86.3	19.6
BOX	4	3341	835.3	108.8	2	63.5	11.3
OM	4	3552	888.0	103.5	4	96.8	19.6
FODA	3	2622	874.0	126.3	2	68.5	7.8
BBR	5	4059	811.8	90.6	3	90.3	31.1
subtotal	28	24835	887.0	102.6	19	83.3	17.2
totals	60	54564	909.4	80.3	42	76.1	20

Individuals differed in their singing behavior seemingly without respect to site membership or nesting state (building, incubation, nestlings). Some birds that sang copiously (1500+ songs in one day) were not observed to feed any chicks, indicating they are in an intermediate stage of breeding where the last nest's chicks are independent but the next one has not hatched yet, or, less likely, that they are unpaired and still attempting to advertise for a mate. Feeding chicks did not preclude singing, however, and many males gave songs either immediately before or after delivering food to the nest.

Song accumulation curves (Fig 3.2) show that the number of new song types discovered levels off sharply after 500 songs. Rock wrens still introduce new song types sporadically up to 2000 songs but relative repertoire size remains constant at almost any number of songs after ~300, implying individual rock wrens introduce new song types at a fairly constant rate and do not sporadically increase or decrease this rate. Additionally, rock wrens sing ~10 songs per minute, so 500 songs represent nearly an hour of continuous singing. Most wrens do not sing continuously for this long, so this represents over an hour of assessment from an individual in the field.

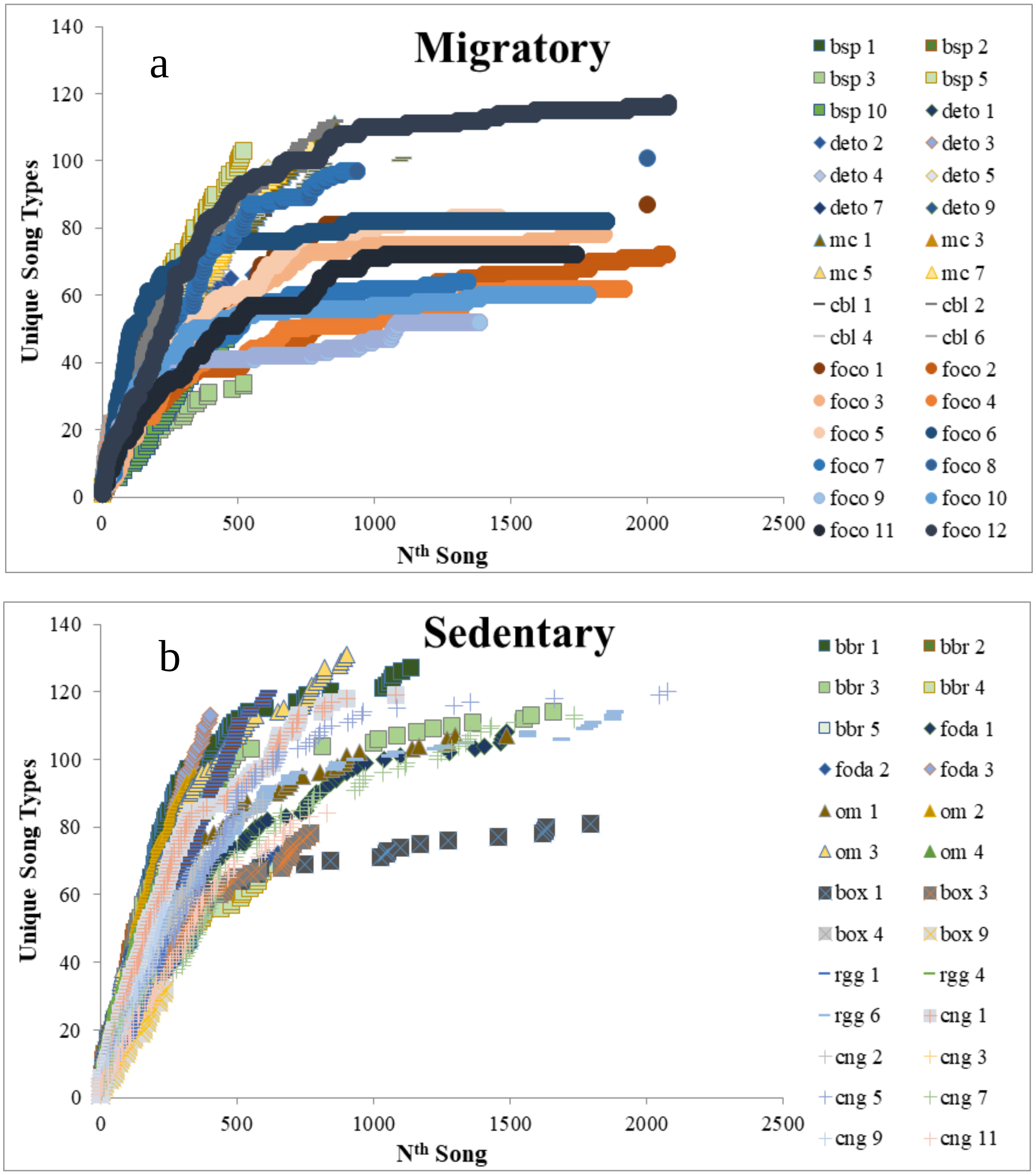


Figure 3.2. Song repertoire accumulation curves for individual rock wrens recorded in northern, migratory sites (a) and southern, sedentary sites (b).

The number of songs recorded is strongly correlated to the number of song types introduced ($p<0.0001$, $R^2=0.21$) up to approximately 300 songs, after which this correlation is not significant ($p=0.17$, $R^2=0.01$). After 500 songs the p-value grows to 0.74. Therefore, only birds with at least 500 songs ($n=42$) analyzed were included in subsequent analyses. I used two main song comparisons: song repertoire size at the 500th song and total song repertoire size.

Taken as a group, the southern, sedentary populations have significantly larger mean repertoires (mean total repertoire size=102.6±20.8, mean repertoire size at 500th song=83.3±17.2) than northern, migratory populations (mean total repertoire size=87±23.0, mean repertoire size at 500th song=68.0±18.8) (Wilcoxon signed rank test p=0.023) (Fig 3.3).

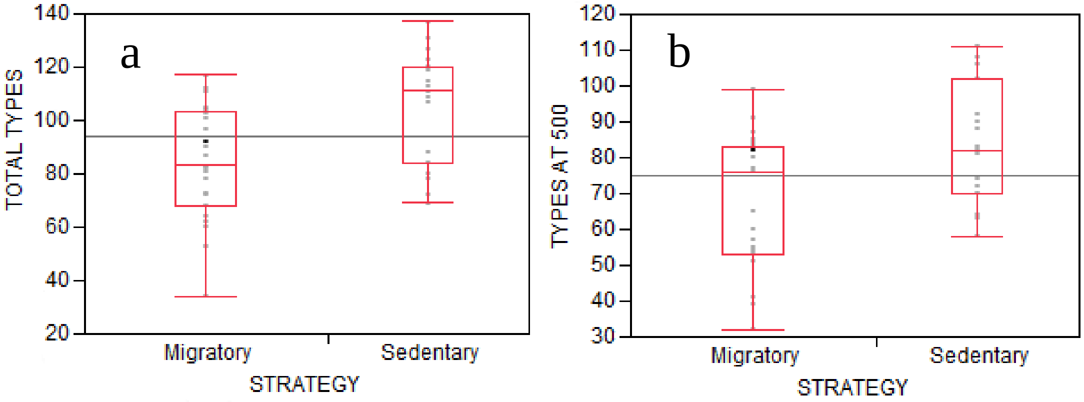
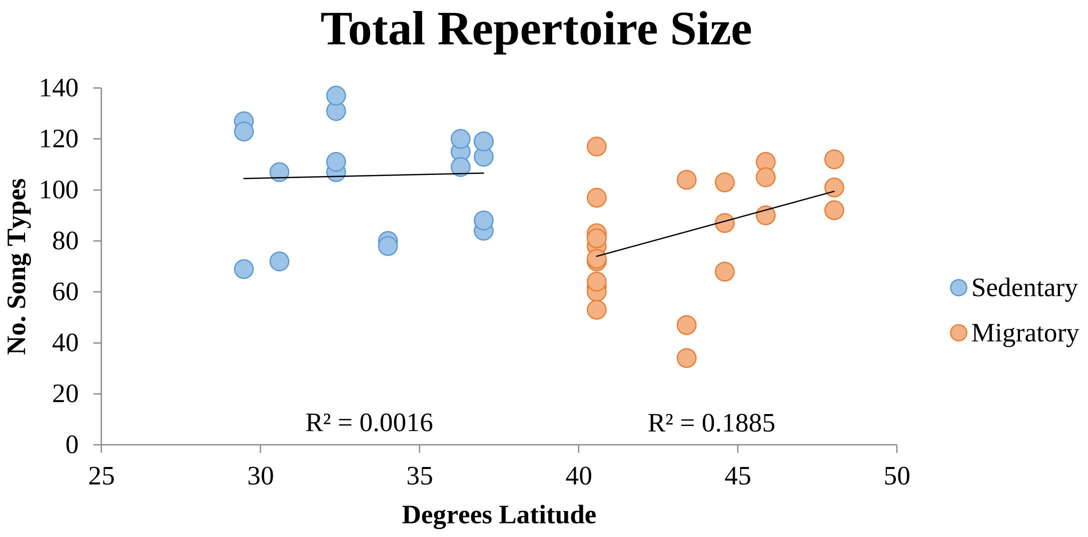


Figure 3.3. Wilcoxon signed rank test of difference in (a) mean total song repertoire size (number of song types a bird can sing) between migratory (87±23) and sedentary (102.6±20.8) birds, and (b) mean repertoire size (number of song types) at the 500th song between migratory (68±18.8) and sedentary (83.3±17.2) birds.

Latitude is correlated with repertoire size in migratory populations (R^2=0.30, p=0.006, F_{23}=9.2) but not sedentary populations (R^2=0.013, p=0.63, F_{23}=0.23) (Fig 4). This is not due to a difference in the distances between northern and southern sites (mean northern site distance 1.5° latitude, mean southern site distance 2.2° latitude, p=0.33, two-tailed t-test). Generalized linear mixed modeling also supports a relationship



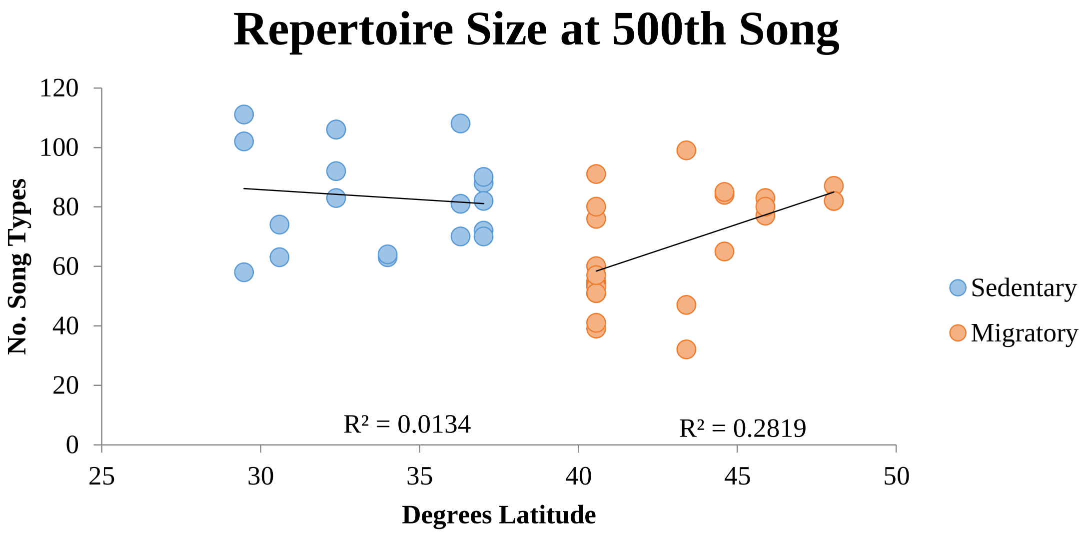


Figure 3.4. Scatterplot of latitude versus total repertoire size at three days for all individuals with at (a) least 500 songs recorded and (b) repertoire size at the 500th song.

between migratory strategy and latitude and song repertoire size (Table 3.4). Interestingly, mean migratory repertoire size does not catch up with mean sedentary repertoire size until 48° latitude (Coal Banks Landing, MT).

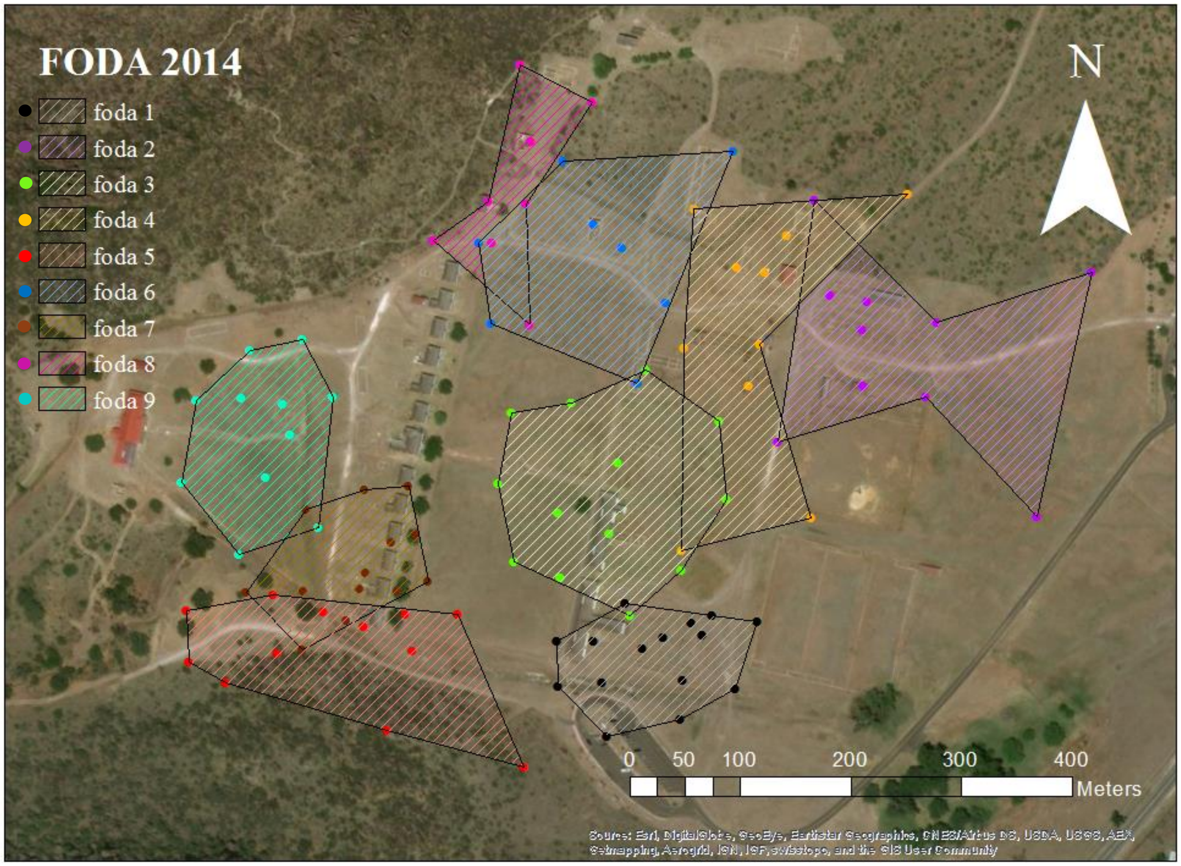
Table 3.4. General linear mixed model of number of song types at 500th song with strategy (migratory or sedentary), site, and strategy x population as fixed effects.

Rank	**Model variables**	***P***	**AICc**
1	strategy x population, strategy	0.0042	376.3
1	strategy x population, population	0.0042	376.3
2	strategy x population, strategy, population	0.0042	376.3
3	strategy	0.0012	379.5
3	population	0.0015	389.6
4	strategy, population	0.0015	389.6

Overall mean territory size ranged from 0.6-2.3 ha per site (10 sites, 53 territories, Table 3.5). Territory size was not significantly associated with strategy (one-way ANOVA, p=0.46, F_{44}=0.54), site membership (one-way ANOVA, p=0.50, F_{44}=0.92), year (one-way ANOVA, p=0.11, F_{44}=2.28), or number of points used to estimate territory size (p=0.30, F_{44}=1.09). Using a stepwise linear regression, no combination of model effects resulted in a better model than the null model. The territories measured at one site, Fort Davis National Historic Site, are illustrated in Figure 3.6 as an example of how rock wrens space themselves. Not all rock wrens present at Fort Davis are represented on the map. More territory maps are presented in Appendix B.

Table 3.5. Mean territory sizes from 10 populations. I measured three populations in two different years, indicated in gray.

Population	**# individuals**	**mean area (ha)**	**median area (ha)**
CBL	8	2.24	2.14
2014	4	2.96	3.25
2016	4	1.52	1.37
MC	7	1.88	1.77
DETO	2	0.49	0.49
BSP	3	1.29	1.21
CNG	5	1.77	1.73
RGG	1	1.32	1.32
BOX	7	2.37	1.83
2014	3	2.86	1.92
2015	4	2.01	1.81
OM	2	0.64	0.64
FODA	15	1.91	1.52
2014	8	2.55	2.88
2015	7	1.18	1.34
BBR	3	1.42	1.52
northern	20	1.48	1.49
southern	33	1.57	1.52



Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Figure 3.5. Exemplar rock wren territories from Fort Davis National Historic Site, Jefferson County, Texas, April 2014.

