2013

Levels Of Selection In A Polymorphic Species

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EDUCATION AND CERTIFICATIONS

Jan 2007 – May 2013	PhD Candidate. Department of Biology Indiana State University, Advisor: Dr. Elaina Tuttle
2006	M.S. Life Sciences, Indiana State University, Advisor: Dr. Elaina Tuttle
2002	B.S. Environmental Biology, California State University Northridge

PROFESSIONAL POSITIONS

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San Diego Zoo Global Institute for Conservation Research, Genetics Division, Escondido CA

Teaching

Graduate Teaching Assistant, Department of Biology, Indiana State University, August 2004-May 2012

Research

Research Assistant to E.M. Tuttle: Genetics and behavior of white-throated sparrows (August 2004 – May 2012)

Research Assistant to D.A. Gray, Molecular Ecology Lab, California State University Northridge: Molecular and song analysis of *Gryllus rubens* and *Gryllus texensis* (February 2002 – April 2003)

HONORS AND AWARDS

2006 – Nominated for the Education Excellence Award; Presented by the College of Arts and Sciences for exceptional instruction both inside and outside the classroom.

2007 – Awarded Education Excellence Award; Presented by the College of Arts and Sciences for exceptional instruction both inside and outside the classroom.

2007 – Awarded Lilly Endowment Graduate Fellowship "Fulfilling the Promise: Pathways to Pre-eminence" for the 2007 – 2008 academic year

2010 – Awarded Outstanding Graduate Assistant, Presented by the School of Graduate and Professional Development, Indiana State University

RESEARCH GRANTS

Funded Intramural Proposals:

2006 – School of Graduate Studies, Indiana State University. Sex Ratio, Relatedness, and Alternative Reproductive Strategies: A Study of Familial Conflict. $480

2006 – School of Graduate Studies, Indiana State University. $300 for presentation of research at the Annual Animal Behavior Meeting, August 2006.

2008 - Familial Conflict in the White-throated sparrow (*Zonotrichia albicollis*); morph influences on nestling behavior School of Graduate Studies, Indiana State University. $498

2008 – School of Graduate Studies, Indiana State University. $600 for presentation of research at the 12th International Behavioral Ecology Congress, August 2008.

2009 - School of Graduate and Professional Development, Indiana State University. Evidence for multiple mechanisms of segregation distortion in the white-throated sparrow. $600

2010 - School of Graduate and Professional Development, Indiana State University. $600 for professional development

2011 - School of Graduate and Professional Development, Indiana State University. $500 for professional development "Candidate gene mapping in the White-throated sparrow"

2012 - School of Graduate and Professional Development, Indiana State University. $300 Investigation of single nucleotide polymorphisms (SNPs) in the serotonin receptor 1e gene

Funded Extramural proposals

2005 – Sigma Xi, Grants in Aid of Research. Parent-offspring conflict and sibling rivalry in a polymorphic passerine the White-throated Sparrow (*Zonotrichia albicollis*) $300

2006 – Indiana Academy of Sciences. Sex Ratio, Relatedness, and Alternative Reproductive Strategies: A Study of Familial Conflict $900

2009 - Indiana Academy of Sciences. Evidence for multiple mechanisms of segregation distortion in the white-throated sparrow $1388

PUBLICATIONS

Original Articles

Tuttle, E.M., **Korody, M.L.**, and Ko, T.S. *In Prep.* Testing adaptive models of sex allocation in a polymorphic species. *Behavioral Ecology and Sociobiology*

Korody, M.L., Formica, V.A., Gonser, R.A., and Tuttle, E.M. *In Prep.* Patterns of parental investment in sex ratio: A birds-eye view using geographic information systems. *Molecular Ecology* (***Presented as a poster at the 43rd annual Animal Behavior meeting August 2006***)

Romanov, M. N., E. M. Tuttle, M. L. Houck, W. S. Modi, L. G. Chemnick, **M. L. Korody**, E. Stemel, K. C. Jones, S. Dandekar, J. Papp, Y. Da, N. C. S. Program, E. D. Green, V. Magrini, M. T. Hickenbotham, J. Glasscock, S. McGrath, E. R. Mardis, and O. A. Ryder. 2009. The value of avian genomics to the conservation of wildlife. BMC Genomics. **10**(Suppl 2): S10

Jamison, A.P, Tuttle, E.M., **Korody, M.L.** and Gonser, R.A. *In Prep.* The influence of Haemosporidian parasites on the white-throated sparrow (*Zonotrichia albicollis*). Wilson Journal of Ornithology.

Tuttle, E.M., **Korody, M.L.**, Rathbun, N.A .,and Gonser, R.A. *In Prep.* Morph ratio variation in the polymorphic white-throated sparrow. *PLoS Biology.* ***(Presented as a poster at the 12th International Behavioral Ecology Congress August 2008)***

Tuttle, E.M., **Korody, M.L.**, Gonser, R.A., Romanov, M.N., Houck, M. and Lear, T.L. *In Prep.* Candidate gene mapping of the white-throated sparrow (*Zonotrichia albicollis*).

Korody, M.L. and Tuttle, E.M. *In Prep*. Evidence for Segregation Distortion in Sperm of the White-throated Sparrow. *Proc Royal Soc B*

Theses

Korody, M.L. 2006. Factors influencing sex ratio variation within the polymorphic white-throated sparrow. M.S. Thesis, Department of Biology, Indiana State University, (Dr. Elaina M. Tuttle, advisor)

CONFERENCE RESEARCH PRESENTATIONS

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2008 - Tuttle, E.M., **Korody, M.L.**, Rathbun, N.A., and Gonser, R.A. Morph ratio variation in the polymorphic white-throated sparrow. (Presented as a poster at the 12th International Behavioral Ecology Congress)

2010 - **Korody, M.L.** and Tuttle, E.M. Evidence for Segregation Distortion in Sperm of the White-throated Sparrow. (Presented as a poster at the ISU research Symposium 2010)

2010 - Hrgich, B.A., **Korody, M.L.** and Gonser, R.A. A molecular analysis of genetic variation in white-tailed deer (*Odocoileus virginanus*) from Southern Maryland. (Presented as a poster at the ISU Research Symposium 2010)

2011 – **Korody, M.L.**, Tuttle, E.M., Gonser, R.A., Romanov, M.N., and Lear, T.L. Candidate gene mapping of the white-throated sparrow (*Zonotrichia albicollis*). (Presented as a poster at the ISU Research Symposium 2011)

2012 – **Korody, M.L.**, Sperry, E.E., Romanov, M.N., Messier, W., Gonser, R.A., and Tuttle, E.M. Investigation of single nucleotide polymorphisms (SNPs) in the serotonin receptor 1e gene within the genus Zonotrichia (Presented as a poster at the ISU Research Symposium 2012)

PROFESSIONAL DEVELOPMENT

Fall 2010 – 5 weeks spent at the University of Kentucky, Maxwell H. Gluck Equine Research Center under the supervision of Dr. Teri Lear. Learned all aspects of fluorescent *in situ* hybridization (FISH).

Spring 2011 and 2012 – 6 weeks spent at the University of Kentucky, Maxwell H. Gluck Equine Research Center under the supervision of Dr. Teri Lear. Continued working on fluorescent *in situ* hybridization (FISH).

OUTREACH ACTIVITIES

2005 Workshop Leader - Led workshops on molecular ecology and evolution and ancient DNA analysis techniques, Indiana Junior Science and Humanities Symposium

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	2011	2012	
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Levels of Selection in a Polymorphic Species

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Marisa L. Korody

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ABSTRACT

Phenotype is affected by many factors, including but not limited to environment, conspecifics, and genetics. Evidence of phenotypic variation is everywhere, some of which is controlled solely by environment, and others that are fixed by genetics. Genetic polymorphisms are rare, but very useful for the study of selection and genetics. These genetic polymorphisms provide a phenotypic link to the underlying genetics and are even more useful when there are associated behavioral differences. I examine multiple levels of selection that are acting upon a polymorphic passerine, the white-throated sparrow (*Zonotrichia albicollis*). Males and females of this species occur in two morphs, white or tan, based upon the color of their crown strips. This plumage polymorphism is absolutely correlated with a complex chromosomal rearrangement on the second largest autosome. Within this dissertation I explore how climate needs to be addressed in ecological studies to fully understand the mechanisms behind variation. I explore whether sexual selection is acting within this species and the differences between the morphs through the use of Bateman Gradients. Darwin suggested that sex ratios influence sexual selection, but what about morph ratios? I examine the frequency variation of morphs within this species. Variation in morph production may be favored by a potential tradeoff between the number of males and the number of white offspring produced in a clutch that suggests greater costs associated with producing white morph individuals. Mendelian segregation is inconsistent in this species, and transmission distortion may contribute to morph ratio variation. I show that white male sperm varies in production from 0% - 100% white sperm/individual consistent with

transmission distortion. Finally, candidate gene mapping was used to identify the genes sequestered in this rearrangement that may be responsible for the polymorphism and the evolution behind the rearrangement.

PREFACE

Selection acts at multiple levels through variation in fitness. The effects are seen from the whole group down to the genes within individuals. Variation in fitness is impacted by many factors including environment, conspecifics, and predation. Darwin and Fisher indentified the impact that sex ratio variation has on sexual selection (Darwin 1871, Fisher 1930). As breeding sex ratios are skewed, competition for mates will increase, and individuals will become choosy, increasing sexual selection. Research has shown that adult sex ratios are highly variable across taxa, resulting in the "opportunity" for sexual selection. What impact does variation in other factors such as polymorphisms have on selection?

Understanding animal behavior has long been a goal of researchers. However, behavior results from complex interactions between genotype, phenotype and environment. To understand behavior we need to understand all of these interactions. Tracking the genetics behind behaviors is facilitated by a phenotypic link between the genetics and behavior. An ideal model for the study of these links is the white-throated sparrow (*Zonotrichia albicollis*). In this species there is a large chromosomal rearrangement that encompasses approximately 1000 genes and is linked to plumage (white or tan crown stripes; i.e. morph) and behavior differences (Knapton and Falls 1983, Tuttle 2003, Thomas *et al.* 2008, Romanov *et al.* 2009). This rearrangement has resulted in a large group of linked genes that provide a starting point for identifying the genes responsible for many behaviors including parental care and promiscuity. This is a comprehensive study of the white-throated sparrow, examining 13 years of field and

molecular data. I examine selection from the population level of climate change, down to the genetic variation between the morphs of this species.

My first chapter will focus on the effects of climate change on boreal forest communities, with a review of the use of climate data in ecological studies. The white-throated sparrow is an indicator species for the boreal forest and should be carefully studied for the effects of the changing climate on our ecosystem. Any current study should include the implications of climate on the organism. Climate impacts individuals and communities at many levels, including physiology, phenology, predation and competition. Climate affects all levels of selection on an organism and varies depending upon the habitat they occupy. The speed of climate change is applying strong selection pressures on birds, and traits need to evolve to survive these changes (Gienapp *et al.* 2008). In order to understand the effects of climate change we need to include multiple species aspects to build a comprehensive picture including genetics and ecology (Pertoldi and Bach 2007). Climate changes will change migration patterns and influence the population sizes during breeding seasons and change competition for mates, territories and food (Schaefer *et al.* 2008), also increasing selection. Currently there are few long term bird studies that will provide statistically significant climate data and should be expanded. Climate and species interactions are very complicated and difficult to tease apart the effects at each level. We need to remember this complexity and include all aspects of the study organism in any model we use to examine climate. More work is currently needed.

Sexual selection has led to exaggerated traits such as feather ornamentation and color as indicators of quality. However, color variation can occur in both sexes and not signal quality, yet approximately 3.5% of avian species display genetic plumage polymorphisms that follow Mendelian laws of inheritance (Roulin 2004). In my second chapter I will examine whether

there is the "opportunity for selection" (Bateman 1948, Webster *et al.* 1995, Webster *et al.* 2007) within a genetically polymorphic species that exhibits alternative reproductive strategies. Using molecular data (5-8 microsatelite markers) from 13 years of field study I examine "apparent" vs. "actual" reproductive success between the morphs. Overall, lifetime reproductive success does not vary between the two male morphs. However the difference in the contribution of extra-pair young to within pair young between the morphs provides varying selection pressures on the males.

For the third chapter I examine frequency variation of this genetic polymorphism in the white-throated sparrow. At the population level the polymorphism is stable due to a pattern of disassortative mating between the two morphs (*i.e.*, white males mate with tan females and tan males mate with white females). However, offspring proportions vary between clutches and between years, following a frequency-dependent cycle with the adult population. Variation in morph production may be favored by a potential tradeoff between the number of males and the number of white offspring produced in a clutch that would suggest greater costs associated with producing white morph individuals.

As evidenced by the seasonal and yearly morph variation, Mendelian segregation is inconsistent in this species. Transmission distortion occurs when there is an unequal transfer of genetic information from one generation to the next. Examples of distortion of an autosome are rare. The most well studied example is the *t*-locus in mice (Lyon 2003). In Chapter 4 I will show that white male sperm varies in production from 0% - 100% white sperm/individual which is consistent with transmission distortion. The distortion appears to be mediated by social environment, suggesting selection from conspecifics maybe acting within this species.

Additionally, since the rearranged chromosome is being differentially transmitted, there may be addition selection at the genic or chromosome level that may be effected by this distortion.

Finally, I have performed candidate gene mapping to identify the genes sequestered in this rearrangement that may be responsible for the multiple aspects of the polymorphism. In Chapter 5 I address the genes we are mapping within this rearrangement to understand which genes are responsible for the differences in plumage and behavior between the morphs of this species. Our mapping has confirmed previous results indicating the complexity of this rearrangement (Thomas *et al.* 2008) and has increased the number of genes mapped to the area. Several genes that are implicated in hormone and behavior differences in other species have been identified within the rearrangement. These include estrogen receptors, vasoactive intestinal peptide gene and serotonin receptors. We have also identified gene locations that suggest the rearranged chromosome may in fact be the ancestral form, which is counterintuitive and has vast implications for the evolution of chromosome structure. Mapping of more genes continues as well as sequencing to identify differences in the genes between the morphs.

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Webster, M. S., S. PruettJones, D. F. Westneat, and S. J. Arnold. 1995. Measuring the effects of pairing success, extra-pair copulations and mate quality on the opportunity for sexual selection. Evolution **49**:1147-1157.

Webster, M. S., K. A. Tarvin, E. M. Tuttle, and S. Pruett-Jones. 2007. Promiscuity drives sexual selection in a socially monogamous bird. Evolution **61**:2205-2211.

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CHAPTER 1

CLIMATE CHANGE AND ECOLOGY AND BOREAL FOREST BIRD COMMUNITIES: A REVIEW

Abstract

Many recent studies have been examining the effects of climate on life history traits of birds. These studies have found varying impacts of climate on reproduction, migration and other life history factors. Unfortunately there is not currently a standard for examining climate impact on birds. Some studies use large teleconnections such as the NAO and ENSO, a few use climate envelopes, while others examine various temperature changes and other local measures of weather. Which is the best approach for examining climate at the organismal level? I examine all current approaches to measuring/identifying climate impact on birds. Additionally, I review the current work on climate and develop an ecologically relevant definition for use in organismal research. Current work attempts to create an overarching definition of climate change impact on organisms for conservation purposes. I will address the benefits of building species and location specific guidelines for measuring climate change. Research and conservation techniques need to be addressed on a species specific level, taking into account breeding ecology and food supply fluctuations. Small and large scale changes in climate also need to be incorporated to create a comprehensive view of climate impact on species. Finally, population genetics need to be examined to determine whether animals are evolving to the changing climate or being

phenotypically plastic. Any conservation measure would be incomplete without including the impact of climate change on genetic variability.

Introduction

Climate change encompasses any significant and lasting changes in temperature, rainfall, humidity and other weather patterns. Responses by various communities and populations to climate change are complicated and difficult to untangle and differentiate between. Climate interacts with multiple levels of communities to impact diversity. As climate change increases there is more variability in the environment which will result in lower species richness. Meta-analyses have shown that there is a significant shift in species ranges towards the poles and an advancement in phenology (Parmesan & Yohe, 2003). However, most short term changes in species are often attributed to natural fluctuations and local changes in human impact, making it difficult to attribute climate change impacts to observed changes in species (Parmesan & Yohe, 2003). Determining the impact of climate change is further confounded by delays of several years in the impact of recruitment to communities (Ottersen *et al.*, 2001).

Those species that are most affected by climate change are those with narrow range requirements, limiting their ability to respond and increasing extinction risk. Communities in the northern latitudes with lower species richness are expected to have the greatest impact from the changing climate (Jetz *et al.*, 2007). Understanding the variation in species richness along the latitudes has been an observed pattern, but one without a working explanation. Current work has found a correlation between the vegetation index NDVI as a measure of energy and species richness. For example, the amount of available energy was correlated with the number of breeding birds in a community (Hurlbert & Haskell, 2003).

Climate has and will continue to impact individuals and communities at many levels. Individuals are affected through impacts on physiology and reproduction, while communities (and indirectly individuals) are impacted through changes in predator prey interactions and levels of competition (Stenseth *et al.*, 2002). Climate change is a complex set of interactions, making it difficult to determine cause and effect. Community responses will be diverse, with changes in range and frequency creating new communities and interactions (Poloczanska *et al.*, 2008).