Discussion

My results are broadly consistent with the hypothesis that migrants experience different selection pressures than non-migrants. If latitude is a good proxy of migratory distance in rock wrens, then repertoire size seems to be correlated with distance migrated. However, it is not commonly predicted that sedentary populations will have larger repertoires than migratory ones.

Song must transmit through its environment in order to function effectively as a signal. The observed patterns of repertoire size change may therefore be indicative of concordant changes in habitat. While trees can be present in rock wren habitat, in general rock wrens are thought to live in “open” (as opposed to “closed,” i.e. forested or covered) habitats. I did not explicitly test the acoustic properties of my study sites in large part because they seem unlikely to play a major role in shaping the size of the song repertoire. Rock wrens sing from the tops of rock formations, cliffs, hills, and short trees and do not generally live in forested areas. The lack of sound attenuating barriers means that rock wren song can be fairly complex without compromising its integrity. Even at high latitudes rock wrens do not live in forests but rather near forests on rocky slopes and canyons. The acoustic adaptation hypothesis (Morton 1975) predicts that each song type is adapted to transmit well through their habitat. Why this would result in smaller or larger repertoires is less clear. Perhaps birds with “small” repertoires appear to be so because they sing only the subset of song types that are best adapted for the habitat they are in. If this is the case, it’s not obvious why they wouldn’t sing other, different song types that do transmit well but preserve whatever signal that repertoire size conveys (condition, genetics, etc.).

A key assumption uniting most hypotheses on the relationship between song complexity, latitude, and migration is that sexual selection pressures are more pronounced at high latitudes and in migrants than at low latitudes or among residents (Catchpole 1982, Morton 1986, Fitzpatrick 1994, Mountjoy and Leger 2001, Weir et al. 2012, Bolus 2014). My results simultaneously agree and disagree with the basic predictions these authors have laid out. Rock wren song repertoire size does seem to increase with latitude in migrants, a prediction made in seven of eight hypotheses (only Bolus’s (2014) panmictic migrants hypothesis predicts otherwise), but sedentary rock wrens have the largest song repertoires overall, a prediction not explicitly made by any authors (Chapter II). Other species where sedentary populations had more song complexity than migratory ones are common yellowthroats (*Geothlypis trichas*, Bolus 2014), eastern towhees (*Pipilo erythrophthalmus,* Ewert and Kroodsma 1994), song sparrows (*Melospiza melodia*, Peters et al. 2000) and fringillid finches (Handley and Nelson 2005, but see Cardoso et al. (2012) who examined the same group and found the opposite pattern).

Sedentary rock wrens have longer breeding seasons, initiating nesting up to two months earlier than migratory wrens. Sedentary populations regularly fledge three successive nests, while migratory wrens are fairly consistently constrained to fledging two (Lowther et al. 2010). This may impose differential selection pressure on migratory and sedentary populations. An extra clutch may alleviate some of the pressure from nest failure or fledgling mortality. The length of the breeding season is inversely correlated with latitude, so the most northern birds are under the greatest pressure to successfully raise their offspring and the loss of a nest may be even more punishing. Without population-level data on clutch size, it's not clear whether the number of offspring reared per nest is similar among migratory and sedentary wrens.

How this pressure translates to repertoire size is an open question that cannot be successfully answered without understanding the signal a large or small repertoire conveys. Recent research suggests repertoire size is more important as a signal between males than as a signal to females (Pitt 2018). Among migratory populations, the expected trend holds. Perhaps larger song repertoires at high latitudes indicate that acquiring and defending a territory is somehow more difficult for migrants at high latitudes. Territorial intrusions may be more common and carry greater risk to the territorial male (e.g. not enough insects available for chicks since neighbors are pilfering them). Song repertoire size may be indicative of some measure of quality in the bird (e.g. condition as a chick, current nutritional status) and is therefore communicating something about the territorial male's ability to fend off intruders (Nowicki et al. 2002). Only the individuals in the best condition can successfully migrate long distances, maintain a territory, and find enough food to fledge offspring.

However, sedentary populations had larger repertoires than migratory ones with no latitudinal gradient, suggesting sedentariness alone is sufficient to maintain this difference. This is contrary to most discussion of this subject (Chapter II) and, consequently, there has been very little literary space dedicated to exploring this idea. If song repertoire size is a largely male-to-male signal, perhaps sedentary individuals face even more challenges acquiring and defending a territory than migrants. Sedentary songbirds vary in their winter territoriality, from highly territorial males and females defending a space with song (e.g. Salomonson and Balda 1977) to nomadic mixed species flocks roaming about a landscape (e.g. Gram 1998). The only mention of rock wren wintering behavior suggests rock wrens become less territorial overwinter (Lowther et al. 2010), although these observations are limited to Kansas where wintering rock wrens are rare and are likely overwintering migrants. Habitually sedentary populations are essentially unstudied. Perhaps sedentary populations aggressively defend their territories year-round, something only the highest quality individuals can do (e.g. Young 1996). This is in direct contrast to a hypothesis put forth by Mountjoy and Leger (2001) who suggested that sedentary individuals acquire their territories largely by chance and that once a territory is acquired it will belong to that male indefinitely. The reality is there are still very few data on how “hard” it is for sedentary birds to acquire and keep their territories, or whether territorial males can be ousted.

Territory size was not different between migratory and sedentary sites, suggesting it is not an important factor determining song repertoire size (Table 3.5). I did not measure any other aspects of territoriality, such as the time to acquire a territory, tenure, or quality in terms of nest site or food availability. One or several of these aspects may be a more important predictor of song repertoire size in rock wrens.

A study of one population did not find that mate choice is influenced by male repertoire size, but inter-sexual selection may be present via other mechanisms. Rates of extra-pair paternity in rock wrens are completely unknown, but sexually-selected signals are thought to be influential in the choosing of extra-pair mates or guarding ones’ mate against them (Spottiswoode and Møller 2004). If repertoire size indicates something about a male’s genetic quality, females may be more likely to seek extra-pair fertilizations with large repertoire males. This could also be threatening to other males who are at risk of losing some or all of their paternity. Repertoire size would thus be a dual signal to both males and females about a male’s genotype, and may not be signaling ability to defend a territory.

While oscine passerines like rock wrens are thought to be fairly flexible in their learning of song, there are many aspects of oscine passerine song that is partially or entirely innate (Beecher and Brenowitz 2005). For example, western marsh wrens (*Cistothorus palustris)* have larger repertoires and HVC[1] volumes than eastern marsh wrens, and this size difference is present at hatching (Kroodsma and Verner 2013). It may be that while individual song types are learned, the number of song types an individual can know is inherently constrained. Migration in passerines is also thought to be largely under genetic control (Pulido 2007). Perhaps the suites of genes controlling migration and song are somehow linked such that migration alleles also tend to be inherited with a particular set of song alleles. Some amount of linkage equilibrium could result in repertoire size being more constrained in migrants. Many genes are thought to be associated with migration and song and it is unlikely that some or even most of them only have two alleles, “migratory vs sedentary” or “large repertoire or small repertoire.” Thus any connection between migration and song alleles will be complicated, but potentially highly informative.

Understanding rock wren migratory behavior is further complicated by the fact that migration is probably not an all-or-nothing trait, and some individuals may overwinter in a place vacated by their migratory conspecifics. Overwintering rock wrens from the migratory part of their range have been reported (eBird 2018), but nothing is known about why these individuals did not migrate. They may have inherited alleles that resulted in the “sedentary” phenotype, they may be flexible in deciding whether to leave or stay, or these individuals might have dispersed from a faraway sedentary population and be inherently sedentary themselves. Future work could attempt to elucidate the genetic connections between migration and song via genome-level sequencing to find the similarities and differences among behavioral types.

This study attempted to document if a migratory or latitudinal gradient in song complexity exists within a species, and whether any observed patterns of song complexity conform to the basic prediction that high latitudes and/or being migratory will result in greater complexity. The results are unexpected – latitude correlates with song repertoire size in migratory, but not sedentary, rock wrens, while sedentary individuals have larger repertoires than most migrants. These results are novel and shine a light on our poor understanding of the life history of passerines in general, their annual cycles in particular, and how these relate to behavior. The best avian species models for song are not models for migration, which are not models of territoriality, which creates difficulty in answering questions that unite these topics. Our expectations of a passerine's life history are based on a theoretical composite bird whose traits come from studies of disparate groups like the sparrows, warblers, and thrushes. It should not be a surprise that studying any one species will result in findings different from our "expectation.". However, such studies are valuable because they increase our understanding of how functional traits actually interact in a species, how they might interact differently among species, and help to dispel the myth of the "average bird."

I have documented a novel pattern of song repertoire size change in rock wren populations that cross a migratory divide. While I have offered some thoughts on what might be driving the observed patterns, much more research is needed to truly begin to unpack and understand these results. Past studies of song complexity within a species almost all suffer from the same problem: Only a few populations are assessed, no patterns are found, and claims are then made that latitudinal gradients and migratory divides are not associated with song complexity. Better study design and sampling may reveal a different scenario for each of the previously studied species. A conservative view on the state of the literature is that we have only just begun to understand how migration and latitude might interact with song complexity in any given species.

Many assumptions of these hypotheses turn on our understanding of a species' migratory behavior. Rock wrens are not well-studied and almost nothing is known about their migration. While some populations disappear over winter and are clearly migratory, it is not known where they go, whether they are philopatric, or if all regions with year-round populations are indeed inhabited by the same individuals year-round. Only 1,093 rock wrens have been captured at banding stations in Canada, the USA, or Mexico since 1960 and none have been recovered (USGS 2018). Any discussion on rock wren life history and song complexity must be tempered by an acknowledgment that we have very little definitive information on their migratory habits. That being said, it is nevertheless interesting to speculate on the potential causes of the differences in repertoire size among migrants and non-migrants.

CHAPTER IV

MIGRATORY MORPHOLOGY AND POPULATION GENETICS OF ROCK WRENS

Contribution of Authors and Co-Authors

Manuscript in Chapter IV

Author: Nadje A. Najar

Contributions: Conceived study design, provided samples and funding, conducted lab work, analyzed genetics and morphological data, wrote the manuscript.

Co-Author: Dr. Garth Spellman

Contributions: Conceived genetics methods, provided samples and funding, generated SNP dataset, assisted in genetics data analysis.

Co-Author: Dr. Lauryn Benedict

Contributions: Helped conceive study design, provided samples and funding, edited drafts of the manuscripts.

Co-Author: Jenna McCullough

Contributions: Aided with genetics lab work.

Co-Author: Dr. Mike Andersen

Contributions: Provided reagents and lab space for genetics lab work.

Abstract

Song repertoire size varies with both latitude and migratory status in rock wrens, but very little is known about their migration. Migration and geography are thought to impose strong selection pressures on bird morphology, physiology, genetics, and behavior. The goal of this study was to test whether prior classification of rock wren populations as migratory or sedentary is supported by morphological or genetic data. We sampled rock wrens at 11 sites along a latitudinal transect spanning a migratory divide and measured hand-wing index, mass, and the lengths of the tarsus, culmen, wing chord, the first secondary, and tail. DNA was collected for genome scans for SNPs using targeted sequence capture to assess population genetic structure. Putatively migratory populations had smaller wing chords, tails, and culmens, and larger tarsi than putatively sedentary populations. Some population genetic structure was resolvable using outlier loci, but did not yield groups consistent with prior predictions. The combination of morphological traits diverging along a migratory divide without corresponding genetic structure suggests migration does not pose a barrier to gene flow.

Introduction

In the previous chapter, I focused on the relationship between song repertoire size, latitude, and migration in rock wrens. Because few wren species migrate (del Hoyo et al. 2018), we know very little about the routes taken by those that do (Taylor et al. 1983, Johnson and Wise 1999) and essentially nothing is known about rock wren migration (Lowther et al. 2000). However, the ecological and sexual selection pressures imposed by migratory behavior and high-latitude living are the cornerstones of nearly every hypothesis proposed to explain why these forces should shape song complexity (Chapter II). If the assumptions of these hypotheses are correct, then we should expect to see the effects of selection for migratory or sedentary behavior in rock wrens.

Adaptation for migration is fundamentally adaptation for efficient locomotion. In birds, this generally means the flight apparatus (wings and tail) is modified in some way. Migratory species are most often characterized by wing morphology with long distal primaries, short proximal primaries, and short secondaries (Rayner 1988, Winkler and Leisler 1992, Egbert and Belthoff 2003) and shorter, more squared tails (Leisler and Winkler 2003, Hedenström 2008). Wing shape and size can directly impact factors associated with flight efficiency, such as wing loading and drag (Hedenström 2008).

Body size and mass are critical to volant species, although the relationships between migratory behavior, latitude, and body size are not as clear cut. Migratory species are generally larger and occur farther north than sedentary species, which are smaller-bodied, a pattern held up by many studies and between many taxa, including North American birds (Blackburn and Gaston 1996b). A variety of hypotheses have been proposed to explain this observation (Blackburn et al. 2008). Larger bodied birds may be less susceptible to starvation (Lindstedt and Boyce 1985), better able to conserve heat in typically cooler environments (Bergmann 1847), or are better able to disperse long distances (Newton and Dale 1996). Of course, it is always possible that mass is linked to some other trait that is advantageous at high latitudes or in migratory species (Blackburn and Gaston 1996a) and within-species or phylogenetically controlled comparisons do not always find this pattern (Blackburn et al. 2008), making it difficult to predict what effect, if any, migration or latitude will have on body size within a given species.

Migration requires a suite of adaptations for navigation, efficient metabolism, and flight (Chapter I). These requirements may impose a barrier to reproduction between migratory and sedentary birds since the "wrong" alleles could result in a costly, possibly fatal, phenotype in offspring (Berthold 1990). That being said, it is also possible that most, if not all individuals, migrant or not, have the required genetic background to migrate given certain environmental triggers, and migratory or sedentary behavior can evolve rapidly in birds (Berthold and Helbig 1992, Pulido and Berthold 2010). Partially migratory species, where not all individuals migrate, are particularly interesting to study because they may represent an evolutionary transition from one state to the other (Pulido 2011). Pulido et al. (1996) proposed a threshold model of genetic variation to explain the amazing lability of this behavior given the frequently uniform expression of the phenotype (i.e. all birds in a region do or do not migrate), wherein migratory "liability" is a normally distributed continuous trait tied to a gene, such as hormone concentration. A bird exhibits migratory behavior once the liability concentration exceeds some threshold. Pulido (2011) expanded on this model to allow environmental variation to raise or lower the threshold, although what exactly is the liability is presently unknown.

While obligate migrants can show strong genetic differentiation along migratory divides or between migratory flyways (e.g. Kelly et al. 2005, Rolshausen et al. 2009), this is not always the case (Linossier et al. 2016). Differences in song may (MacDougall-Shackleton and MacDougall-Shackleton 2001) or may not (Lougheed and Handford 1992, Wright and Wilkinson 2001) be associated with genetic population structure, a situation likely influenced (and confounded) by the fact that song is learned in oscines. The interaction among genetics and song in a facultative migrant may be even more complicated and difficult to predict.

Rock wrens disappear each winter from most of the northern half of their range, their destinations remaining a mystery (Lowther et al. 2000). Rock wrens are rarely banded and have never been recovered at a location other than where they were initially marked (USGS 2018 pers. obs.). Without knowing the specifics of their migrations, it is difficult to estimate just how strong of a selective force migratory behavior imposes. At least three scenarios could result in the observed breeding and wintering distributions of rock wrens. Migrant rock wrens could be dispersing evenly throughout the wintering distribution, such that migrants and residents are intermixed (Fig 4.1a). Rock wrens could be ‘leapfrog’ migrants, where the southernmost migrants travel a very short distance to mix with residents, and the northernmost migrants travel very far south (Fig 4.1c). Perhaps most rock wrens are migratory and they all migrate some short or intermediate distance, so that northern birds displace southern birds (Fig 4.1b). For any migratory route scenario, rock wrens may be highly facultative migrants and any given individual may or may not migrate in a given year.

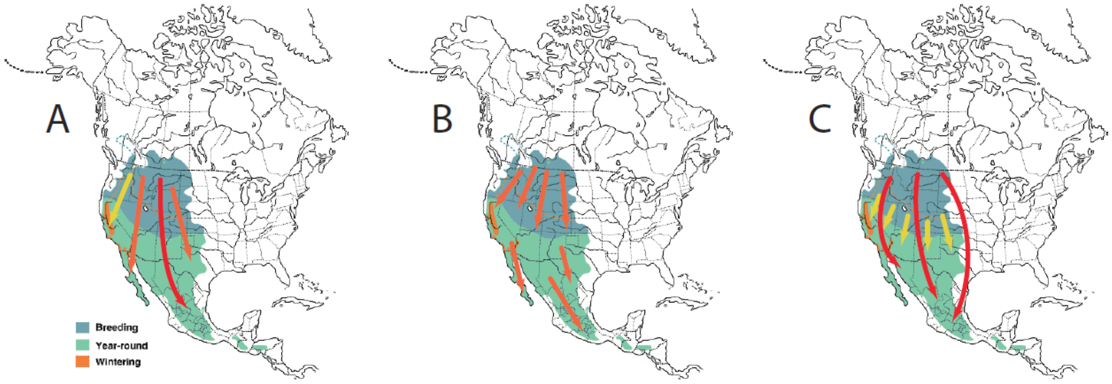


Figure 4.1. Three possible strategies for migration in rock wrens. Even mixing of residents and migrants (a), universal equidistant migration (b), and leapfrog migration (c). Rock wren range map modified from the Birds of North America online (birdsna.org).