On average, the temperature on earth has increased by 0.6ºC within the last century and is expected to continue rising. However, organisms are not as affected by global change as they are by changes within their geographic regions (Walther *et al.*, 2002). Understanding the impacts on organisms within their region can help to preserve these species. Changes in biodiversity are of key interest as the climate changes, since as environments are lost due to temperature, so are organisms (Thomas *et al.*, 2004; Lovejoy & Hannah, 2005), resulting in a reduction of biodiversity. Species can either adapt to these changes in the environment or become extinct.

Due to the heterogeneity of the planet, climate change has impacted the various regions and species differently, changing precipitation rates, temperature, or other weather variables uniquely to each area. Climate change affects all levels of the ecosystem, from the individual to the community. Implications of climate change for the organisms include changes in phenology, range shifts or shrinkage, species interactions and composition in the various niche levels, and changes in the ecosystem as a whole. Phenology refers to the timing of events in an organism's life, including but not limited to breeding, migration, flowering, or the ending of hibernation. Timing changes within species can increase competition for resources and can also cause mistiming between breeding and peak food abundance (Walther *et al.*, 2002; Both *et al.*, 2006; Jonzen *et al.*, 2007). Many of these changes in phenology can be traced to changes in spring

temperatures or the severity of winter as measured by the North Atlantic Oscillation (NAO) (Walther *et al.*, 2002). To further complicate the determination of the impact of climate on these factors, genetics, population dynamics and photoperiod my also play a role in phenology. Variations between species located within the same sites also complicate the impact of climate (Walther *et al.*, 2002).

Climate and weather can impact many aspects of an organism's life history. The various climate differences around the world help to create the diverse ecosystems found, each relying on different combinations of factors for survival. Through the influx of heat and moisture from the different weather patterns, most of the energy for an ecosystem is obtained, changing the types of organisms that live in each area (Bonan, 2002) due to the different factors required for survival. Thus through climate, selective pressures on ecosystems change, resulting in changes of the composition of the species in that system (Greenland *et al.*, 2003).

Currently many authors are attempting to address the impact of climate patterns on organisms (for review see: Stenseth *et al.*, 2003). Unfortunately, there are many interactions at the species level, making it difficult to determine which climate factors are actually impacting changes in species (Walther *et al.*, 2002). Birds are useful but complex indicators of climate change because they are influenced by many different factors and climate locations throughout their life cycle. Conditions in the breeding grounds and wintering grounds can vary significantly, but the impacts of both conditions are interrelated. Due to this fact more studies need to examine the entire life cycle of the species instead of just studying individual life-history factors (Adahl *et al.*, 2006). The purpose of this review is to examine the current work on climate change in respect to birds, determining useful measures and definitions of climate and

weather for ecologists. Also, identify variables for measuring avian response and discuss why they are important. Finally, try to apply the current work on climate change to conservation.

Weather and Climate

The terms weather and climate are often used interchangeably by ecologists. While they are obviously related, depending on the measure of interest within the species, weather, climate or a combination of both groups of variables may be more useful than just one measure.

The weather in an area includes measures and fluctuations of local temperature, precipitation, humidity, air pressure and wind (Stenseth *et al.*, 2003), averaged over days or months for an individual location. Climate refers to the average measure of weather in an area, based on long periods of statistics for the locale. The measures include the extreme deviations from the norm, as well as the average values, generally reported as an index of climate. There are many different ways to measure climate and weather, and each combination of these measures may apply differently to different ecosystems (Greenland *et al.*, 2003; Stenseth *et al.*, 2003). A variety of climate and weather factors can be used in combination in order to accurately define the weather patterns at a given time and place. In addition to examining the climate patterns and the many variables within, extreme deviations should also be considered (Greenland *et al.*, 2003). Extreme deviations may be more of a limiting factor for some species than other indicators may lead you to conclude (Williams & Middleton, 2008).

In order to apply climate to an ecological perspective, it is vital for researchers to understand how the weather changes at the location of interest in response to the changing climate index. These relationships are not always easy to determine. Geographic location changes the effect of the climate index on different areas due to changes in topography. The seasons experienced at the study site may also impact the relationship of local weather with the

climate index. Finally, it is most important to research and apply the most relevant climate index to the study site (Stenseth *et al.*, 2003). Many recent studies simply chose the North Atlantic Oscillation (NAO) as a measure of climate because it is a very common index. However, it may not be the most relevant index for the current study, thus finding no significant correlation with climate and the study species. NAO and the El Niño Southern Oscillation (ENSO) have been used widely by ecologists, but there are other indices that are also available that might be useful depending upon the location and migration path of the study species (Greenland *et al.*, 2003). In addition to the large scale indices, local weather must also be taken into account for an accurate representation of the impacts of climate on organisms.

Measures of Climate and Weather

Climate Indices vs. Weather Variables

The use of indices breaks down complex weather patterns into usable, interrelated measures (Stenseth *et al.*, 2003) such as the NAO, that can be more easily correlated to ecological parameters. Teleconnections influence the frequency of storms, temperature and precipitation. These measures also represent the interconnectedness of the climate within the observed hemisphere or area, allowing for comparisons across wide species ranges. Another benefit of using large-scale climate indices is standardized measures are currently easily accessible through government websites. These indices have also been extrapolated into the past through tree ring information (Cook *et al.*, 1998; Stahle *et al.*, 1998) and ice-core data (Appenzeller *et al.*, 1998). However, due to the complexity of the interactions, using local weather measurements might make it easier to determine the mechanisms behind the changes in the systems, rather than extrapolating from complex indices.

North Atlantic Oscillation (NAO)

The major force of winter climate in the North Atlantic area is the NAO. The NAO is one of the most common teleconnection patterns used by ecologists since it covers such a large portion of the globe, impacting both North America and Europe. Various temperatures, pressures and changes in the amount of storminess can be easily correlated with either negative or positive NAO measures (Stenseth *et al.*, 2003). This teleconnection is also highly variable even within a positive year. A positive NAO results in warm ocean water in the North Atlantic which provides mild wet winters to North America. Negative NAO however results in cold and snowy winters. Current work with the NAO suggests that temperature variations may be one of the most important factors impacting ecology (Ottersen *et al.*, 2001). Wintering song birds may be the most impacted by changes in nighttime winter temperatures (Root, 1988), which can easily be applied to an area through the NAO measures.

El Niño Southern Oscillation (ENSO)

This teleconnection index can affect climate worldwide. ENSO is another commonly used index by ecologists since it does impact climate across the globe, especially areas of migration flyways. ENSO is the link between the Southern Oscillation (SO) and El Niño and the fluctuations between them. Changes in sea surface temperatures (SST) and sea level pressures (SLP) are used to calculate this index. "El Niño" and "La Niña" refer to the warm or cool phase of the SO respectively (Stenseth *et al.*, 2003). The cool phase of El Niño results in the areas around the South Pacific and Mexico experiencing cool wet winters. While the opposite, La Niña, provides hotter and drier winters. ENSO is of particular interest for Neotropical migrants since it impacts their wintering grounds in the south pacific and coasts of Mexico.

Other Teleconnections of Interest

In addition to the NAO and ENSO, there are several other teleconnections that may be of interest to ecologists depending on the system they are working on. Some of these include the Arctic Oscillation (AO), Antarctic Oscillation (AAO), Pacific Decadal Oscillation (PDO), and the Pacific-North American (PNA) (Stenseth *et al.*, 2003). Ecologists and other researchers should collaborate with climatologists to determine the most useful teleconnection or combination of teleconnections for use with their study species. These combinations should be determined by wintering and breeding grounds in addition to migration routes since the conditions at all locations can combine to impact reproductive success.

Mobile Indices

Another option is to examine the movement of these teleconnections, following the location of the focal points or nodes. The benefit of this type of teleconnection is the ability to track the changing nodes of the teleconnection through the changing seasons allows the strength of correlations between seasons to be maintained. Currently, the NAO is the only index for which a mobile version has been calculated. This NAO shows relationships that were currently lost when looking at seasons other than winter (Portis *et al.*, 2001). By taking the movement into account the correlations between the summer and winter measures are stronger due to elimination of some of the variance. When tracking the impact of climate on organisms it is important to be able to determine the impact of climate throughout the year, not just during the winter months. Having a more accurate measure of the NAO for the other seasons allows for interpretations to be drawn for more organisms where previously no correlations were observed.

Weather Variables

Climate at the local level is referred to through measures of weather as daily or monthly averages. These include temperature, precipitation, sea surface temperature, pressure and wind. When being used to examine species the averages as well as the maximum and minimums should be used. When examining the impact of climate on species the average temperature for the month may not necessarily be an accurate indicator of the impact of weather on the organism. If there are extreme temperature changes within the time frame that are lost in the averages, these extremes might have more of an impact on the species though bottlenecks of food supply than the averages would (Williams & Middleton, 2008).

Climate Envelopes

In order to predict the impact of climate on organisms many different models are being developed. Climate envelopes are a grouping of factors for a specific species that identifies the tolerance levels of the species (Walther *et al.*, 2002; Jiguet *et al.*, 2007; Beale *et al.*, 2008). Each climate envelope needs to be designed with the organism of interest in mind and include life history traits. Life history traits are various aspects of a species life cycle including reproduction, generation time, phenology. These aspects must be included in any climate model to make it relevant to the species of interest; otherwise important correlations may be lost. These traits help to determine the tolerance for climatic change that a species has. As these boundaries of tolerance shift, so does the species distribution. Changes in distribution are important for planning and implementing conservation measures for species.

According to Jiguet *et al.* (2007), the use of climate envelopes can be a good first approximation of the response of species, but when inferring results, the complexity of the system must be remembered. Ideally, a climate envelope model would only include climate

variables, but this type of model is not feasible (Pearson & Dawson, 2003). Other factors of the study species must be included. If enough variables are used, and they are chosen based on the species being examined, climate envelopes can be effective. The traits of the study species (such as ecological niche and life history traits) need to be included in the model, determining which traits make them sensitive to climate (Jiguet *et al.*, 2007). For some species the thermal range might be the limiting factor, but for others the thermal maximum might be their sensitivity to climate. Determining these ranges and habitats for birds is even more difficult since most species have larger ranges due to migration (Jiguet *et al.*, 2007). Once a climate envelope model has been developed for a species, phylogenetic relatedness can be used for applying the model to other closely related species (Jiguet *et al.*, 2007).

When used carefully, with individual species traits and the complexity of the system included, climate envelope models can a useful starting point for modeling response to climate change (Pearson & Dawson, 2003). However, there are three main criticisms of climate envelope models; biotic interactions, evolutionary change, and species dispersal. The interactions between individuals of the same species and individuals of cohabitating species may impact the response of species to climate change. These interactions make predictions into the future through models inaccurate (Pearson & Dawson, 2003). Additionally, models ignore the fact that changes in the environment can select for or against different phenotypes, potentially directing evolutionary change of the species (Pearson & Dawson, 2003). Finally, species dispersal can impact the response to climate change in a climate envelope model since dispersal has not been included as a factor. The range of a species dispersal can provide a buffer to climate change, increasing their chances of survival. Climate envelopes attempt to predict future ranges under climate change, not patterns of dispersal (Pearson & Dawson, 2003). The use of

climate envelopes can provide a good foundation at the macro-scale for species response to climate, but the usefulness at an individual species level needs to be addressed for each group.

Recent Studies on Birds

Distribution and Abundance

Climate change forces species to adjust their ranges (Parmesan & Yohe, 2003), or adapt to new conditions. If they cannot adjust to the new variables in their environment then they will be forced to extinction. As usable habitat is destroyed, the size of the population that can be supported may also decline. A current study examining rainforest birds compared species richness and abundance. They found that changing seasonality in rainfall had the most impact on tropical birds (Williams & Middleton, 2008). Species richness did not change throughout the study, however species abundance was impacted. Changes in rainfall caused by ENSO, alternating between years of severe drought and heavy rainfall, created resource bottlenecks for the species, limiting the food supply and thus population size. Similar work has been done in a species of bat (*Myotis myotis*) (Zahn *et al.*, 2007), also finding that climate seasonality impacts the major food supply (insects), impacting the abundance of the organism. Changes in climate that impact the distribution and timing of resources are an important factor limiting species abundance (Williams & Middleton, 2008).

Migration Timing

The timing of migration is influenced by a variety of factors. In some species the timing of migration (circannual rhythms) is actually genetically determined, identified through cross breeding experiments, but can be modified by external factors (Gwinner, 1996; Berthold, 1998). These species are especially vulnerable to variability in the environment if they do not have time to evolve to the changing conditions. A new study on geese has found evidence for photoperiod

triggering the start of migration, but the temperature variations along the migration route allow for adjustment in the speed at which they reach the breeding grounds (Bauer *et al.*, 2008). Climate conditions at the breeding grounds may not coincide with the migration conditions, impacting stress levels and fitness and resulting in arriving in unfavorable conditions. Thus the timing of migration can have a large impact on the successful reproduction of the species (Pulido, 2007).

A study in the pied flycatcher (*Ficedula hypoleuca*) has found advancement in laying date, but not in migration time. This species has been able to adjust the timing of reproduction due to climate change, but not in migration timing which is controlled by day length changes (Both & Visser, 2001). However, the adjustment of breeding time has brought the start of laying close to the arrival time, leaving the pied flycatcher with little variability in the onset of reproduction causing some to breed after the peak of insect abundance (Both & Visser, 2001).

Long- and short-distance migrants are impacted differently. Short-distance migrants traverse fewer climate gradients and may therefore be better able to estimate climate in the breeding grounds (Both & Visser, 2001). Long-distance migrants are impacted by climate changes at every stopping point along the migration route, especially wind (Pulido, 2007). These migrants must constantly be making decisions about the timing of arrival as they proceed along their migration route. Additionally, if long-distance migrants have a trigger other than climate change (such as genetics or photoperiod), migration may place them at the breeding site at non-optimal times (Both & Visser, 2001). A recent study has found that long-distance migrants in Europe are declining in population size more quickly than short-distance migrants (Sanderson *et al.*, 2006) due to the inability of the species to adjust o timing changes. Also, those species that

have been unable to adjust their phenology (timing) of migration are also on the decline in Europe (Moller *et al.*, 2008).

Another study has found correlations between a positive NAO index and the arrival time of both long- and short-distance migrating species. However, the mechanism for the advancement may be different for each group. The long-distance migrants were affected by improved conditions in wintering grounds, while the short-distance migrants were affected by the conditions at the stopover sites during migration (Forchhammer *et al.*, 2002). Studies of this type reinforce the observation that climate impact must be determined on a species by species basis to observe the relevant mechanisms of change.

Originally, it was assumed that the timing of migration was fixed and, that birds cannot predict what the conditions are like at the breeding grounds (Lehikoinen *et al.*, 2004), suggesting that they determine migration time by changes in temperature (Wilson, 2007) or in photoperiod (Gwinner, 1996; Berthold, 1998) at the wintering grounds. A recent study however has suggested that the interconnectedness of the climate in the wintering and breeding grounds may give the birds a clue about future weather conditions, allowing them to adjust migration timing based on the assumed weather in the breeding grounds (Saino & Ambrosini, 2008).

Physical conditions during winter may also impact timing of migration (Pulido, 2007). Individuals need to be in optimum condition throughout migration to survive and reproduce once they reach the breeding grounds. Better physical condition leads to earlier arrival and territory establishment, earlier breeding, and thus earlier fledging of offspring which conveys a higher survival rate to the offspring (for review see: Pulido, 2007).

Continued research is needed in determining the true climate factors that impact avian migration, whether it is a combination of teleconnections between wintering and breeding

grounds or a combination of different weather variables from each location (Gordo, 2007). The determination whether the changes observed are genetic or phenotypic plasticity also need to be discerned (Gienapp *et al.*, 2007).

Population Genetics

While determining the impact of climate on birds, the question is posed: Is the response simply phenotypic plasticity, or are there evolutionary changes occurring within the species? In order to determine the function of evolution in these changes, long term genetic data needs to be available (Parmesan, 2006). Current work suggests that species will not evolve rapidly enough to continue living in current distributions if the habitat's climate changes too much. However, evolution may aid the species adaptation to the changes (for review see: Parmesan, 2006). The speed at which the climate is currently changing should be exerting strong selection pressures on species, allowing for microevolution of traits to deal with these changes (Gienapp *et al.*, 2008).

In order to make estimates of population change we need to look at genetics. With shrinking population sizes, we need to examine genetic variability to determine if the populations have enough genetic diversity to respond to and survive the climate change. Examining the impact of climate on population genetics is a fairly new line of inquiry that needs to be examined much more intensely in the next few years. By combining multiple lines of study, including population genetics and ecology we can work on developing a comprehensive image of species response to climate change (Pertoldi & Bach, 2007). Long-term data sets of populations are needed in addition to experimental manipulation in order to determine genetic and phenotypic differences in response to climate change (Pulido, 2007). Currently many of the responses to climate are phenotypic with only rare indications of genetic changes (Garant *et al.*, 2008;

Gienapp *et al.*, 2008; Teplitsky *et al.*, 2008), many more studies are necessary to determine climate impact on population genetics.

Use in Conservation

Current emphasis on species conservation has boosted research on climate change and species response, which interact in a complicated fashion. Many more studies involving a wide variety of species are needed. In order to develop useful conservation measures the responses of each unique species under observation need to be understood. Unfortunately, this means that there is not one general measure of climate change that can be applied across the globe, making it difficult to show the public definitive signs of climate change impacting species diversity and survival. Work needs to be done to identify an index that will be easily conveyed to the general population (Hansen *et al.*, 1998). Many traits such as the type of food eaten, number of young produced and frequency of reproduction, lifetime, size and migration distance all impact an organism's response to climate change (Jiguet *et al.*, 2007). Those organisms that are specialized in the types of food they eat are often more impacted than those that have a wider range of foraging habitats.