I attempted to determine whether the classification of migratory and sedentary rock wrens I outlined in Chapter IV was concordant with the expectations outlined in previously published literature. To that end, I used morphological measurements and SNP data from individuals captured at the same 11 populations in which I recorded rock wren song (see Chapter II). If migratory strategy imposes differential selection pressures on migratory versus sedentary birds, then I expect that migratory rock wrens and sedentary rock wrens will be morphologically and genetically distinct. Specifically, I predict that migratory, high-latitude rock wrens will have longer wing chords, larger hand-wing indices, longer tarsi, larger masses, and shorter tails than sedentary, low-latitude rock wrens. Migratory and sedentary rock wrens will be identifiable as separate genetic clusters using population genetics approaches, and the migratory divide will be located in central Colorado between Comanche National Grasslands and Horsetooth Reservoir.

Methods

Handling

Rock wrens were captured using mist nets in 2015 and 2016. Birds were lured into the net with playback of conspecific song and, on occasion, a taxidermied rock wren mount. Each wren was marked with a unique combination of plastic color bands and a USFWS metal band. Mass and the lengths of the culmen, tarsus, wing chord, first secondary, and tail were measured. Approximately 15µl of blood was taken by puncturing the brachial vein with a 26-gauge hypodermic needle and drawing blood with a capillary tube. Blood was stored in Longmire's solution without refrigeration in a chest cooler until the end of the field season, after which samples were frozen at -20°C. Finally, a photograph of the outstretched wing was taken against a 1cmx1cm grid. The bird was then released.

I was able to capture 109 rock wrens. Of these, 107 were adults and 2 were juveniles. Of the 107 adults, 96 were males and 11 were females. The Horsetooth Reservoir population was managed by Dr. Benedict, who captured 15 individuals in 2015 and 2016 (Table 4.1).

Table 4.1. Demographics of captured rock wrens.

Abbreviation	Locality	Total captures	Males	Females	Juveniles
CBL	Coal Banks Landing, MT	9	7	1	1
MC	Milligan Canyon, MT	12	12	0	0
DETO	Devils Tower, WY	12	9	3	0
BSP	Boysen State Park, WY	12	12	0	0
FOCO	Horsetooth Reservoir, CO	15	14	1	0
CNG	Comanche National Grasslands, CO	12	9	3	0
RGG	Rio Grande Gorge, NM	12	11	1	0
BOX	The Box National Recreation Area, NM	12	11	1	0
OM	Desert Peaks National Monument, NM	9	9	0	0
FODA	Fort Davis National Historic Site, TX	13	10	2	1
BBR	Big Bend Ranch State Park, TX	6	5	1	0

Hand-Wing Index

As flight is the means by which migrants reach their breeding sites and reproduce, migration is thought to represent a strong selection pressure on the morphology of bird wings. It has long been noted that migratory birds have “pointier” wings (i.e. the feathers proximal to the leading edge of the wing are longer than those distal to it, like a dove or falcon wing) than non-migrants (Swaddle and Lockwood 1998, Hedenström 2008). Wing-pointedness is highly correlated with migratory distance and can be used to infer whether one population migrates farther than another population. To infer wing-pointedness, I visualized photographs of outstretched rock wren wings and measured the lengths of the wing chord and first secondary in imageJ (version 1.8 Schneider et al. 2012) using the line measure tool after scaling. Each feather was measured three times and the average was used as the final measurement. Kipp’s hand-wing index (Kipp’s index, Kipp 1959) was calculated using the following formula: ((Wing length-1st secondary length)/1st secondary length)*100. While numerous indices have been proposed to describe wing shape, and in particular, wing pointedness, Kipp’s index is most suited to measuring overall proportions of the handwing and is closely correlated with aspect ratio (Lockwood et al. 1998). Kipp’s index is significantly confounded with body size, but this is largely a problem for interspecific comparisons (Lockwood et al. 1998), whereas I am comparing measurements among individuals from the same species and largely from the same age and sex class.

Statistical Analysis of Morphology

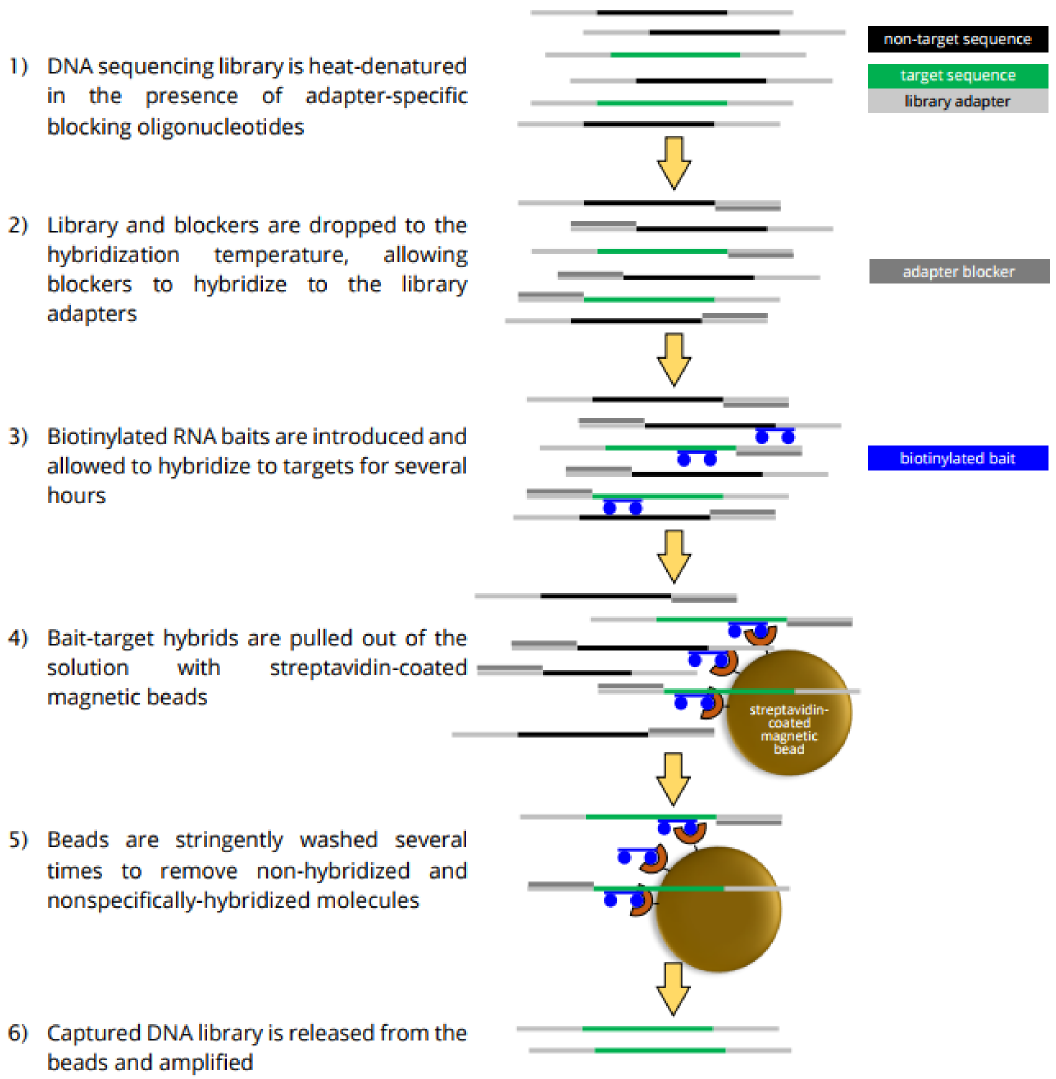
I used bivariate analysis and one-way ANOVA to identify significant associations between morphological measurements and migration strategy (alpha=0.05). To correct for multiple comparisons I used the Benjamini-Hochberg procedure with a false-positive rate set at 10% (Q=0.1).

Deoxyribonucleic Acid (DNA) Enrichment

DNA was extracted from blood samples with DNeasy kits using the tissue protocol (Qiagen, Inc.). Whole, extracted DNA was prepared for target sequence capture using a MyBaits kit (MYcroarray, Inc.) (Fig 4.2) containing both custom and pre-designed ultraconserved element (UCE) probes. Genes thought to be associated with migration, morphology, and song were discovered via literature search (Appendix C). Probes to capture the custom exons were designed and manufactured for this project by MycroArray using the annotated zebra finch genome (*Taeniopygia guttata*) as a reference. A total of 3,000 randomly selected UCE loci and 246 custom loci using ~12,000 unique probes were included in the final probe set. This project was conducted in collaboration with Dr. Garth Spellman at the Denver Museum of Nature and Science, who contributed 56 samples and funding while we (myself and Dr. Benedict) contributed 118 samples, funding, and lab work. While Dr. Spellman was working on a different rock wren project, the following steps were performed on all 174 samples together.

Whole genomic DNA was sheared on a Covaris M220 focused-ultrasonicator to generate approximately 500bp sized fragments at 10ng/µl. All sample concentrations were determined with a Qubit fluorometer using the high-sensitivity kit (Life Technologies, Inc.). Samples were end-repaired, adenylated, and dual-indexed following a KAPA Hyper Prep kit protocol (KAPA Biosystems) using 0.8X SPRI bead clean-ups and Illumina TruSeq adapters (Illumina, Inc.). Each sample was dual-indexed with a unique combination of iTru5 and iTru7 series adapters using the following PCR protocol: 98°C for 45s; 10 cycles of 98°C for 15s, 60°C for 30s, 72°C for 30s, with a final extension of 72°C for 1 minute.

Libraries were pooled in lots of 8 individuals for up to 500ng of total DNA (up to 62.5ng per individual) and concentrated to 7 µl in ddH_2O using a SpeedVac. Blocking mix was assembled according to the MyBaits protocol, with the exception of the substitution of block 1 (which was provided in the kit) with Chicken COT-1. Hybridization mix was assembled according to the protocol. Samples were incubated at 65°C for 24 hours and then bound to streptavidin dynabeads (Invitrogen, Inc.). Beads were washed and then 15µl captured DNA was amplified with 25µl KAPA HiFi HotStart ReadyMix, 5µl ddH_2O, 2.5µl each of Illumina library primer (at 10µM) using the following PCR protocol: 98°C for 2 minutes; 16 cycles of 98°C for 20s, 60°C for 30s, and 72°C for 60s, with a final extension of 72°C for 5 minutes. After a final 1.2X SPRI bead clean-up, 2µl of sample was used to quantify the concentration with a Qubit fluorometer. Small (<150bp) fragments were removed using a GeneRead Size Selection kit (Qiagen, Inc.). Of 174 starting samples, 167 were successfully amplified and pooled. These samples were shipped to the Oklahoma Genomics Resource Facility for sequencing on an Illumina HiSeq 3000 on one lane.



1) DNA sequencing library is heat-denatured in the presence of adapter-specific blocking oligonucleotides

2) Library and blockers are dropped to the hybridization temperature, allowing blockers to hybridize to the library adapters

3) Biotinylated RNA baits are introduced and allowed to hybridize to targets for several hours

4) Bait-target hybrids are pulled out of the solution with streptavidin-coated magnetic beads

5) Beads are stringently washed several times to remove non-hybridized and nonspecifically-hybridized molecules

6) Captured DNA library is released from the beads and amplified

Figure 4.2. Summary of the target capture process. Blockers are hybridized to library adapters, then baits hybridized to their targets for 24 hours. Streptavidin-coated beads bind to biotinylated baits and non-bound DNA is washed away. Captured DNA is amplified via PCR. From the MYbaits Manual v. 3.02 (MYcroarray, Inc.).

Genetics Analysis

A total of 158 samples were successfully sequenced. Demultiplexed FASTQ sequences were cleaned for adapter contamination and low quality sequences using ILLUMIPROCESSOR (Faircloth 2013) and TRIMMOMATIC (Bolger et al. 2014). Cleaned sequences were assembled into contigs using the ABYSS program (Simpson et al. 2009) executed in the PHYLUCE package (Faircloth 2015), which removes low quality sites in addition to assembling contigs. We generated 75% and 95% complete matrices in PHYLUCE using the ‘match contigs to probes’ function to identify the four individuals with the best coverage. These individuals were aligned to the UCE and exon probes sets in GENEIOUS (version 11.1.4., Kearse et al. 2012) to generate pseudoreferences. All other individuals were mapped to the psuedoreferences to identify variable sites for the UCE and exon probe sets using BWA and SAMTOOLS. Variants were called in GATK (version 4.0, Van der Auwera et al. 2013) to output two single-nucleotide polymorphism (SNP) datasets, a 75% and a 95% set (i.e. 95% or 75% of individuals share that variable site). At least three individuals had to have a polymorphic site relative to the pseudoreference for a locus to be called as a SNP.

The rest of the analyses are on the subset of 96 individuals sequenced from my focal transect of 11 populations. I used the R package OutFLANK (Whitlock and Lotterhos 2015) to identify outlier loci with the minimum heterozygosity of an allele set at 10% and the q threshold (false positive rate) set at 0.05. Outlier analysis is extremely sensitive to the number of populations you specify so I varied the number of “populations,” with two (i.e. migratory vs sedentary), three (migratory, sedentary, intermediate), and eleven (site membership) groups. I noted which SNPs were identified as outliers in multiple grouping schemes. I used the output from OutFLANK to identify which loci had any outlier SNP, which loci had multiple SNPs, and which loci were marked as outliers in multiple population classification schemes. I also used the output of the eleven population outlier analysis to generate a new SNP dataset consisting only of outliers. Outlier loci are interesting to study because they are likely under some selective pressure (Storz 2005).

I used FASTSTRUCTURE (Raj et al. 2014), which implements a variational Bayesian method, to assess genetic population membership among my eleven sampled populations. Using the full 75% data set and the outlier set I ran K=1 through K=15 with a logistic prior and used the built-in cross-validation function to identify the best supported value of K. I used the R package ADEGENET (Jombart and Ahmed 2011) to run discriminant analysis of principal components (dapc) on the full and outlier data sets as an alternative method to identify the best supported group number. For each dapc, I chose the number of principal components that describes ~95% of the data since this analysis is susceptible to overfitting. I used VCFTOOLS (Danecek et al. 2011), SAMTOOLS (Li et al. 2009), and PLINK (version 1.9, Purcell et al. 2007) throughout the data analysis process to convert amongst data input formats and rename and remove samples as needed.

Ethics and Permitting

All research was conducted with institutional, federal, and state permissions as required, and permitting agencies were accordingly reported to. All birds were handled according to Guidelines to the Use of Wild Birds in Research (Fair et al. 2010). The following is a list of permits received:

Federal – 23741 B Najar

IACUC – UNCO: 1105C-LB-Birds

NPS: IMR_FODA_Najar_RockWren_2015.A2,

IMR_DETO_Najar_RockWren_2016.A2

Montana – 2016-040

Wyoming – 33-1005

Colorado – trB2041

New Mexico – Najar 3582

Texas – SPR-0315-031

Results

Morphology

The results of morphological analyses are presented in Table 4.2. Tail length was correlated with migration strategy ($p=0.0001$, $F_{107}=18.02$, one-way ANOVA) and latitude ($p=0.0003$, $F_{107}=9.64$, $R^2_{adj}=0.10$); birds at higher latitudes had shorter tails. Tail length was associated with the other direct feather measurement, wing chord ($p=0.0001$ $F_{107}=63.23$, $R^2_{adj}=0.34$), so that birds at high latitudes had both shorter tails and smaller wing chords. Wing chord was correlated with migration strategy ($p=0.05$, $F_{128}=4.64$, one-way ANOVA) and latitude (migrants have shorter wing chords, $p=0.03$, $F_{128}=4.64$, $R^2_{adj}=0.028$). Tarsus was significantly associated with migration strategy ($p=0.0001$, $F_{127}=18.75$, one-way ANOVA) and overall latitude (migrants have larger tarsi, $p=0.024$, $F_{127}=5.l9$, $R^2_{adj}=0.032$). Mass and hand-wing index were not associated with any other metrics after correcting for multiple comparisons using the Benjamini-Hochberg procedure.

Table 4.2. Summary of morphological measurements of migratory and sedentary rock wrens. All measurements are of adult males. WC = wing chord length, S1 = first secondary length, HWI = hand-wing index.

	Migratory	Sedentary	Strategy		Latitude	
	mean (n)	mean (n)	*P*	F_{crit}	*P*	F_{crit}
WC	70.1 (72)	70.7 (56)	**0.05**	3.89	**0.03**	4.64
S1*	59.8 (46)	60.1 (55)	0.57	0.31	0.97	0.0008
HWI*	16.46 (46)	15.44 (55)	0.13	2.3	0.24	1.39
Tail	50.5 (52)	52.7 (55)	**0.0001**	18.02	**0.0003**	13.69
Tarsus	21.9 (71)	20.7 (56)	**0.0001**	18.75	**0.024**	5.19
Mass	15.8 (69)	15.6 (56)	0.18	1.81	0.25	1.31
Culmen	18.4 (72)	19.1(56)	**0.0048**	8.23	0.77	0.08

*measured digitally using imageJ.

Table 4.3. Predictions versus outcomes for morphological measurements. Predictions are on the left column with a gray background and outcomes are on the right with a white background. WC = wing chord length, S1 = first secondary length, HWI = hand-wing index.