Natural ecosystems are very complex, attempting to model and predict changes to these systems at an accurate level is currently impossible. However, the use of climate envelopes and other modeling techniques can be used to predict impacts on species and develop methods of conservation. Conservation actions need to be addresses in all areas of the species life cycle, not just the breeding grounds. Areas that are part of the migration and wintering habitats also need to be protected, which may be difficult across various countries (Sanderson *et al.*, 2006).

Climate Change

Global temperature has increased ~0.6 °C over the past 100 years and is anticipated to continue increasing 1.1-6.4°C over the next century (IPCC, 2007). Climate change attributed to anthropogenic effects has now been documented around the globe and has been shown to affect most taxonomic groups (Parmesan, 2006) and in diverse ecosystems and is estimated to impact 41% of all species (Parmesan & Yohe, 2003). Changing and increasing temperatures are not the only result of global climate change. Additionally associated with climate changes are changes in precipitation, severity of storms and sea level variation are also expected (IPCC, 2007).

Climate is responsible for supplying the ecosystems with solar energy and water. Changes in the supply of these will affect ecosystems in varying ways, impacting and changing the organisms that survive in the different environments. Areas of very different climate regimes will support widely different species and communities. The earth's surface is very heterogeneous, resulting in a wide range of effects of climate change around the globe, varying the impact on species in those areas (Walther *et al.*, 2002). The impacts these changes have on organisms need to be addressed by ecologists.

Grinnell (1917) first identified the role of climate in determining the extent of species range. Species will only exist in areas that fit physiological needs and limits. As changes occur in temperature and precipitation the areas that fit these physiological needs will change, resulting in range shifts. Changing climate has been shown to impact species distribution and abundance, phenology, and survival (For review see; Seavy *et al.*, 2008). By looking at the past and using historical species data researchers are attempting to predict the changes we can expect in the future. Shifts in geographic range and decreases in species diversity as well as changes in community composition are expected to occur in the future of climate change and have already

been documented in Africa and North America (Seavy *et al.*, 2008) and very well documented in Europe (Parmesan, 2006; Seavy *et al.*, 2008). Additionally, we need to understand how the changing climate will impact the population dynamics of species. Ecological climate is the combination of multiple weather factors that determine the boundaries between ecosystems. It is a complex interaction between energy, temperature and precipitation. Additionally, small variations in topography will affect the climate in small sub areas of the larger ecosystem. Changes in the frequency and severity of climactic events are also occurring with the changing climate (IPCC, 2007). These changes also impact organisms and change biodiversity even if they don't demonstrate large, long-term temperature changes. Some organisms may be impacted by the length of cold or warm periods, while other organisms may be affected by a single, severe change in temperature. Ecosystems and communities are impacted by climate at a variety of levels. Climate may change interactions with a delayed reaction, changing sizes and survival of cohorts between years. Large cohort years will in the future also be large groups, increasing the size of age classes in the community. Climate also impacts differential mortality of sexes and ages of organisms in the community, changing population dynamics in the area (Stenseth *et al.*, 2002).

When examining climate there are many factors to address and terms to differentiate. Depending upon the focus of the study, the study species and location the variables of interest may change. Weather refers to the current state of the atmosphere at a specific point in time including such aspects as temperature, air pressure, precipitation and wind. While the term climate refers to the long term weather characteristics for a given area based on past averages. Large scale teleconnection indices are often used to describe climate trends in given regions; these include the NAO (North Atlantic Oscillation), ENSO (El Niño Southern Oscillation) and

the PDO (Pacific Decadal Oscillation). These represent shifts in pressure systems across large areas (ENSO can impact climate around the globe) and can be predicted from long-term climate data as well as interactions with land masses and oceans. These patterns impact local weather through wind speed, humidity, storm tracks, and how the air masses move through the area. Finally, a micro climate refers to the various different climates that can occur within the different ecosystems across a region.

Climate variables are all interrelated which makes determining specific cause and effect difficult. These interacting effects may in some instances be more important than individual weather characteristics in impacting the biological community (Stenseth *et al.*, 2003). Temperature related variables, especially the temperature of the coldest winter months, are important climate variables for determining species richness (Kivinen *et al.*, 2008). Finding the right scale for the current study of interest is important in determining the impact of climate on various species. Using large scale teleconnections such as the NAO has proven to be more accurate than local climate in about half the studied cases of life history traits and population dynamics (Stenseth & Mysterud, 2005). This accuracy suggests that in the study of ecology these teleconnection indices may be a more useful than individual climate variables since organisms respond in complex ways.

Climate impacts how well individual organisms perform and will change their distribution and abundance (Stenseth *et al.*, 2003). Changes in climate will vary depending on the changing characteristics of the habitat such as fragmentation and elevation (Saether *et al.*, 2004). Depending on the types of species in each area the response time will vary, some responding within seasons (e.g. insects) and others across decades (e.g. trees). Understanding the impacts of climate change is difficult and complex due to the many interacting effects of the

ecosystem and the environment. The interactions of climate and organisms are very complex because organisms are not stationary, have varying generation times and are impacted by multiple factors of weather such as temperature and precipitation. Due to the multiple interacting factors, no individual variable will accurately describe the impact of climate on a single organism, suggesting that the use of climate indices may be more useful in ecological studies (Stenseth & Mysterud, 2005). Understanding the various ways population dynamics are impacted by climate will help to determine the best scale of climate for each ecological study.

A bioclimate envelope is a method for determining the climate criteria or niche for each species and is based upon the ecological niche of the organism. These examine life history traits for the species of interest and determine which climactic factors are most important for a given species. They identify limits of individual species and determine where these limits will fall within the changing environment (Walther *et al.*, 2002). By identifying these limits broader models may be developed. Life history traits of various species are expected to influence the species ability to respond to climate change (Jiguet *et al.*, 2007).

Community and Population Dynamics

Populations refer to single species groups while communities are groups of organisms that interact and affect the population dynamics of the others within an area. There are two hypotheses to describe the population fluctuations observed from climate change, the tub-hypothesis and the tap-hypothesis. The tub-hypothesis examines how the overall population fluctuates with the impact of climate variation on the non-breeding population; i.e. examines the number of individuals that survive. The tap-hypothesis associates climate impact with fluctuations in the size of the breeding population and the number of new recruits that are brought into the population (Saether *et al.*, 2004). Changes in population size can either be

dependent or independent of the population density and can impact survival of the adult population or the fecundity of the population. Biotic interactions are affected by climate change, moving through the trophic levels and impacting communities. Climate change can have either a positive or negative effect on species depending upon the interactions that are impacted (Poloczanska *et al.*, 2008). Studies have shown that two similar species can have opposite effects of climate change, altering community position (Poloczanska *et al.*, 2008). Population carrying capacities are affected by the supply of resources available (Ricklefs, 2008) while these resources are impacted by the energy being added to the area through climate.

There are differences in latitudinal gradients with species richness, as the latitude increases and there is more variability in the environment, species richness declines. As species ranges move with climate, community composition can change, especially as the range shifts far northward. Additionally, small changes in the topography can change how the climate impacts the species, and alters the area of suitable habitat. As the changing climate impacts the range of species, the rate of migration and gene flow between populations can change, potentially reducing the effective population size in areas that become isolated (Pertoldi & Bach, 2007). Small populations are at greater risk due to genetic drift resulting in loss of genetic variability, reducing the ability of the population to evolve in response to the changing conditions. Only large populations in areas that are experiencing limited climate change will be able to evolve with the climate (Pertoldi & Bach, 2007).

Weather factors such as temperature and precipitation can have large impacts on survival and reproduction and should be considered when examining the populations of species. The changing climate has caused a reduction in usable habitat for some species, while others adapted their range. Those species that are unable to expand their ranges fast enough or are prevented by

geographic barriers will be forced to extinction or have already gone extinct (Parmesan, 2006). Dispersal ability can impact species distributions and allow for non-native species to colonize new habitats (Walther *et al.*, 2002). Species that have been able to expand their ranges as dispersers are more successful at colonizing areas previously outside the normal range for that organism (Parmesan, 2006). Researchers need to understand the distribution, abundance and interaction of species, but these patterns will vary depending on the scale of observation (Leibold *et al.*, 2004).