	Migratory		**Sedentary**		**Latitude**		**As Predicted?**
WC	larger	smaller	smaller	larger	increases	Decreases	no
S1*	**no diff**	**no diff**	**no diff**	**no diff**	**no diff**	**no diff**	yes
HWI	larger	no diff	smaller	no diff	increases	no diff	no
Tail	smaller	**smaller**	larger	**larger**	decreases	**decreases**	yes
Tarsus	larger	**larger**	smaller	**smaller**	increases	**increases**	yes
Mass	larger	no diff	smaller	no diff	increases	no diff	no
Culmen	no diff	smaller	no diff	larger	no diff	**no diff**	no/yes

SNP Discovery and Outlier Analysis

Two datasets were generated using the pipeline described in the methods, a 95% completeness set with 32,478 SNPs, and a 75% completeness set with 185,504 SNPs. No rock wren in the transect had more than 3% overall missing data (n=96), so I used the larger SNP set for subsequent analyses.

OutFLANK identified 636 SNPs out of 185,504 (0.3%) as candidate outliers using a prior population assignment of 11 (each sampled population is a separate comparison group) (Table 4.4). I used the outliers identified from this “sampling location” grouping for subsequent analyses, in large part because this assignment scheme is a natural one and resulted in a moderate number of outlier loci. Other schemes resulted in 18-5313 outliers (Table 4.4). Of the “sampling location” outliers, 358 (57%) were located on targeted sequences (e.g. exons and introns) and 278 (43%) were located on UCEs. A total of 98 targeted genes had at least one outlier locus (39.8%), and the distribution of outliers is laid out in Figure 4.3. Of 3,000 UCEs sequenced, 216 had at least one outlier (7.2%).

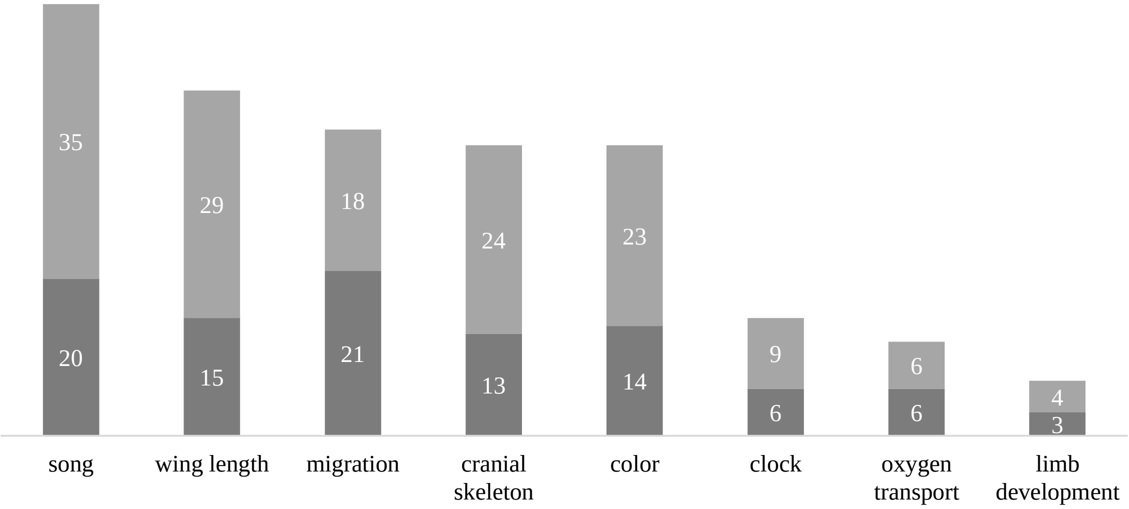


Figure 4.3. Proportions of genes with outliers versus number of genes sequenced for a given function. The number on the bottom, green portion of a bar is how many genes had outliers, the number on the top, gray portion of the bar is how many genes did not have outliers. This distribution is for outliers identified from population assignment A.

Population Assignment

I ran FASTSTRUCTURE on two datasets; the full SNP set with 185,504 loci and the reduced set with 636 outliers. The best supported K for the full set was K=1, suggesting panmixia. The best supported K for the reduced outlier set is K=10 (marginal likelihood=-0.263), closely followed by K=3 (marginal likelihood=0.269) and K=2 (marginal likelihood=0.279) (Figure 4.4).

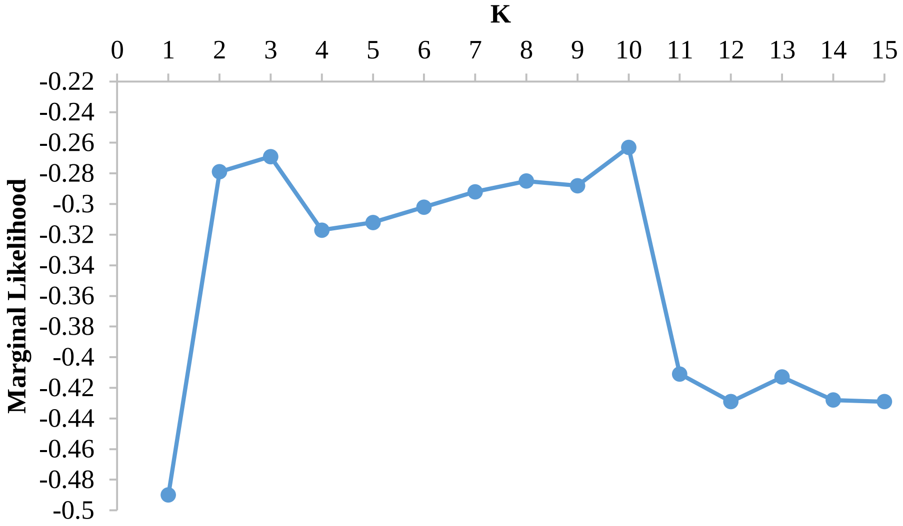


Figure 4.4. Summary of marginal likelihoods for each prior population assignment (i.e. K) for FASTSTRUCTURE assignments using outlier loci.

Population structure elucidated from the outlier dataset is illustrated in Figure 4.5. At K=2, the largest group includes all individuals except three (two FODA and one CBL), and 10 individuals are classified as “admixed,” mostly belonging to the southern, sedentary end of the transect, and seems to be dominated by individuals from Fort Davis National Historic Site. At K=3, the group split from K=2 remains, but a new group containing all of Big Bend Ranch, two individuals from Horsetooth Reservoir, one individual from Boysen State Park, and all of Coal Banks Landing is resolved. At K=10, there are four main groups, not ten. The largest group includes all of The Box, Comanche National Grasslands, Horsetooth Reservoir, and Devils Tower, and all but one individual from each of Milligan Canyon and Boysen State Park. The second largest group includes all but one individual from each of Rio Grande Gorge and Coal Banks Landing, the third group includes all but one individual from Big Bend Ranch, and the fourth group is highly admixed but is mostly represented by individuals from Fort Davis.

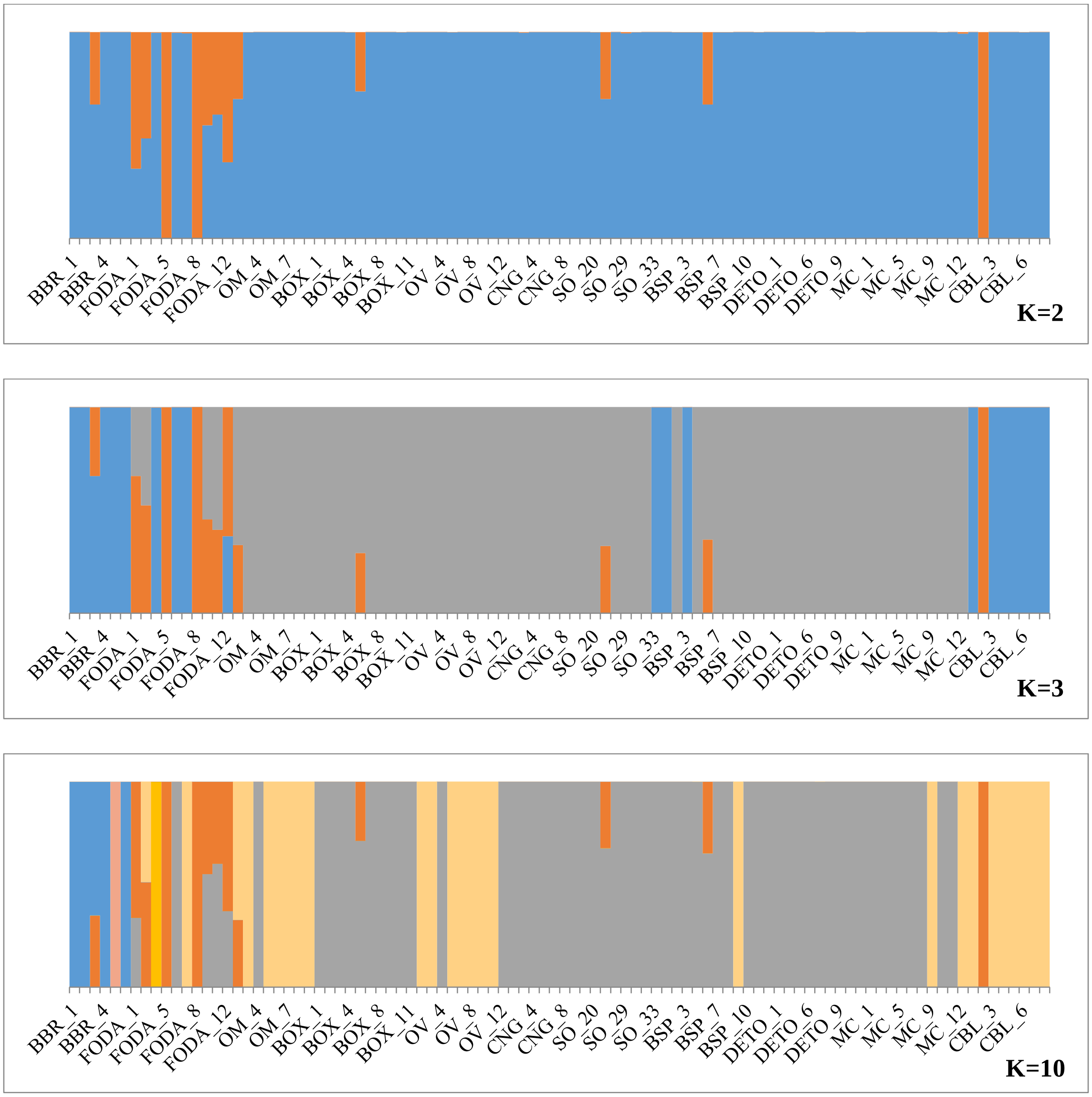


Figure 4.5. FASTSTRUCTURE plots for K=2, K=3, and K=10. Organized by population, with the populations progressing from south to north going left to right. Colors represent group assignment. I hypothesized the migratory divide is between CNG and FOCO (SO) in the middle of the plots.

The best groupings from discriminant analysis of principal components (dapc) were K=3 and K=4 (Figure 6, Appendix D). At K=3, dapc recovers BBR, CBL, MC, RGG, most of BOX, and three individuals from CNG as belonging to one group (group 1, green), the rest of the BOX, MC, and CNG, BSP, DETO, OM, and all but one individual from FOCO, and a few individuals from FODA, and one RGG bird as belonging to group 2 (navy blue). Group 3 is comprised mostly of FODA along with one individual from each of BBR, BOX, BSP, CBL, OM, and FOCO.

At K=4 all of BBR, all but one CBL, and one RGG individual are separated as a group (group 8, purple). The largest group is comprised of all of the BOX, BSP, CNG, DETO, MC, OM, RGG, and all but one FOCO along with three FODA individuals (group 7, sky blue). The smallest group is only two individuals from FODA (FODA5 and FODA8) (group 5, orange), and the final group is comprised of the same individuals as K=3 group 3 and contains the other half of FODA along with one individual from each of BBR, BOX, BSP, CBL, OM, and FOCO (group 6, brown).

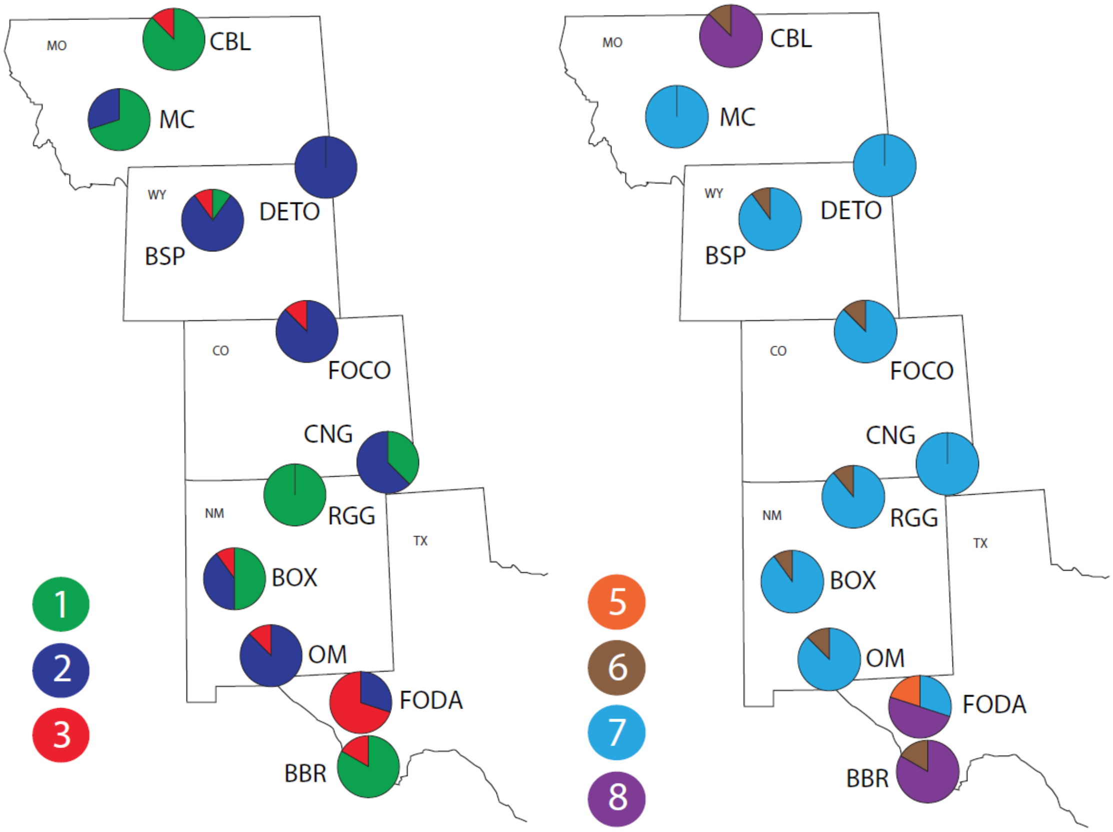


Figure 4.6. Graphical illustration of population assignments by dapc. K=3 (groups 1-3) on the left, K=4 (groups 5-8), on the right.

There is no clearly identifiable split between migrants and residents (statistically or otherwise) and no analysis recovered a purely sedentary or migrant group (with the exception of dapc K=4 group 5, which only includes two FODA birds). Groups are also not generally defined by their geographic locations, and even at K=10 I did not recover 10 populations but rather four larger groups that span the entirety of the transect.

Discussion

Migratory Morphology

Migrants are thought to be under strong selective pressure to optimize their flight efficiency, and common adaptations in migrants are longer, pointier wings, shorter tails, and larger body sizes (Pulido 2007, Hedenstöm 2008). Migration is thought to be largely genetically controlled (Berthold 1990), although environmental factors may play a major role in whether an individual will migrate (Pulido 2011). Rock wren morphology and population genetics are partially consistent with these predictions. In practice, however, which genes are most important, how heritable they are, and the conditions that regulate them are effectively unknown. Migratory status or latitude predicts some morphological measurements in rock wrens. The two direct feather measurements, wing chord and tail, are most strongly associated with both migratory status and latitude (Table 4.1). Tail and wing chord length are also very strongly associated with each other, suggesting the same mechanism controls overall feather length. Hand-wing index was not correlated with strategy or latitude.

Of the two body size measurements (tarsus and mass), tarsus was significantly correlated with both latitude and strategy, while mass was not significantly correlated with either. Overall, the difference between migratory and sedentary tarsus size is associated with latitude, it is much more strongly correlated with strategy alone (Table 4.1). Looking within each group (migratory versus sedentary), tarsus increases with latitude in residents (although not significantly so) but is essentially constant among migrants. This is only partially in line with my prediction that tarsus will be most strongly predicted by latitude, and as a consequence will also be associated with strategy. These results suggest a divide between migratory and sedentary individuals. While latitudinal gradients in mass are commonly studied (Blackburn et al. 2008), mass is not as good an indicator of body size in birds because it can fluctuate from day to day, while the tarsus is a bone and highly correlated with the overall size of the skeleton (Senar and Pascual 1997).

Feather measurements, while associated with migration and latitude, were not correlated in the direction I predicted. Migrants have smaller tails and wing chords than residents, despite having larger tarsi. While I predicted migrants should have smaller tails, I also predicted they should have longer wing chords. I did not measure wing area, but the combination of shorter wing chords and secondary feathers (Table 4.1) strongly suggests that wing area is smaller in migrants. Even if migrants did not gain mass to migrate, they would have higher wing loading (mass per wing area) than sedentary birds. Most notably, hand-wing index does not differ at all between migrants and residents, suggesting wing size but not wing shape is under selection.

This is a peculiar state completely contrary to my predictions about how wing shape should change with migratory strategy. Wing shape is most often studied in species that undertake long distance and/or non-stop migrations (e.g. *Phylloscopus* warblers, Marchetti et al. 1995, *Acrocephalus* warblers, Peiró 2003), although some short distance migrants have been studied as well (e.g. dark-eyed juncos, Mulvihill and Chandler 1990). Overall, it is most common to find that migrants have longer and/or pointier wings than sedentary birds (Hedenström 2008). Most wrens are not migratory and morphologically are classic examples of adaptation to terrestrial, closed environments requiring maneuverability and rapid take-off (Norberg 1995). Rock wrens are almost certainly not making flights over open water or through vast stretches of inhospitable habitat since they are adapted to forage in rocky hills, plains, and desert with little regard for tree cover or water (Lowther et al. 2000). In that case, they may be less constrained by the need to reach particular stopover sites than other migratory species, resulting in little to no selection for the typical migratory wing phenotype.

Genetic Structure

In contrast to morphology, genetics do not seem to predict migratory behavior in any manner. Neither program (FASTSTRUCTURE or ADEGENET) clustered individuals by sampling location or (presumed) migratory behavior. The full data set consisting of 185,504 loci could not resolve any population structure (best K=1), and only by reducing the data set to outlier loci could any structure be found. Both programs broadly agree in their assignment of groups, with BBR and CBL falling out as one population, FODA comprising another, highly admixed population, and the rest belonging to one or two very large populations. Critically, there is no switch or transition from one population assignment to another in either Colorado population (CNG or FOCO) that would correspond with a switch in migratory strategy. These programs cluster genetic data in very different ways, with FASTSTRUCTURE using a Bayesian method to generate an optimized model of evolution, and ADEGENET using discriminant function analysis of the principal components of the data, which has no a priori model of evolution. This suggests these results are not an artifact of the methodology employed but rather accurate reflections of genetic similarity.

The inability of these programs to resolve population structure from the full data set, and only detecting structure using outlier loci presumably under selection, strongly suggests that all 11 rock wren populations in my transect are highly admixed and effectively constitute one large population. If migration indeed imposes a selective filter such that only certain migratory alleles can pass through, then I can only conclude that I did not find those alleles.

The structure resolved from outlier loci could be indicative of several possibilities. First, the more isolated and sedentary a population is, the more divergent we expect it to be from other populations. This could mean FODA, BBR, and CBL, are short-distance/poor dispersers while the other populations are all migratory and/or long-distance dispersers. This scenario would suggest the migratory divide is actually further south than I assumed, and birds overwintering in New Mexico and southern Colorado are migrants from further north (similar to the possibility depicted in Fig 4.1b). This could explain why FODA is the most admixed population, with individuals assigned to all other groupings present. I did not sample birds in the winter so I could not test the potential migratory connectivity of wintering and breeding populations.

Second, if outliers are under selection, then CBL and BBR might be grouping together because they share alleles adapted for some common selective pressure. I have assumed that, as my northernmost population, CBL birds are migrating the farthest. It is possible that these individuals are not migrating very far or at all. There are not many rock wrens in central Montana, and most of them are concentrated on the sand banks of the Missouri River (eBird 2018). This particular spot is not commonly visited by birdwatchers in the winter and even common winter birds are not reported here. While it is not very likely that these birds are sedentary in Montana in general since they are insectivorous, it is possible that they are wintering along the river where it is slightly warmer or migrating a relatively short distance to Idaho or Washington where rock wrens are regularly documented to overwinter along rivers (eBird 2018). However, FASTSTRUCTURE clusters CBL with RGG and OM at K=10 and not BBR.

Perhaps CBL and BBR are grouped together by the chance sharing of alleles that are under selection for something other than migration. CBL birds had among the largest song repertoires of any migratory population, so perhaps it is similarity in alleles on genes associated with song that is driving this grouping. We also sequenced a random assortment of 3000 UCEs, some of which had outlier loci. It could be these UCE outliers that are driving the grouping of CBL and BBR.

While the ends of the transect keep falling out as somewhat unique, the analyses consistently had difficulty differentiating among sampling locations in the middle. Bayesian assignment generally did not split up sampling populations (i.e. all individuals from DETO were classified in the same group) suggesting that individuals from any given sampling location are relatively indistinguishable from their neighbors, but that they are also indistinguishable from individuals sampled over 1500 km away. This pattern suggests high levels of gene flow among these populations, even more so than amongst all rock wrens in the transect, and possibly common selective pressures.

Migratory Syndrome in Rock Wrens

While the pattern of morphological measurements is, overall, consistent with the hypothesis that there is some ecological difference between migratory and sedentary rock wrens, the genetic population structure does not support this idea. If I have misclassified which populations are migratory and which are sedentary, then it is difficult to explain the pattern of both song repertoire size (Chapter IV) and morphology. I suspect my classification of migratory and sedentary individuals is largely correct, but that the assumption that migration is so strongly genetically controlled that it is sufficient to isolate these two groups is not. We cannot rule out the possibility that migration is entirely facultative in rock wrens and that any rock wren, even one transported from a southern site, could successfully navigate to suitable wintering grounds. It is fairly well established now that migration is not all-or-nothing and can be highly influenced by local environmental conditions. Many factors, such as local food availability, competition, or physical condition, may influence whether a given individual will migrate or not (Pulido 2011). There are many reports of rock wrens overwintering even at high latitudes (Lowther et al. 2000, eBird 2018) and it is entirely possible that this is a common occurrence.

I set out to assess whether I would see a pattern of morphological measurements and genetic structure concordant with my predictions of how they should be influenced by migration. The reality is more complicated than I expected. This may be due, in part, because we tend to study mostly obligate migrants with discrete wintering and breeding ranges, like thrushes (Ruegg et al. 2006, Ruegg et al. 2014) and warblers (Paxton et al. 2007, Ruegg et al. 2014). These are the species most likely to exhibit more extreme adaptations for migration, probably because they have been obligate migrants for a long time. Warblers, for example, are thought to have evolved in North America from migratory ancestors, with sedentariness being a derived state (Winger et al. 2014). In contrast, the position of rock wrens in the most recent wren phylogeny is somewhat ambiguous, with rock wrens either the most basal wren, or sister to the most basal wren (Barker 2017). This makes interpreting the origins of migration in wrens more difficult, although given the rapid modern expansion of rock wrens (eBird 2018) and the general lack of migratory wren species (del Hoyo et al. 2018) I suspect that rock wren migration is derived. While rock wren tail and tarsus length change with latitude and migratory status as I expected, wing length and hand-wing index do not. The overall lack of population genetic structure reveals a highly admixed population, and even reduction of the data set to outlier loci does not seem able to resolve migrants from residents, possibly suggesting a relatively recent gain of migratory behavior.

CHAPTER V

SYNTHESIS AND CONCLUSIONS

Song Complexity

Examples of complex signaling systems are all around us. Even seemingly simple signals, like chickadee alarm calls, have hidden depths of meaning that only recently have we started to understand (Ficken 1990, Templeton et al. 2005). Despite several decades of work (Searcy and Nowicki 2005), research of signal complexity is still essentially in its infancy. The best-studied signals are unimodal and naturally selected, like alarm or feeding calls (Hebets and Papaj 2005). Many studies are correlative, and we as a community of scientists are still searching for patterns – we are far from fully understanding the processes that generate them. Multi-modal and/or sexually selected signals are, by their nature, even more difficult to pick apart and only very recently have researchers attempted to tackle them (Hebets et al. 2016). For example, scientists do not fully understand all the factors influencing the evolution of the peacock’s courtship display, perhaps the most famous of all complex signals (e.g. Thavarajah et al. 2016).

Many hypotheses have been proposed to explain variation in signal complexity in birds: sociality (Freeberg et al. 2012) and social rank (Spencer et al. 2004a), developmental conditions (Spencer et al. 2004b), habitat structure (Briefer et al. 2010), and signaling efficacy (Galván 2008) are a few examples. Social signals are those that are not adapted for mate attraction, such as territorial soft song (Searcy et al. 2006) or feeding calls (Elgar 1986). Sexual signals, where reproductive opportunities are at stake, can be so diverse and elaborate that many hypotheses deal only with this "special case" of communication (displays as handicaps – Zahavi 1975, nutritional stress – Nowicki et al. 2002, good genes – Fitzpatrick 1994, parasite resistance – Hamilton and Zuk 1982, etc.).

Bird song is the best studied signal, both social and sexual. Perhaps we can relate to the birds – they are largely diurnal and social, using vocal and visual communication like us. Their sounds are mostly described as pleasant and musical, and we often remember their vocalizations by pretending they are saying some phrase. White-winged doves ask "Who cooks for you?" while mountain chickadees really want a "Cheeseburger!" and white-throated sparrows proclaim their love for "Oh sweet Canada Canada Canada Canada!" Barring the great apes, birds come the closest to human speech and language abilities. African grey parrots have famously been taught the meanings of hundreds of words and can use them in simple sentences (Pepperberg 1987). We strongly associate birds and their songs with feelings and places: how many movies have you seen where the yodel of a loon, the scream of a piha, the chirps of a house sparrow, or the croak of a raven sets the scene without any other cues?

Perhaps we can best sum up the main question we are asking like this: why do birds sound so different from each other? There are so many possible factors influencing bird vocalizations: phylogeny, environment, sociality, learning mechanism, mating system, drift, none of which are mutually exclusive with each other. A small backwater field of bird song complexity research has looked at this question from a very large scale, suggesting the selection pressures associated with high latitude, temperate habitats may ultimately be responsible for major differences both among and within species (Chapter

II). These are fairly grand claims, supported mostly by observations of correlations with song complexity and latitude or migratory behavior between species. Whether this is universally (or even mostly) true for birds remains an open question, and some have justifiably called into question the fairly well-accepted expectation that bird song complexity increases with latitude. Many studies suffer from poor geographic coverage and problems with comparability, and I think it is fair to say that we still have not documented this pattern very well, if it exists at all. The same processes that are hypothesized to drive the evolution of complexity at high latitudes between species should theoretically operate within species. However, single species studies are often poorly designed, with few individuals or populations for comparison (Chapter II). In this dissertation, I used a more systematic approach to studying latitudinal gradients and migratory splits in song complexity in a single species to document whether such a pattern exists and what hypotheses the results are consistent with.

Pattern of Repertoire Size

Rock wrens are small, monomorphic, partially migratory passerines with large, variable song repertoires. Despite high variance within any given population, there is a fairly large difference in repertoire size between migratory (87±23) and sedentary (102.6±20.8) rock wrens and repertoire size is correlated with latitude in migrants but not residents (Fig 3.3). This outcome has never been explicitly predicted before, probably in large part because of a systematic bias towards predicting sedentary populations or species are somehow lower quality or experience less intense selection pressure.

That being said, this pattern is partially consistent with some of the hypotheses described in Table 2.1. The only hypothesis that predicts sedentary birds will have higher song complexity is the panmictic migrants hypothesis (Bolus 2014). This hypothesis posits that higher dispersal in migrants renders them more similar to each other than residents are. Increased complexity in residents comes from local dialect formation. I did not assess whether the identities of the song types in migratory populations were more similar to each other than among resident populations, so I will not rule out this hypothesis as a possible mechanism generating song complexity in rock wrens. However, increased local dialect formation does not necessarily translate to more song types. Looking for cultural similarities in lieu of genetic ones is an avenue of research I will pursue in the future.

Table 5.1. Summary of support for published hypotheses (from Chapter II).

Hypothesis	**Prediction**	**Support**
Sound space	Latitude: complexity increases Migration: no prediction	Partial – complexity increases with latitude, but only in migrants
Rapid pairing	Latitude: complexity increases Migration: complexity higher	Partial – complexity increases with latitude, but only in migrants
Temporal isolation	Latitude: no prediction Migration: complexity higher	Not supported
Panmictic migrants	Latitude: no prediction Migration: complexity lower	Partial – complexity lower in migrants, but latitude important as well
Good migrations	Latitude: no prediction Migration: complexity higher	Not supported
Ranging	Latitude: no prediction Migration: complexity higher	Not supported
Territory Lottery	Latitude: no prediction Migration: complexity higher	Not supported

Two hypotheses are partially consistent with my findings in that they predict a correlation between latitude and song complexity. The sound space hypothesis proposes that changes in habitat type along a latitudinal gradient will result in more complex song in birds since more ‘sound space’ is available at high latitudes (Weir et al. 2012). This hypothesis predicts a fairly gradual cline in complexity from tropical to temperate habitat. The rapid pairing hypothesis suggests that shorter breeding seasons at higher latitudes result in selection for increased song complexity as a cue for quick assessment of potential mates (Catchpole 1982). Fundamentally this hypothesis is driven by latitude, and migrants are affected because they breed in places that have shorter breeding seasons. The outcome that song complexity increases with latitude is consistent with these two hypotheses, but the higher song complexity of residents is not. Given the proposed mechanisms of these two hypotheses (habitat structure and length of the breeding season) this result seems to exclude them as real possible explanations.

The remaining four hypotheses all make the same basic prediction that migrants should have higher song complexity than residents, albeit for different reasons. They make no prediction about the effects of latitude. Since I did not find that migrants have higher song complexity than residents it is fairly simple to reject these hypotheses. The predilection towards predicting migrants have higher complexity reveals how pervasive this paradigm is among researchers studying this. Half of the hypotheses suggest increased sexual selection pressure in migrants should render “better” sexual signals adaptive, while only one surmises the opposite. Within this small body of literature there is almost no way to explain how sedentary populations or species evolve more complex songs, despite this pattern being found repeatedly in past study (Chapter II).

Migratory Syndrome…?

Essentially nothing is known about rock wren migrations beyond the observation that the populations of some northern areas disappear in the winter (Lowther et al. 2000). Since the breeding and wintering distributions for rock wrens are contiguous, it is impossible to know without more explicit testing what routes rock wrens take on migration, and therefore how difficult that migration is in terms of distance travelled, longest nonstop flights, overall time spent travelling, etc. However, this information is critical to these hypotheses. While only the good migrations hypothesis explicitly cites selection for "ability to migrate" as the key driver of increased sexual selection, this philosophy seems to be governing the song complexity zeitgeist. Authors don't explicitly predict residents will have increased song complexity because how can they? Not when migration is such a powerful selective force. Thus, it is useful to try and assess somehow whether we can detect any of the telltale signs of selection for better migration on rock wrens to satisfy this basic premise.

Rock wrens in the northern, putatively migratory, half of the transect have larger tarsi, smaller tails, and smaller wing chords than birds in the southern half of the transect (Table 5.2). This is partially consistent with the expectations for migratory morphology in passerines. Past research has noted both larger tarsi and smaller tails in migratory birds, but the smaller wing chord and unchanging hand-wing index is unexpected (Hedenström 2008), although not unique (Huber et al. 2017). Generally, the "more migratory" a species is (i.e. the farther it travels), the longer the wing chord is relative to the length of the secondary feathers, a pattern found in many species (MacPherson 2017). Taken out of any context, the shorter wing chord of migrants would seem to imply that "residents" are actually flying more. I think this highly unlikely, particularly given the other measurements that are concordant with expectations of migration. I think it more likely that selection on wing length in rock wrens is mediated by some other factor I didn't measure.

Table 5.2. Summary statistics of measured features for each population. Red populations are migratory, blue populations are sedentary. Adult males only. Rep @ 500 = number of song types in an individual's song repertoire at the 500th song; Tarsus = tarsus length, Tail = tail length, WC = wing chord, HWI = hand-wing index. Mean, standard deviation, and sample size (in parentheses) reported. Data from Chapter IV.

	Rep @ 500	**Tarsus (mm)**	**Mass (g)**	**Tail (mm)**	**WC (mm)**	**HWI**
CBL	101.7±2.9 (3)	21.1±0.8 (7)	15.1±2.1 (7)	51.1±2.0 (7)	69.6±2.1 (7)	18.3±1.4 (6)
MC	102±3.0 (3)	20.7±0.9 (12)	15.5±0.7 (12)	51.1±2.5 (12)	69.3±1.7 (12)	15.4±1.8 (12)
DETO	86.0±11.3 (3)	21.2±1.0 (9)	16.2±1.5 (9)	50.6±3.3 (9)	71.0±2.5 (9)	17.9±4.1 (9)
BSP	61.7±47.4 (3)	21.0±0.4 (12)	15.7±0.7 (12)	50.3±2.5 (12)	69.7±1.1 (12)	16.3±1.8 (12)
FOCO	76.8±15.6 (12)	23.8±2.0 (32)	16.4±1.0 (29)	51.3±2.7 (12)	70.4±1.7 (32)	15.8±2.8 (7)
CNG	104.6±8.5 (5)	21.5±0.8 (12)	15.7±0.9 (12)	53.3±2.4 (12)	70.5±1.7 (12)	15.8±3.9 (12)
RGG	114.7±19.6 (3)	21.2±0.8 (9)	15.9±0.9 (9)	50.9±2.3 (9)	70.7±1.4 (9)	14.3±2.9 (9)
BOX	79.0±0.7 (2)	20.3±1.3 (11)	15.5±1.0 (11)	53.3±2.3 (11)	71.2±1.5 (11)	13.4±3.8 (11)
OM	121.5±11.3 (4)	20.9±0.7 (9)	15.6±0.9 (9)	50.9±3.1 (9)	69.9±2.3 (9)	16.2±2.0 (9)
FODA	89.5±7.8 (2)	19.9±0.5 (10)	15.7±0.7 (10)	54.7±2.1 (9)	71.8±1.9 (10)	18.7±3.4 (9)
BBR	90.3±28.4 (3)	20.9±1.2 (5)	15.5±0.5 (5)	53.6±3.9 (5)	69.8±1.6 (5)	13.9±5.3 (5)

Migration is thought to be largely genetically controlled (Pulido et al. 1996), so there should be selection against migrants and residents interbreeding (Berthold and Helbig 1992). Such mixing could lead to an intermediate, less fit phenotype. I did not detect any genetic differences between migrants and residents, both using a large SNP dataset of mostly neutral variation and a reduced SNP dataset of loci under selection. What genetic structure I could resolve suggests that the ends of the transect are distinct from the middle (Chapter IV). Taken together this indicates there is high amounts of gene flow among populations, suggesting rock wrens (both migrants and residents) may be relatively good dispersers and are probably facultative migrants.