Communities are groups of different species that live in the same area at the same time (Ricklefs, 2008). Communities can be grouped together by larger regional biota and create a metacommunity where local communities can exchange immigrants (Leibold *et al.*, 2004), aiding in their ability to survive environmental changes. Habitat fragmentation may cause patches to be separated by distances larger than the dispersal ability of the species, preventing them from colonizing new habitats even if they are suitable (Thomas, 2000). Changes in ranges lead to community reassembly as the species in the area change (Walther *et al.*, 2002; Schaefer *et al.*, 2008), colonization, and local and global extinctions also cause reassembly (Barry *et al.*, 1995) which might lead to changes in species richness. Changes in migratory behavior of birds; reduction of migratory behavior or expansion of migratory ranges will create colonization opportunities by increasing the ranges and possibly extinction of previous residents as migrants become permanent residents (Schaefer *et al.*, 2008). Community compositions are in flux from climate change, gaining and losing residents.

Biodiversity

Biodiversity is the cornerstone of a functioning ecosystem. In order for ecosystems to function correctly there must be diversity in its residents, without diversity the system will not

function properly. Biodiversity is the different number of organisms living in an area and varies across the globe. Species richness is a common measure for biodiversity (Purvis & Hector, 2000) and is determined from the number of species found in the area. Species richness varies geographically and is affected by multiple mechanisms which makes determining a cohesive picture of species richness across all areas very difficult (Carnicer *et al.*, 2007). Climate change can positively or negatively affect the biodiversity of an area. Some systems with high biodiversity may be able to adapt to the changing environment due to a more stable system and increases in productivity. However, if the changing environment is outside the tolerance limits of the community then diversity will decrease through extinction or emigration (Pertoldi & Bach, 2007). Community size has also been found to impact species richness in an area. In order to assess the biodiversity for a given area you also need to take into account the species that are already in danger of extinction. Climate and habitat quality or type determine distribution patterns of species and level of threatened status (Kivinen *et al.*, 2008).

Available energy in the ecosystem is important for determining species richness. Areas that have high energy input are able to support more individuals, thus increasing species richness (Hurlbert & Haskell, 2003). However, as variability in energy increases, species richness decreases; suggesting that more stable environments support higher species diversity (Rowhani *et al.*, 2008). More specifically, areas that are more temperature stable have higher species richness. Precipitation also influences richness, however, if the temperature is extreme, precipitation has less impact than temperature (H-Acevedo & Currie, 2003). Lower stability in the environment increases the probability of extinctions and increases the size of the niche required. The energy available in the environment has been used to describe the variation in the patterns of global biodiversity. Species-energy relationships predict a positive relationship

between the amount of energy in the system and the number of different species found (Rowhani *et al.*, 2008). Energy is provided by climate through the amount of vegetation in the habitat or primary productivity. As primary productivity increases so does species richness. Habitat fragmentation, disease, and anthropogenic disturbance also help explain some of the variability in species richness. As climate change continues to grow, extreme weather events are much more likely (IPCC, 2007), resulting in a much higher variability in vegetation and an impact on species richness (Rowhani *et al.*, 2008). More research is needed into the changing climate and the impact on ecosystem energy to prevent local extinctions.

Current thinking is that climate is the main limiting factor in species distributions (Araujo & Luoto, 2007) and explains the variation in species richness and rarity across the globe (Ohlemuller *et al.*, 2008). Areas that support small range species are limited by the types of climate, thus shaping the distribution patterns and rarity of these species (Ohlemuller *et al.*, 2008). Species diversity increases in areas that are geographically and climatically diverse, creating diversity hot spots. These areas may be more impacted by the changing, warming climate, resulting in extinction hot spots (Ohlemuller *et al.*, 2008).

Temperature and amount of precipitation has the greatest impact on species richness. An increase in species richness is expected for northern latitudes and higher elevations as species begin to shift their ranges northward, while there will be a decrease in species richness in arid areas (Bohning-Gaese & Lemoine, 2004). As species ranges change there can be changes in species richness, the changes can either be an increase or a decrease depending on the extent of colonizations and extinctions in the population (Bohning-Gaese & Lemoine, 2004).

There are significant differences in the strength of response to climate change across taxonomic groups, making broad assessments of climate change impact difficult (Parmesan,

2007). Changes in biodiversity due to climate change have been documented through studies of distribution and abundance, changes in phenology, and changes in community composition (For reveiw see; Devictor *et al.*, 2008). Within bird species, migratory species are impacted the most by changes in energy availability at the peak in winter and summer regions, while non-migrants are most impacted by the lowest level of energy in the area (Hurlbert & Haskell, 2003).

Indirect Effects

Changes in climate will also impact avian communities indirectly through changes in vegetation, food supply and competition or predation. All organisms depend directly or indirectly on primary production, which is impacted by climate (H-Acevedo & Currie, 2003). Precipitation impacts vegetation, changing structure and composition which can impact avian distribution and abundance. There is also evidence for changes in the phenology of invertebrate populations (Seavy *et al.*, 2008). Changes in temperature also impact all trophic levels through changes in the metabolic rate of organisms lacking thermal regulation (Ottersen *et al.*, 2001). These invertebrates are an important food supply for birds, especially during the breeding season. Mismatches in timing between avian reproduction and invertebrates will impact breeding success (Stenseth & Mysterud, 2002). Evidence of these indirect effects may be visible after only a few years with the invertebrates, but may take decades to see in vegetation structure (Seavy *et al.*, 2008). Phenological changes interact with other aspects of species life history traits and distribution to determine the species ability to respond to climate change (Parmesan, 2006).

The effects of climate change may not directly impact the species, but rather its food and habitat requirements. Phenological changes cause mismatches in timing across species between vegetation and food supply and between predators and prey. Smaller organisms such as insects

may more quickly adapt to changing climate, impacting timing of reproduction and causing a mismatch with the reproduction of larger organism such as birds that rely on them for food supply. Thus, varying trophic levels may impact migratory bird species as timing between levels is asynchronous (Jones *et al.*, 2003). Individual differences in species responses to climate change results in the high variability in wild populations response to climate change (Parmesan, 2006). Researchers need to remember to examine both direct and indirect effects of climate change, complicating the identification of factors (Stenseth & Mysterud, 2002).

Birds and Climate

On average, Northern hemisphere birds are shifting their range northward with a few also contracting their southern border. These shifts may be limited by the individual species tolerance for winter nighttime low temperatures (For review see; Parmesan, 2006).

Environmental variables can be correlated with the occurrence and richness of species across areas. Temperature has been found to be very important in species richness in addition to land cover (Kivinen *et al.*, 2008). Birds are very mobile and are therefore able to track changes in available resources and respond quickly to changes in the environment (Rowhani *et al.*, 2008). Migratory and non migratory birds will respond differently to changes in the environment, non migratory birds are exposed to harsh northern conditions all year while migrants have changes in breeding and non breeding grounds as well as conditions on the migration route to contend with. Migrants will either develop residency or non-migrants will begin migrating. Migration rate is determined by the severity of climate in the wintering areas and the availability of food in the breeding season (Hurlbert & Haskell, 2003). Changing climate will impact the number of migratory and non migratory bird species, changing the dynamics of the populations, varying population densities and changing the competition rates for food, nesting sites and territories as

the species composition of an area changes depending on migratory behavior (Schaefer *et al.*, 2008). Changes in migration patterns will vary depending on the local climate regime for the population of study (Schaefer *et al.*, 2008). Schaefer et al (2008) have shown with their model that with climate change, bird communities will undergo community reassembly and behavior adaptation, impacting their communities.

Climate change in birds has been well studied, documenting changes in their ranges and rates of colonization. Changes in precipitation and temperature have so far been found to explain differences in avian species richness (H-Acevedo & Currie, 2003). Using NDVI (normalized difference vegetation index) to estimate the productivity of the plants which birds directly or indirectly utilize has been correlated to species richness (Hurlbert & Haskell, 2003). Productivity has a direct relationship on the species richness and community size of birds (Carnicer *et al.*, 2007). Primary productivity of an area (measured in NDVI) is a proxy measure for the amount of food (IPCC, 2001) an area supplies to the community. As community size increases so does the population density, with a corresponding reduction in the extinction rate. Productivity and habitat availability are important factors in determining species richness and community size. High levels of productivity will have limited impact, while low levels of productivity will constrain population sizes and reduce species richness in areas (Carnicer *et al.*, 2007).

Climate impacts survival rate during the winter months and impacts population change during the breeding season (Saether *et al.*, 2004). Avian population responses to climate change are based on life history and ecological traits that may help buffer the effects of the changes (Saether *et al.*, 2004). Changes in temperature affect all steps leading to recruitment into a population (Ottersen *et al.*, 2001). Climate change will impact the position and size of bird

ranges, richness and composition of bird communities, changes in the migratory behavior of species will result in the changes of the composition of communities (Bohning-Gaese & Lemoine, 2004).

Understanding how climate affects bird species is very important. Currently, statistically significant relationships between field data on bird communities and climate are few and need to be expanded. Birds are an important aspect of all communities, providing disease vectors, pollination and seed dispersal, impacting the food web of the community and changing community interactions (Bohning-Gaese & Lemoine, 2004).

Boreal Forest Communities

Climate warming has had an even larger impact on the boreal region, with an increase in temperature of 4°C over the past century (IPCC, 2001), indicating that higher elevations are indeed experiencing greater impact of climate change. However, variation between geographic regions and species is too complex to be explained simply by latitude, continuing to challenge the identification of causative agents (Parmesan, 2007). Boreal forest is highly susceptible to climate change due to the ecological characteristics of the area. The boreal forest covers approximately 10 - 14% of the earth's land surface and is the second most extensive community after tropical rainforests. However, biodiversity is lower in these areas due to the extremes of temperature and precipitation. Since the organisms in this biome are adapted to low temperatures they are extremely vulnerable to the changing environment. Slight changes in temperature may result in great changes in the makeup of the biome. Additionally, the boreal forest impacts many areas around the world; supplying fresh water, stabilizing heat transferred by the oceans, aiding in solar energy absorption, release of energy from decomposing organic matter, and finally water, energy and gas exchange with the atmosphere (for review see; Chapin

et al., 2006). Climate differentiates the boreal forest biome from other biomes by the changing climate in northern latitudes. Climate can change resource supply, as the climate warms the length of the growing season is increasing in the boreal forest. As the length of the growing season increases, so does the amount of carbon stored by the biome (McGuire & Chapin, 2006). Any impact of the changing environment on one species can have an impact on the entire community (Jones *et al.*, 2003).

Boreal regions around the world vary greatly in climate, with large variation in temperature, precipitation and soil conditions (i.e. permafrost to no permafrost) (Hinzman *et al.*, 2006). A meta-analysis of lower range boundaries in the Northern Hemisphere has shown they have moved north (or upward for mountainous regions) on average 6.1 km/decade (Parmesan & Yohe, 2003), impacting species distribution. Tree lines have also been moving northward (Luckman & Kavanagh, 2000). Winter temperature is an important variable to examine. Coldest night temperatures can impact survival. The changing climate has been shown to impact northern temperate altricial birds during the non breeding season with a weather dependent loss of birds during the winter months. NAO and ENSO have been used to explain a lot of the variation (Saether *et al.*, 2004). As birds change their migration patterns with climate change, reducing the distance of migration we can initially expect an increase in species richness in the boreal forest (Bohning-Gaese & Lemoine, 2004). However, as climate change continues and the species ranges continue to shift north those species that cannot shift will gradually become extinct, resulting in gradual species loss in these areas.

Summary

Climate change is a complex interaction of many factors that affects all levels of an ecosystem. Changes in temperature and precipitation will have different impacts on every

organism. Shifts in many species ranges due to climate warming have been well documented through history (For review see; Parmesan, 2006). However, bird range shifts have been supported with very few field studies. Additionally, it has taken many years of research to convince the population that there is currently a trend for global warming. To date, there is limited statistical evidence for changes in species richness due to climate change. Evolutionary responses of organisms can help mitigate the influence of climate on species, allowing them to inhabit a wider range of habitats (Parmesan, 2006) adding to the difficulty of distinguishing response to climate change.

Shrinking ranges, extinctions, and declines in species richness are indicators climate change (Bohning-Gaese & Lemoine, 2004). Climate change impacts at all levels; individual, population and community, affecting all trophic levels through productivity changes and population dynamics (Ottersen *et al.*, 2001). We need to examine how the bird communities are changing and determine the impact this will have on the ecosystem. Birds are crucial to a functioning ecosystem since they provide necessary pollination and seed dispersal, climatic impact on their communities will impact all levels of the ecosystem (Bohning-Gaese & Lemoine, 2004). Understanding how the changing climate affects the boreal forest is of great importance since the boreal biome has such an impact on the climate around the world and it shows the impact of climate change earlier and more dramatically than other ecosystems.

Climate impact on birds is a very large, complicated pattern without an easy solution or model. Much more work is needed examining life history traits and climate change. No single model will be applicable to all species, and it is this lack of a simple model that makes conveying information to the public in an understandable manner difficult. Additionally, long term biological data is scarce, making strong correlations with climate difficult. Researchers must

also remember to examine variation within- and between- years to determine which factors of climate impact the species the most (Williams & Middleton, 2008). Long-lived species will continue to be impacted by the conditions during winter months through the breeding season and into the following winter, making the determination of the actual mechanism difficult.

The majority of studies on climate change are conducted at a relatively small scale, using local study sites and short term data collection (Parmesan, 2006). In order to determine the real impact on species their entire range and life cycle need to be addressed, which is complex, especially for migrating species. Equating climate data with ecological data is difficult due to this limit on available ecological data. Climate data can be found for several centuries due to the development of proxy indices, where as many organismal studies attempt to infer changes in species with only a few years of data. These years could easily overlap with a major, but short term shift in climate variables. All species studies should interpret available climate data for the area of the study and determine where fluctuations in the climate are occurring to determine if their sample size is an accurate representative of climate change impact on species and not just a response to an unusual extreme shift.

The majority of studies that have found evidence of the influence of climate on species has come from some measure of phenology change (Parmesan, 2006), mainly due to the large amount of data surrounding agriculture and changes in plant phenology. However, many other factors interact with phenology, making it difficult to determine the impact of climate on individual species (Parmesan, 2006). There have also been many studies on the timing of migration and spring arrival, however there is still no conclusive evidence for the mechanism underlying these observed changes (Pulido, 2007).

Complex interactions of species and communities with climate make it difficult to predict how ecosystems will respond (Walther *et al.*, 2002). Ecologists need to remember to not only account for changes in climate such as temperature increases and changes in precipitation levels, but to also include the degree and frequency of variability. Changes in extremes of climate and the frequency of the swings between the extremes can dramatically impact species. Short-lived species across taxonomic groups are expected to be more drastically impacted by high variability in climate than long lived (Morris *et al.*, 2008). Species that rely on one reproductive event each year will be the most impacted by these events, causing complete reproductive failure in some individuals, potentially leading to extinction. While examining the potential for species extinction we need to remember to include generation time and reproduction as factors for modeling climate impact on organisms (Morris *et al.*, 2008).

Many biological researchers attempt to measure climate change, when in actuality they are measuring a small subset of weather variables on a local scale. Instead, researchers need to examine both climate indices for a wide range of areas and local climate variables to create a complete picture of climate impact on birds. Ecologists need to remember that all aspects of an organism's life cycle are impacted by climate change and need to be examined and included in the models. Additionally, they need to use species specific measures to determine the impact on their organism of study. Species all differ in their ecological niche requirements and tolerance to change. These variations within species are mirrored in the variations in response to climate (Parmesan & Yohe, 2003).

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CHAPTER 2

THE OPPORTUNITY FOR SEXUAL SELECTION IN THE SOCIALLY MONOGAMOUS WHITE-THROATED SPARROW (*ZONOTRICHIA ALBICOLLIS*)

Abstract

Sexual selection has been examined for many years and across many groups, however the results have been inconsistent. Most birds are socially monogamous; however, they also show a variety of exaggerated sexual characteristics. White-throated sparrows are a unique group in which to study sexual selection. Within this species there are two plumage types, white or tan, that are caused by a genetic polymorphism on the second largest autosome. Males occur in both plumage types and use alternative reproductive strategies, allowing us to compare varying levels of sexual selection within one species. Using microsatellite data we examined 13 years of parentage data, 27.5% of the chicks were extra-pair offspring of white males while only 4.6% of the offspring were extra-pair young sired by tan males. Using Bateman gradients to examine the measure of sexual selection, we determined that tan males are under stronger sexual selection from the production of with-in pair young. This is most likely due to differences in mate quality between the pair types. However the white males are under stronger sexual selection from extra-pair mates.

Introduction

The concept of sexual selection has been around since Darwin (1871) and refers to the variation in reproductive success that can occur between competing males. In the general model, some males in a population may monopolize most or all of the females for mating while others will be excluded and fail to mate at all. This will result in variation of characteristics between the males (*i.e.* development of secondary sexual characteristics) and the removal of "less fit" members of the population as these members fail to breed. In birds, most passerines are socially monogamous which, if it is true genetic monogamy, should minimize sexual selection. It has been suggested that over 90% of birds are monogamous (Lack 1968). However, there are many socially monogamous species that show signs of sexual selection through exaggerated male ornaments. With the advancement of molecular techniques it is now possible to compare "apparent reproductive success" (number of offspring in a nest) to "realized (*i.e.* genetic) reproductive success," accounting for the acquisition of extra-pair matings and loss of parentage in their social nest. Extra-pair young are now known to be quite frequent in birds (Westneat et al. 1990, Westneat and Stewart 2003, Byers et al. 2004), especially in passerines (for review (Griffith *et al.* 2002), which would explain the levels of sexual selection observed in previous assumed monogamous species. However, a male may simply trade extra-pair young (EPY) for within-pair young (WPY) if he fails to father the young in his social mate's nest. Differences in reproductive success through EPY may also explain the effects of sexual selection in other species.