This situation is consistent with Pulido's (2011) environmental threshold model for migration, where "migration" alleles are present in the majority of the species but only expressed in populations living in certain environments. Recent phylogenies reveal that migration is a highly labile trait, with species rapidly gaining and losing migration (Barker et al. 2015), supporting Pulido's (2011) hypothesis that there exists high intraspecific variation in migration alleles. Even obligate migrants with fixed pathways can be somewhat flexible. While coastal and inland subspecies of Swainson's thrushes (*Catharus undulatus*) take different migratory routes, hybrids do exist and have been documented to survive the round-trip (Delmore and Irwin 2014). Their path takes them directly in between the two main flyways across huge swathes of desert, unsuitable habitat for a Swainson's thrush. This is in contrast to the stark predictions of hybrid death based on lab experiments (Berthold 1992).

Summary

Despite not finding population-level genetic differences between migratory and sedentary rock wrens, it does not rule out migration as a selective force per se. The combination of a lack of genetic structure, and morphological and song features that are together best explained by grouping populations by migratory strategy, suggest that morphological and behavioral changes in rock wrens have evolved recently and rapidly.

While rock wrens are thought to be the most basal wren (Barker 2017), it’s possible that they have recently expanded their range northward, resulting in limited genetic divergence, and making migration a relatively new behavior. Additionally, it’s entirely possible that the most important genes regulating morphology and migration behavior were not included in the set we targeted.

Rapid changes in behavioral and morphological traits in birds have been documented occurring over time frames as short as decades. The evolution of beak size in the medium ground finch (*Geospiza fortis*) is perhaps the most well-known example (Grant and Grant 2002). A combination of selection imposed by drought-mediated seed availability and random oscillating drift have influenced beak phenotypes over several decades. Many bird species have responded to noise pollution in cities by altering the spectral characteristics of their song, generally by raising the overall frequency (Ortega 2012). Blackcap warblers evolved an entirely new migratory route and wintering distribution, a feat that astonished the ornithological community when it was first documented by Langslow (1979).

I have documented fairly concordant differences in song and morphology between migratory and sedentary populations of a single species. While researchers have long hypothesized differential selection on migrants and residents, our propensity to predict that trait elaboration will occur in migrants versus these results suggest we still don’t fully understand the tradeoffs associated with these different strategies. For example, year-round territoriality has been characterized as almost simple and carefree for those individuals lucky enough to live in the wonderful places that support it (e.g. Mountjoy and Leger 2001). This view seems almost comically simplistic and wrong – tropical rainforests, where most species are residents, are among the most competitive places on earth (Morris et al. 2004). Rock wrens defend relatively large territories for a small passerine (Warning and Benedict 2015) and are described as “uncommon” in field guides because they are fairly widely spaced. This behavior suggests space might be a key limiting factor among breeding wrens, so ousting a territory holder could be hugely beneficial.

We have barely scratched the surface of the possible migratory and song phenotypes of birds, much less the mechanisms generating and maintaining these phenotypes. With approximately 10,000 species of birds, nearly half of which are passerines, a few studies of sparrows or warblers are not going to reveal everything there is to know. However, rapid advances in tools for song analysis, genetics, tracking, and monitoring of birds are making it possible to understand these traits in any species cheaply and easily. In 2013 a single light-level tracking device cost $500, a prohibitive cost when at least ten are necessary to have a reasonable chance of recapturing one tracked bird. The cost of sequencing a genome’s worth of DNA cost ~$10,000, notwithstanding the additional costs associated with preparing that DNA (Wetterstrand 2018). Now, in 2018, for $5000 you can buy around 40 geolocators and almost guarantee at least a few returns. For $10,000 you can prepare and sequence 9-10 genomes worth of DNA. In the next decade many things we thought we understood about bird migration in particular will change as the cost to study more species goes down.

There remains a huge amount of work to do to better understand partial migration, breeding ecology, and song complexity. My project has been a small part of the push towards understanding not only patterns but the processes underlying them.

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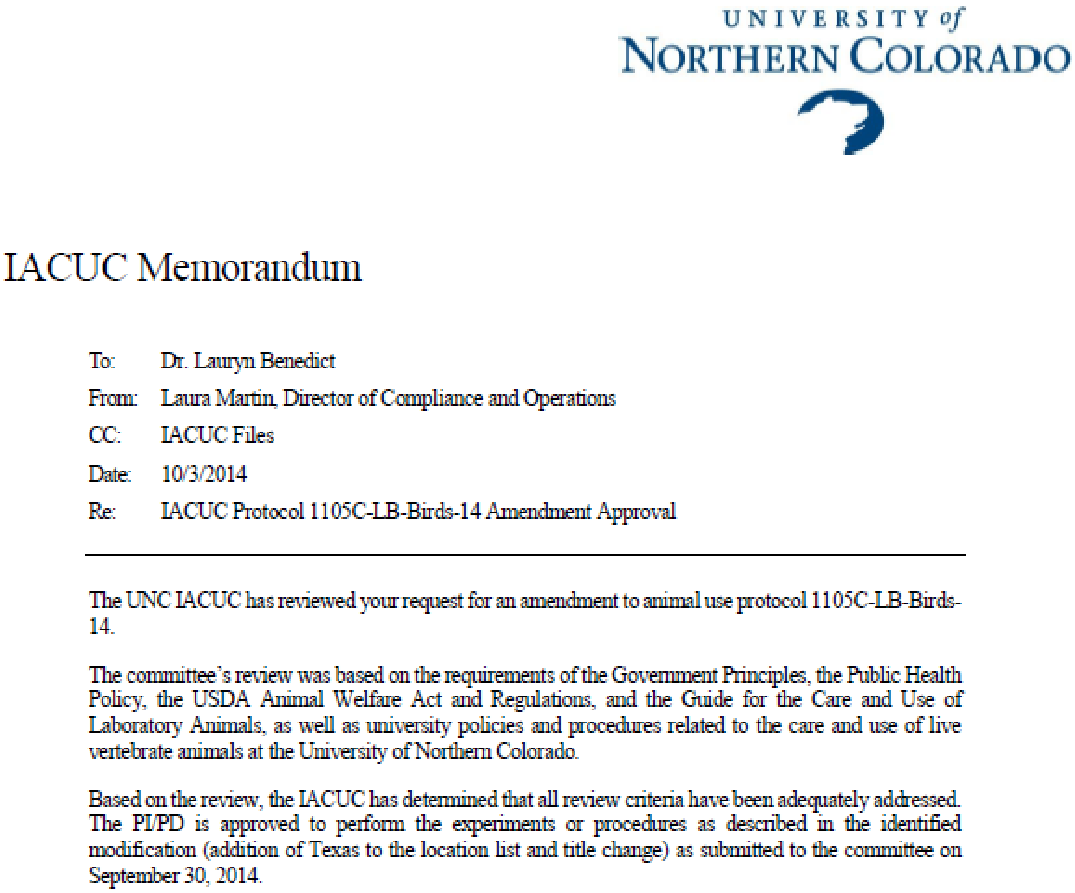
APPENDIX A

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL



UNIVERSITY of NORTHERN COLORADO

IACUC Memorandum

To: Dr. Lauryn Benedict
From: Laura Martin, Director of Compliance and Operations
CC: IACUC Files
Date: 10/3/2014
Re: IACUC Protocol 1105C-LB-Birds-14 Amendment Approval

The UNC IACUC has reviewed your request for an amendment to animal use protocol 1105C-LB-Birds-14.

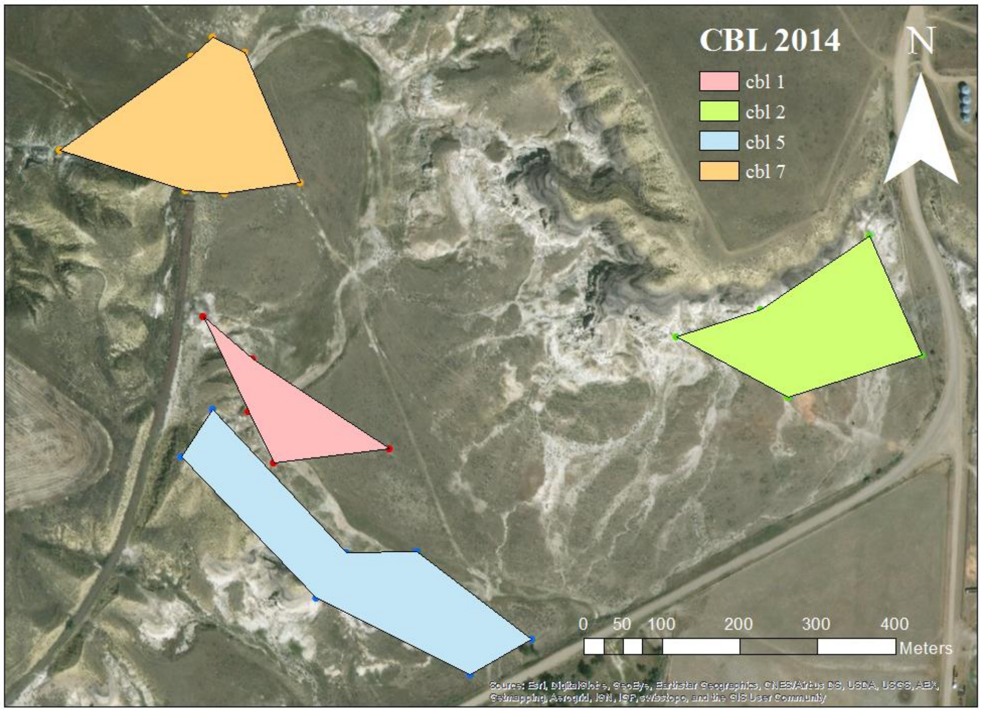
The committee's review was based on the requirements of the Government Principles, the Public Health Policy, the USDA Animal Welfare Act and Regulations, and the Guide for the Care and Use of Laboratory Animals, as well as university policies and procedures related to the care and use of live vertebrate animals at the University of Northern Colorado.

Based on the review, the IACUC has determined that all review criteria have been adequately addressed. The PI/PD is approved to perform the experiments or procedures as described in the identified modification (addition of Texas to the location list and title change) as submitted to the committee on September 30, 2014.

A-1. IACUC approval letter.

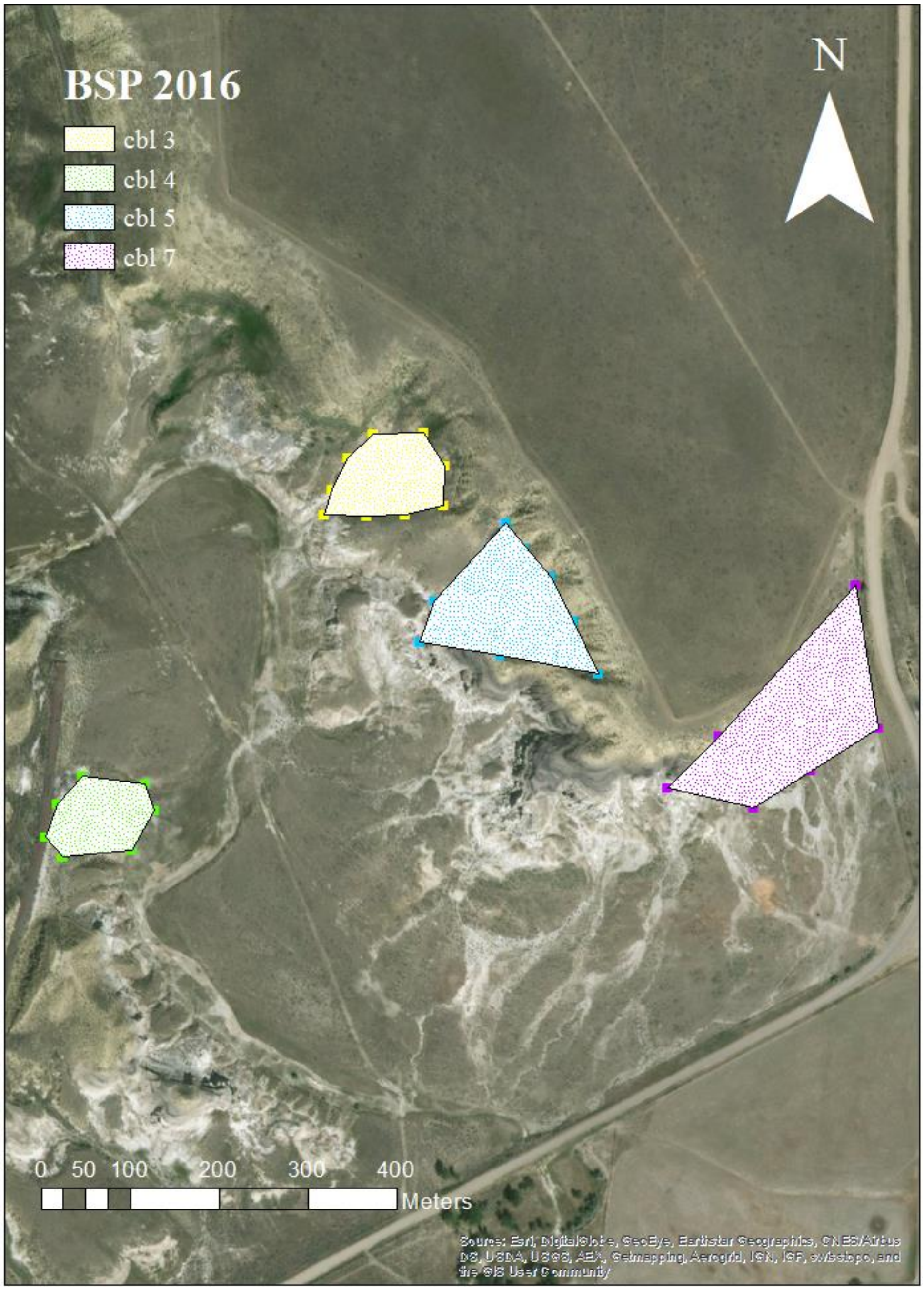
APPENDIX B

TERRITORY MAPS



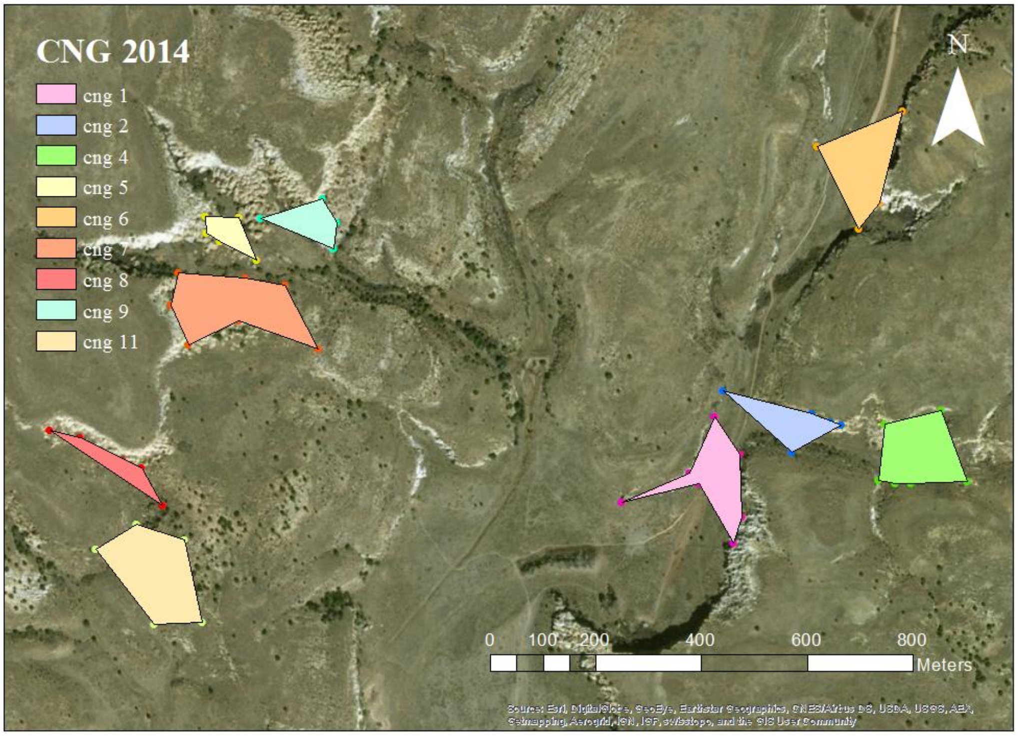
Appendix B-1. CBL 2014.

Appendix B-2. DETO 2015.



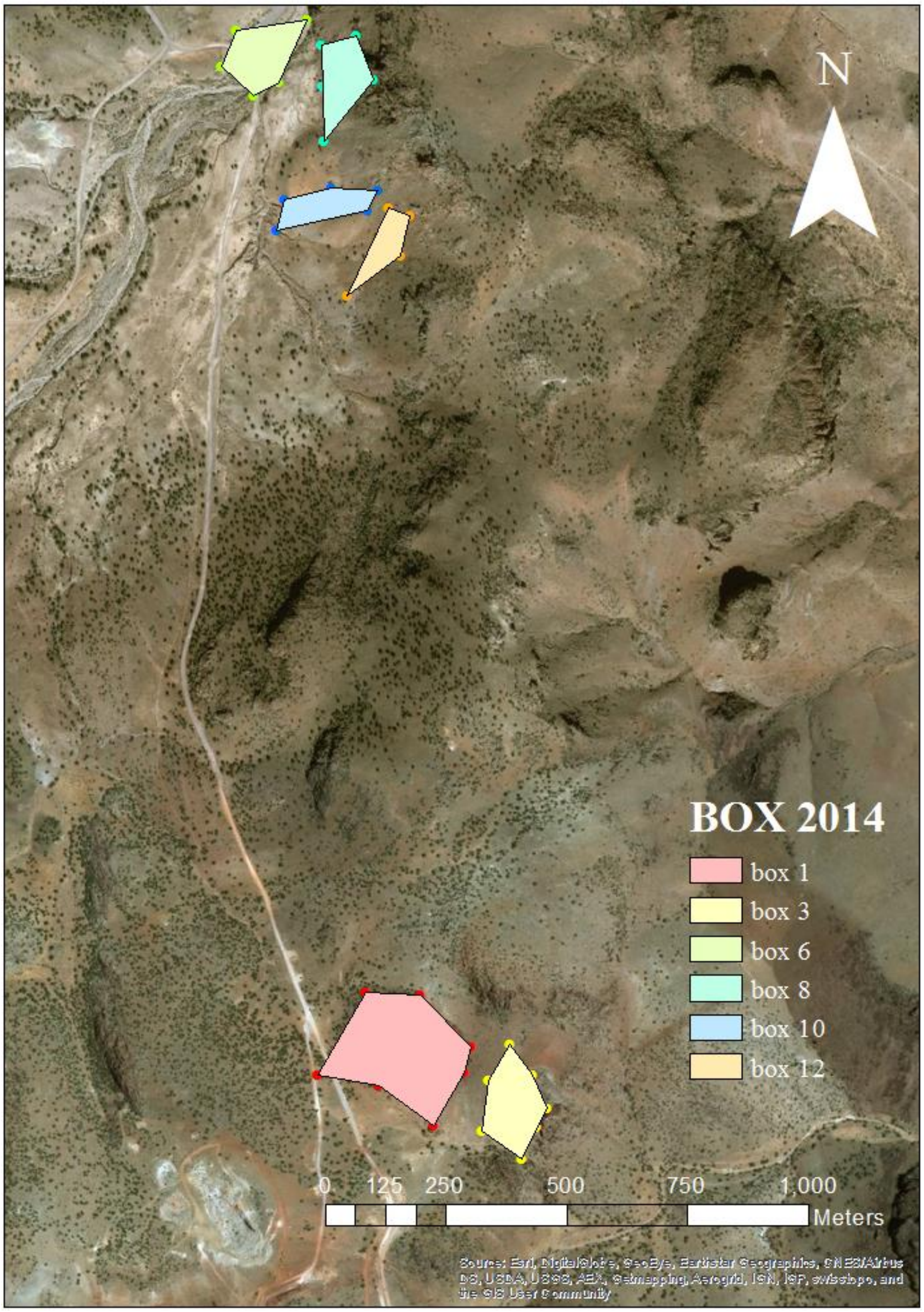
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Appendix B-3. BSP 2016.



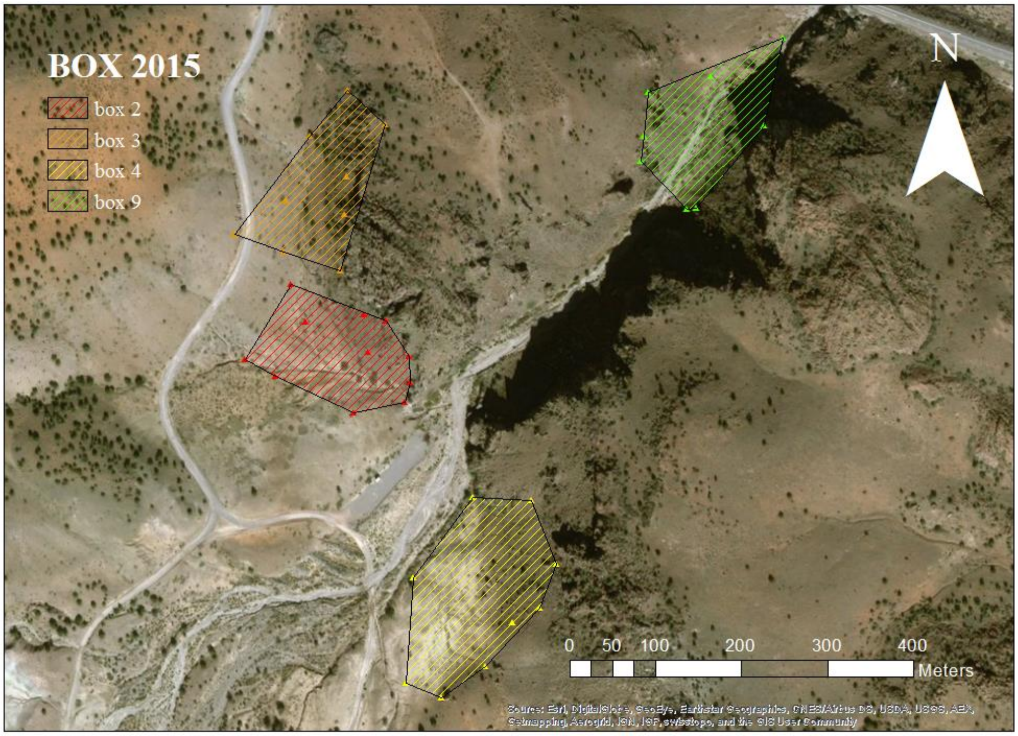
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Appendix B-4. CNG 2014.

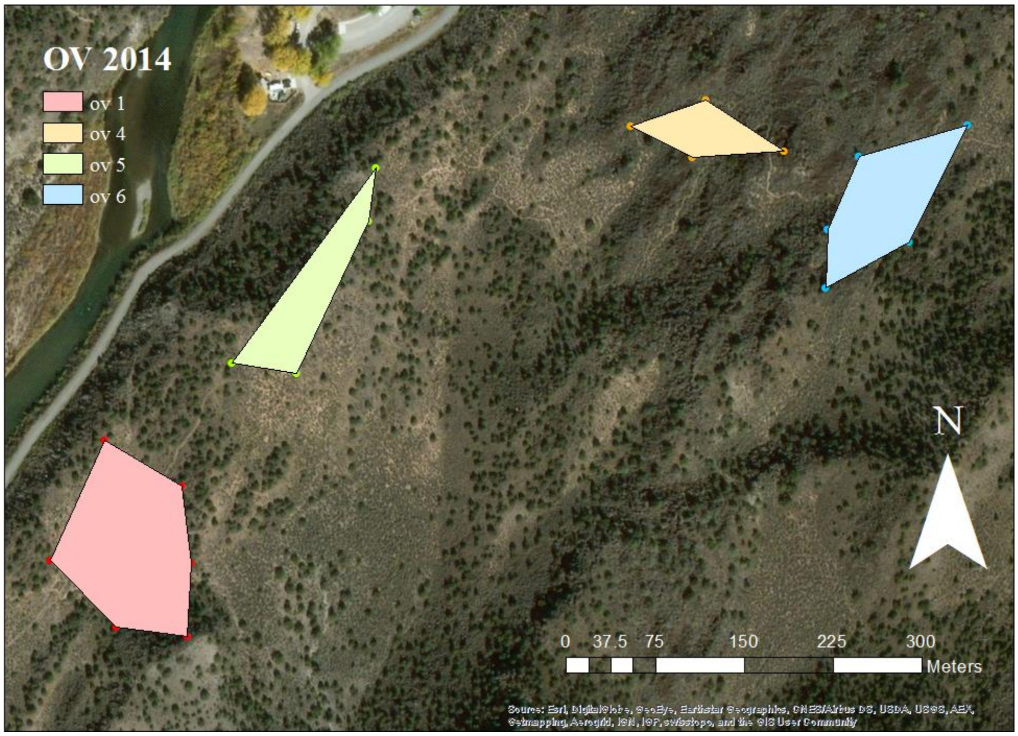


Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

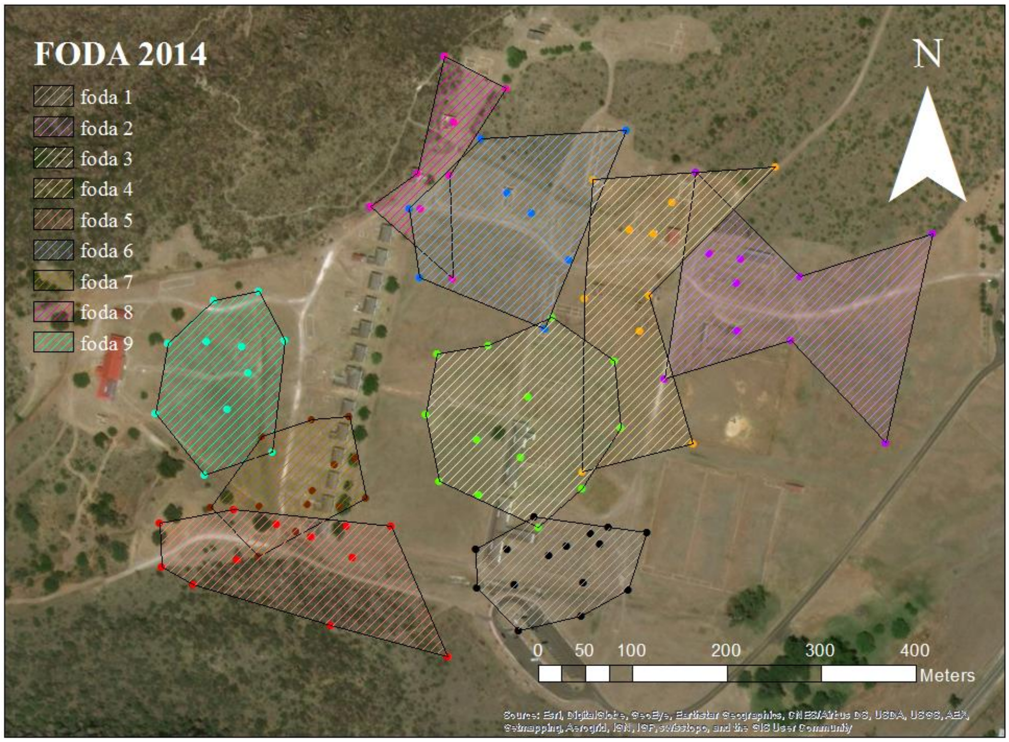
Appendix B-5. BOX 2014.



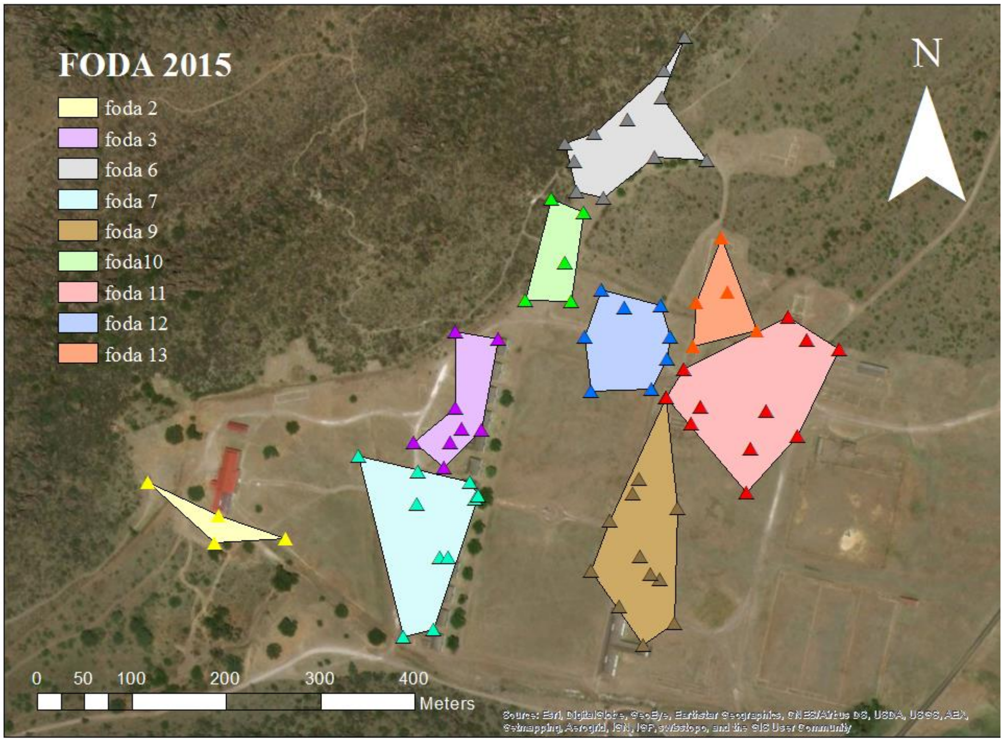
Appendix B-6. BOX 2015.



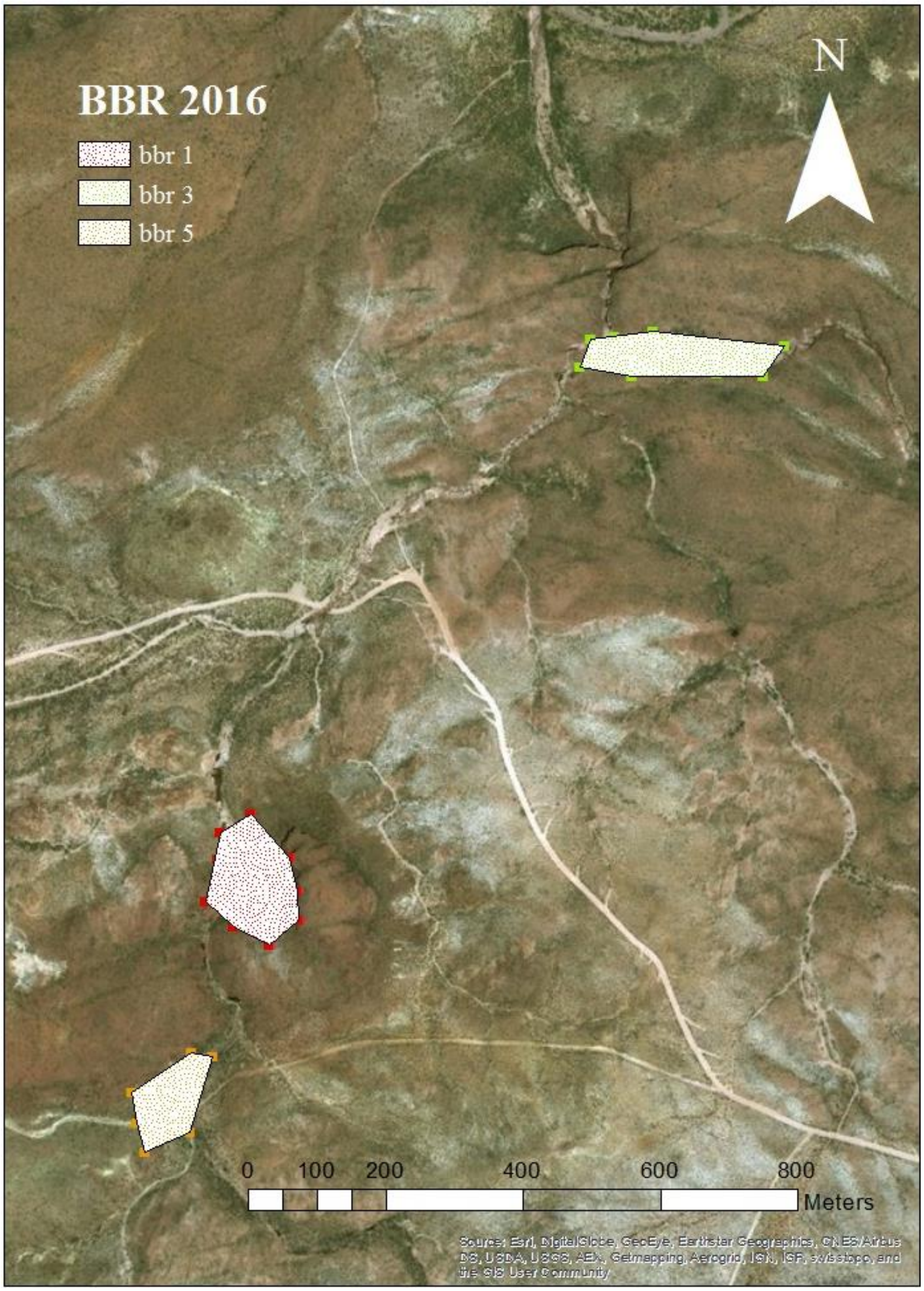
Appendix B-7. OV 2014.



Appendix B-8. FODA 2014.



Appendix B-9. FODA 2015.



Appendix B-10. BBR 2016.

APPENDIX C

TARGETED LOCI

Appendix C. Loci targeted for this project.

Locus name	Function	Chr	Strand	Start	End
FBXL3	CLOCK	1	-1	70,009,422	69,998,367
PHLPP1	CLOCK	2	1	41,293,490	41,356,772
ID2	CLOCK	3	1	98,432,467	98,434,469
PPP1CB	CLOCK	3	1	7,789,309	7,803,934
CRY2	CLOCK	5	-1	22,688,019	22,663,773
ATOH7	CLOCK	6	-1	1,416,252	1,415,797
PER2	CLOCK	9	1	1,071,103	1,097,729
SOX14	CLOCK	9	1	5,492,381	5,493,103
BHLHE40	CLOCK	12	1	20,332,562	20,336,907
PPP1CC	CLOCK	15	-1	3,610,234	3,596,239
USP2	CLOCK	24	-1	2,003,693	1,992,120
CRTC1	CLOCK	28	1	4,881,931	4,907,826
CRY1	CLOCK	1A	-1	53,504,662	53,488,115
NR2F6	CLOCK	Un	1	56,205,335	56,207,348
GNAQ	CLOCK	Z	1	54,689,551	54,800,225
GNAQ	CLOCK	Z	1	54,800,035	54,800,225
DCT	color	1	1	41,739,696	41,758,139
EDNRB	color	1	-1	70,491,343	70,477,329
OCA2	color	1	-1	33,100,570	32,957,625
TYR	color	1	-1	82,044,800	82,001,752
BCL2	color	2	-1	41,520,568	41,427,386
MC4R	color	2	1	40,245,711	40,246,706
GSTA2	color	3	1	90,760,574	90,769,559
KITLG	color	4	-1	43,668,592	43,633,016
HPS6	color	6	-1	21,810,043	21,807,911
FAP	color	7	-1	11,909,785	11,873,282
MREG	color	7	1	3,447,782	3,461,060
ZEB2	color	7	-1	36,173,271	36,138,512
APOD	color	9	1	14,320,362	14,324,106
TRPC1	color	9	1	11,925,313	11,941,925
MYO5A	color	10	1	8,523,659	8,598,997
PLIN	color	10	1	12,853,132	12,853,570
RAB27A	color	10	1	7,711,439	7,717,132
SLC24A5	color	10	1	9,926,860	9,934,773
STARD5	color	10	1	11,998,207	12,002,087
BCO1	color	11	-1	2,500,189	1,529,923
MC1R	color	11	1	11,645,486	11,646,430
ADAMTS9	color	12	-1	14,749,614	14,670,362
WNT5A	color	12	-1	7,954,719	7,946,133
SCARB1	color	15	1	1,818,382	1,833,367
ASIP	color	20	-1	1,865,428	1,861,698
MC3R	color	20	-1	13,443,407	13,442,523
STARD1	color	22	-1	2,795,308	2,793,195

Appendix C, continued.

Locus name	Function	Chr	Strand	Start	End
STARD3	color	27	1	1,747,593	1,761,686
GNA11	color	28	-1	2,747,647	2,740,933
ADAMTS20	color	1A	-1	29,301,663	29,215,410
CD-36*	color	1A	-1	10,156,980	10,137,192
KITLG	color	1A	-1	42,338,833	42,312,985
PMCH	color	1A	1	55,305,965	55,307,397
SOX10	color	1A	1	50,801,260	50,808,581
MEF2C	color	Z	-1	12,221,410	12,150,796
STARD4	color	Z	-1	20,762,542	20,755,392
TYRP1	color	Z	-1	60,977,573	60,967,774
TBX15	cranial skeleton	1	1	91,009,635	91,037,405
CTNNB1	cranial skeleton	2	-1	64,389,067	64,381,473
EXOC2	cranial skeleton	2	1	42,817,144	42,930,716
KIAA1429	cranial skeleton	2	-1	132,589,247	132,563,026
MMP16	cranial skeleton	2	-1	130,161,393	130,000,343
SETD2	cranial skeleton	2	1	915,431	950,540
SHH	cranial skeleton	2	-1	8,970,941	8,961,117
TGFBR1	cranial skeleton	2	-1	75,667,046	75,644,138
TGFBR2	cranial skeleton	2	1	60,123,444	60,183,464
BMP2	cranial skeleton	3	-1	25,964,692	13,034,747
EIF4A3	cranial skeleton	3	-1	330,900	325,675
RUNX2	cranial skeleton	3	-1	108,943,040	108,793,102
SIX2	cranial skeleton	3	-1	17,205,719	17,202,350
PDGFRA	cranial skeleton	4	-1	43,801,290	43,777,990
WDR19	cranial skeleton	4	-1	48,240,398	48,204,115
CALM1	cranial skeleton	5	1	44,694,192	44,703,366
DKK3	cranial skeleton	5	1	1,621,546	1,645,796
DLK1	cranial skeleton	5	1	50,244,126	50,254,271
SIX4	cranial skeleton	5	1	56,478,911	56,483,811
FGF8	cranial skeleton	6	1	22,049,471	22,153,810
FGFR2	cranial skeleton	6	-1	31,392,609	31,316,475
ALDH1A2	cranial skeleton	10	1	6,786,365	6,838,567
ALDH1A3	cranial skeleton	10	1	17,976,236	18,007,605
SMAD3	cranial skeleton	10	1	19,539,755	19,604,218
FOXC2	cranial skeleton	11	1	324,456	325,922
PLEKHF1	cranial skeleton	11	1	14,547,970	14,548,791
WNT9B	cranial skeleton	27	-1	915,456	903,982
SLC39A3	cranial skeleton	28	-1	4,019,922	4,018,363
AKR1D1	cranial skeleton	1A	1	66,994,762	67,029,500
ALX1	cranial skeleton	1A	1	41,176,098	41,194,161
MGAT4C	cranial skeleton	1A	-1	41,522,572	41,515,203
RASSF9	cranial skeleton	1A	-1	41,454,577	41,430,374
PAX5	cranial skeleton	Z	1	72,604,672	72,742,717
SMAD2	cranial skeleton	Z	-1	447,313	407,582
SV2C	cranial skeleton	Z	-1	57,264,946	57,181,716
C2CD3	limb development	1	-1	97,789,427	97,759,187
FGF9	limb development	1	1	46,863,157	46,889,606
GJA5	limb development	1	-1	103,330,387	103,329,275
TULP3	limb development	1	1	88,541,725	88,559,710
NR2F2	limb development	10	1	16,269,224	16,276,151

Appendix C, continued.

Locus name	Function	Chr	Strand	Start	End
SALL1	limb development	11	1	9,790,180	9,802,832
IFT122	limb development	12	1	21,182,241	21,209,637
ATP1A1	migration	1	-1	92,650,502	92,632,416
DCUN1D5	migration	1	1	76,647,200	76,657,497
NHLRC3	migration	1	1	54,040,201	54,046,033
NPAS2	migration	1	-1	30,384,984	30,323,145
ADCYAP1	migration	2	-1	107,405,076	107,400,822
ADCYAP1R1	migration	2	-1	3,466,540	3,349,852
ARPP21	migration	2	-1	28,961,874	28,858,378
COL1A2	migration	2	1	25,921,531	25,964,644
CPNE4	migration	2	-1	62,575,299	62,394,091
HRSP12	migration	2	-1	134,101,651	134,093,022
NRSN1	migration	2	1	73,823,245	73,824,834
PMP2	migration	2	-1	127,674,421	127,670,401
TTR	migration	2	1	111,938,264	111,946,440
FAM49A	migration	3	-1	102,394,341	102,376,601
NEK2	migration	3	1	13,333,421	13,341,138
NRXN1	migration	3	-1	22,789,593	22,119,248
CLOCK	migration	4	1	43,426,057	43,442,175
CREB1	migration	7	1	21,302,445	21,317,966
BRINP3	migration	8	1	649,867	833,451
PARL	migration	9	1	2,355,875	2,368,301
CREBRF	migration	13	1	2,399,119	2,417,215
FSCN1	migration	14	-1	11,359,303	11,353,084
C8G	migration	17	-1	263,048	256,501
HSPA5	migration	17	-1	10,813,273	10,809,175
AANAT	migration	18	-1	7,913,150	7,911,249
TEKT1	migration	19	-1	11,557,916	11,553,528
PER3	migration	21	-1	1,871,191	1,854,675
SLC2A1	migration	21	1	565,487	573,418
HSPA8	migration	24	-1	3,519,892	3,515,929
CSNK1E	migration	1A	1	50,664,894	50,676,441
GRP94	migration	1A	-1	54,684,558	54,674,742
PLEKHA5	migration	1A	-1	67,944,951	67,772,119
SRPK2	migration	1A	-1	12,966,067	12,874,109
bmal1	migration	5_random	-1	1,014,634	990,870
DRD4	migration	5_random	1	7,465,884	7,474,708
Hsp90	migration	5_random	-1	50,813,587	50,806,609
PER2	migration	Un	1	4,296,515	4,299,578
NFIL3	migration	Z	1	6,413,742	6,415,115
SLC1A3	migration	Z	1	41,845,197	41,905,911
TLE4	migration	Z	-1	54,144,138	54,049,308
INHBA	oxygen transport	2	1	33,558,179	33,570,469
ADD1	oxygen transport	4	-1	62,182,065	62,119,898
TET2	oxygen transport	4	1	22,162,473	22,181,744
CAT	oxygen transport	5	1	6,271,419	6,285,186
HIF1A	oxygen transport	5	-1	56,034,204	56,019,200
INHA	oxygen transport	7	1	10,772,704	10,774,697
EIF2AK1	oxygen transport	14	-1	15,120,965	15,108,765
HBAA	oxygen transport	14	-1	2,980,020	2,979,229
HBAD	oxygen transport	14	-1	2,983,107	2,982,248

Appendix C, continued.

Locus name	Function	Chr	Strand	Start	End
HBZ	oxygen transport	14	-1	2,987,641	2,985,737
EPB42	oxygen transport	20	1	7,245,443	7,260,280
MB	oxygen transport	1A	1	51,871,350	51,874,513
GAP43	song	1	-1	94,891,566	94,867,610
VEGFD	song	1	1	16,956,279	16,985,864
ARC	song	2	-1	155,072,629	155,071,445
CNTNAP2	song	2	-1	30,635,452	30,184,705
HIVEP1	song	2	1	68,815,057	68,914,507
NOD1	song	2	1	61,687,899	61,705,843
RIPK2	song	2	1	130,575,920	130,604,795
MAP3K7	song	3	1	77,416,948	77,461,643
NAPB	song	3	1	29,013,677	29,020,672
RPS27A	song	3	-1	28,390,875	28,389,344
SNAP25	song	3	-1	24,657,667	24,595,900
TAB2	song	3	-1	47,349,347	47,330,741
GRIA2	song	4	1	29,268,697	29,316,975
MAPK10	song	4	-1	13,298,023	13,158,992
UCHL1	song	4	-1	47,443,659	47,439,512
FOS	song	5	1	38,401,322	38,403,217
MAPK8IP1	song	5	-1	22,657,931	22,633,421
TH	song	5	1	13,590,579	13,606,604
TRAF6	song	5	-1	17,580,941	17,570,627
novel gene song 5	song	6	1	17,318,879	17,337,895
ARPC5	song	8	1	3,048,560	3,051,309
JUN	song	8	-1	24,262,555	24,261,611
novel gene song 6	song	9	1	981,142	1,023,985
ALDH1A2	song	10	1	6,786,365	6,838,567
ALDH1A3	song	10	1	17,976,236	18,007,605
Novel gene song 1	song	12	1	21,141,673	21,151,763
DRD1	song	13	-1	3,304,109	3,302,748
EGR1	song	13	-1	21,075	18,142
MAPK9	song	13	1	7,794,999	7,811,397
ARPC1A	song	14	-1	11,171,215	11,159,703
MAPK8IP3	song	14	-1	169,558	96,425
novel gene song 2	song	14	1	15,035,480	15,038,080
novel gene song 3	song	14	1	15,054,873	15,057,546
MED15	song	15	-1	12,048,171	12,022,466
BRINP1	song	17	-1	4,368,423	4,306,078
CACNA1B	song	17	1	2,170,313	2,361,057
CACNA1G	song	18	1	9,311,783	9,410,030
MAP2K4	song	18	-1	5,450,029	5,386,249
AUTS2	song	19	-1	2,093,355	1,465,187
novel gene song 4	song	19	1	8,088,812	8,089,895
STX1A	song	19	-1	3,112,218	3,056,752
TRPV1	song	19	1	8,167,455	8,175,198
NEFM	song	22	-1	1,910,169	1,905,167
ALDH1L2	song	1A	1	54,246,635	54,269,122
FOXP2	song	1A	-1	25,773,703	25,373,922
KCNC2	song	1A	-1	37,056,631	36,960,718
PVALB	song	1A	1	51,421,774	51,430,291
TAB1	song	1A	-1	50,327,922	50,294,874

Appendix C, continued.

Locus name	Function	Chr	Strand	Start	End
TAB3	song	1A	1	10,283,061	10,297,852
GRIA3	song	4A	1	10,072,724	10,184,428
NLGN3	song	4A	-1	19,983,222	19,964,176
SRPX2	song	4A	-1	884,702	881,927
novel gene song 7	song	un	-1	2,149,195	2,144,414
novel gene song 8	song	un	-1	21,903,087	21,899,075
ALDH1A1	song	Z	1	16,195,088	16,218,427
EDAR	wing length	1	1	27,189,227	27,214,190
EGFL6	wing length	1	-1	17,947,750	17,923,016
FGF14	wing length	1	1	38,142,274	38,522,023
FSTL1	wing length	1	1	92,211,856	92,321,140
FZD4	wing length	1	1	82,859,635	82,860,975
TSC22D1	wing length	1	1	57,934,978	58,022,594
WNT-NovelA	wing length	1	-1	90,154,374	90,152,358
ARMC3	wing length	2	-1	19,303,049	19,248,903
COL1A2	wing length	2	1	25,921,531	25,964,644
DLX5	wing length	2	-1	26,877,661	26,874,520
DLX6	wing length	2	1	26,862,169	26,865,657
EGFR	wing length	2	1	32,242,358	32,284,289
EN1	wing length	2	1	8,697,342	8,699,687
FZD1	wing length	2	1	24,429,242	24,431,067
FZD6	wing length	2	1	136,145,668	136,179,811
FZD7	wing length	2	1	21,851,851	21,850,142
FZD8	wing length	2	1	14,258,364	14,259,142
HOXA2	wing length	2	-1	52,557,425	52,555,610
WNT3a	wing length	2	1	2,089,241	2,092,721
WNT9a	wing length	2	-1	1,888,001	1,871,851
DLK2	wing length	3	-1	36,936,738	36,931,970
DLL1	wing length	3	-1	41,905,632	41,897,719
EDARADD	wing length	3	1	45,168,656	45,183,126
EYS	wing length	3	1	87,850,683	87,938,554
FZD3	wing length	3	-1	112,516,044	112,498,936
novel-notch	wing length	3	1	87,272,324	87,445,154
novel-notchB	wing length	3	1	87,507,474	87,557,778
RHOB	wing length	3	1	104,625,772	104,626,362
RHOU	wing length	3	-1	43,027,394	43,022,926
TCF21	wing length	3	-1	58,518,539	58,516,413
TGFB2	wing length	3	-1	10,312,880	10,254,830
FGF19	wing length	5	-1	5,283,601	5,279,974
FGF3	wing length	5	-1	5,349,059	5,343,340
RHOV	wing length	5	-1	23,082,375	23,078,064
BMPR2	wing length	7	-1	21,657,354	21,610,072
DLX1	wing length	7	1	15,276,976	15,278,610
DLX3	wing length	7	-1	15,288,523	15,287,457
FZD5	wing length	7	-1	21,384,847	21,383,282
TWIST2	wing length	7	1	1,558,575	1,559,057
WNT6	wing length	7	1	10,265,488	10,277,806
CRELD1	wing length	12	1	12,104,383	12,104,853
WNT7a	wing length	12	1	793,550	826,577
FZD9	wing length	19	-1	188,226	186,664
FST	wing length	Z	1	46,604,913	46,610,442

APPENDIX D

DISCRIMINANT ANALYSIS OF PRINCIPAL COMPONENTS POPULATION ASSIGNMENTS

Appendix D. Population assignments for all individuals from dapc K=3 and dapc K=4. Colors match Figure 4.6.

Population	K=3	K=4
BBR1	1	2
BBR2	1	2
BBR4	1	2
BBR5	1	2
BBR6	1	2
BOX1	1	1
BOX11	1	1
BOX2	1	1
BOX4	1	1
BOX7	1	1
BSP9	1	1
CBL1	1	2
CBL3	1	2
CBL4	1	2
CBL5	1	2
CBL6	1	2
CBL7	1	2
CBL8	1	2
CNG11	1	1
CNG2	1	1
CNG4	1	1
MC1	1	1
MC10	1	1
MC11	1	1
MC12	1	1
MC5	1	1
MC7	1	1
MC9	1	1
RGG1	1	1
RGG10	1	1
RGG12	1	1
RGG3	1	1
RGG4	1	1
RGG6	1	1
RGG8	1	1
RGG9	1	2
BOX10	2	1
BOX3	2	1
BOX8	2	1
BOX9	2	1
BSP1	2	1
BSP10	2	1
BSP11	2	1
BSP12	2	1
BSP3	2	1
BSP4	2	1
BSP7	2	1
BSP8	2	1
CNG1	2	1
CNG5	2	1

Population	K=3	K=4
CNG6	2	1
CNG8	2	1
CNG9	2	1
DETO1	2	1
DETO11	2	1
DETO12	2	1
DETO2	2	1
DETO3	2	1
DETO6	2	1
DETO7	2	1
DETO8	2	1
DETO9	2	1
FODA4	2	1
FODA6	2	1
FODA7	2	1
MC2	2	1
MC4	2	1
MC6	2	1
OM3	2	1
OM4	2	1
OM5	2	1
OM6	2	1
OM7	2	1
OM8	2	1
OM9	2	1
OV5	2	1
SOA	2	1
SOC	2	1
SOD	2	1
SOE	2	1
SOF	2	1
SOG	2	1
SOH	2	1
BBR3	3	3
BOX5	3	3
BSP6	3	3
CBL2	3	3
FODA1	3	3
FODA10	3	3
FODA11	3	3
FODA12	3	3
FODA3	3	3
FODA5	3	4
FODA8	3	4
OM2	3	3
SOB	3	3