White-throated sparrows (*Zonotrichia albicollis*) offer a unique opportunity to examine the opportunity for selection. Within this passerine species both males and females occur in one of two morphs, white or tan, based upon the color of their median crown stripe. These morphs

mate disassortatively (Lowther 1961), a white morph mates with a tan morph ~97% of the time. Associated with the morph differences are behavioral differences, both of which are absolutely correlated with a large chromosomal rearrangement on the second largest autosome (Thornycroft 1975, Thomas *et al.* 2008). White birds are heterozygous for this rearrangement while tan birds are homozygous for the lack of the rearrangement. The rearrangement is inherited in a Mendelian pattern, which combined with the disassortative mating system, maintains the morphs equally across their range. Mate choice experiments have shown that tan males are the preferred male morph and white females are the preferred female morph (Tuttle 1993). These preferences are most likely due to increased levels of parental care by the tan males and the increased levels of aggression and nest defense in the white females. Some level of homozygous disadvantage must be acting to prevent pairing of white-white birds. Differential behavior may also influence the pairing, to create ideal pairs based on personal genotype (Brown 1997).

Behavioral differences include variation in song rates, territory defense and aggression (Ficken *et al.* 1978), and levels of parental care (Knapton and Falls 1983). Most importantly for the opportunity for selection, the different male morphs practice alternative reproductive strategies; white males pursue extra-pair copulations while tan males mate guard to protect their paternity (Tuttle 2003).

Density has also been shown to influence the opportunity for extra-pair paternity. White-throated sparrows differentially settle in different habitat types to control the number of neighbors they have. White males prefer to settle in territories with more neighbors (*i.e.* bog) thus increasing the probability of encountering fertile females. Tan males prefer to settle in low-density areas that are bordered by uninhabitable area (*i.e.* ponds) to limit the number of neighbors (Formica *et al.* 2004).

Thus, due to the behavioral differences within this species we predict differing levels of selection between the two morphs. Through their social choices for high-density territories, white males increase the potential for selection, whereas the tan males are decreasing the potential by limiting exposure to extra-pair copulations. Additionally, if extra-pair copulations are driven by the female, we would expect stronger selection in the white males than in tan males. However, if extra-pair copulations are male-driven, which field observations would suggest (personal observation), then the force of selection would be reduced.

Methods

Field Methods

We studied a population of white-throated sparrows at the Cranberry Lake Biological Station (44.15ºN, 74.78ºW) in the Adirondack Mountains of upstate New York. This site is approximately 2 km^2 (200 hectares) and supports between 20 and 100 breeding pairs each year. This population has been studied since 1988, however the current study encompasses data from 1998 – 2010. Adults were captured through mist netting either passively while foraging, at the nest or with playback of intruding male song. Captured adults were uniquely marked with a Fish and Wildlife numbered band and three colored bands (U.S. Fish and Wildlife Master Banding permit to E.M. Tuttle 22296). Blood samples (approximately 200 µl) were taken from all individuals through the brachial vein for genetic parentage analysis.

Historic territories were monitored and territorial pairs were identified (minimum 80 hours observation/pair). Nests were found through observation of resident pair behavior or systematic searching of the territory. Once found, nests were monitored daily for egg/chick number, hatching date and predation events. All surviving chicks were banded 6 – 7 days post hatching with a unique color combination and aluminum numbered band. Blood samples

(approximately 50 µl) are also taken from the chicks for genetic analysis. All blood samples were separated by centrifugation and hematocrit was stored in Longmire's solution (Longmire *et al.* 1992) at 4ºC until molecular analysis was performed. We are confident that the majority of nests were found or determined to have failed through the behavior of the pairs. Additionally, all un-banded fledglings captured do not match parentage of resident pairs and are assumed to be dispersers from nearby areas.

Molecular Methods

Stored hematocrit was used for DNA extraction with the DNA IQ® magnetic extraction kit (Promega, Madison WI) (years 1998 - 1999, and 2002 - 2010) or by the phenol:chloroform method (years 2000 and 2001) (Sambrook and Russell 2001). Five – eight polymorphic microsatellite markers were optimized from other species and used for the parentage analysis; MME1 (Jeffery *et al.* 2001), Gf01 and Gf12 (Petren 1998), Dpµ01 and Dpµ03 (Dawson *et al.* 1997), Zole_C02, Zole_C07, Zole_H02 (Poesel *et al.* 2009). The primers MME1, Gf1, Gf12, Dpµ01 and Dpµ03 were amplified according to Formica *et al* 2009. While the white crowned sparrow primers were amplified as published by Poesel *et al* 2009. Amplified fragments were run on an ABI 310 Genetic Analyzer® and analyzed with the GeneScan® program (Applied Biosystems, Foster City, CA). Parentage assignments were generated by first checking for binning errors by hand through comparison of social parents and nest mates based on territory observations and then confirmed by using CERVUS (3.0) (Kalinowski *et al.* 2007), and the calculated likelihood score (LOD). Scoring errors are also frequent on the four dinucleotide loci, MME1, Gf01, Gf12, and Dpµ01, causing mismatches in CERVUS, we attempt to eliminate these errors before running the analysis. Additional extra-pair sires identified are also identified by CERVUS that were missed during the preliminary examination.

Statistics

Total numbers of offspring in the nest, number of within-pair young, and the number of extra-pair young for each male were calculated for each nest. Reproductive success (number of offspring) for each male was calculated for each year and for their lifetime. The number of young produced in each nest is considered the "apparent reproductive success" and number of genetically matched young from all territories is the "actual reproductive success" of each male.

Territories were categorized as bog, pond or forest based upon structural characteristics (i.e. located within the bog, bordered on one side by a pond, or located along a forest edge). Densities for each territory were estimated depending upon the number of neighbors each territory has, 1-2 neighbors are low density and three or more neighbors are high density. These categories were used for statistics comparing pair types across the study site.

The "Bateman Gradient" (Bateman 1948, Arnold and Duvall 1994) or the measure of sexual selection is the slope of the regression of the number of genetic offspring against the number of mates. Relative mating success and relative reproductive success were calculated by dividing an individual's lifetime reproductive success by the average for the population. Usually, when calculating relative reproductive success, males and females are calculated separately because females are limited in reproductive potential by clutch size, while males are not. Within this species white males seek extra-pair copulations, while tan males do not. Tan males could potentially have the same level of selection acting on them as a female in another species, but the tan males still have the potential to seek extra-pair copulations. For this reason we calculated the relative mating success and relative reproductive success based on the population level mean and by individual morph means. Statistical analysis was performed using JMP (SAS, Cary NC).

Results

Population Level

Density of the entire population has increased, ranging from 23 pairs (0.115 pairs/hectare) in 1998 to a peak of 111 pairs (0.555 pairs/hectare) in 2007, with an average of 58 pairs per breeding season (Figure 1). Distributions of pair types across years varied significantly to white male-tan female bias during four years of the study; 1998 ($\chi^2 = 4.5455$, $p = 0.03$, $df = 1$), 1999 ($\chi^2 = 5.33$, $df = 1$, $p = 0.02$), and 2005 ($\chi^2 = 5.8824$, $df = 1$, $p = 0.015$).

Overall, there was no difference in the number of chicks to reach banding age ("apparent reproductive success") across the different habitat types (pond $F=1.73$, $df=3, 255$, $p=0.16$; forest $F=0.1066$, $df=3,142$, $p=0.96$; bog; $F=2.08$, $df=2, 258$, $p=0.13$) for both pair types. However, tan male-white female pairs were more likely than white male-tan female pairs to have nests with chicks reach the banding age of 6 days ($F=3.815$, $df=1,651$, $p=0.05$).

Parentage Analysis

We found 729 nests over the 13 years of this study, with 401 clutches successfully producing at least one offspring. We analyzed 1829 individuals, 1425 offspring (including 164 dispersing fledglings), 252 candidate females, and 315 candidate males. There were 45 nestlings that returned in later years and so were included as offspring and later as candidate parents.

CERVUS simulations with 8 loci predicted a 94% assignment rate. We were able to assign parentage to all but 112 of the nestlings from known nests (8.8% of the population), however none of the fledglings were assigned. We hypothesize that the majority of the fledglings are dispersing from natal grounds and are sired by parents other than on our study site. Our lower rate of assignment is most likely to do the inclusion of several nestling that were found dead in the nest and were lower quality DNA samples that did not amplify at all loci. This

was an attempt to identify any trends in predation/abandonment of the offspring. These samples were excluded from any further analysis. We also had several nests where neither, or only one parent was banded, making assignment difficult (31 chicks with only 1 parent banded, 10 chicks with neither parent banded). The remaining nests most likely contain chicks sired by a male located on a territory outside of the study site. See Table 1 for loci statistics.

There were a total of 9 white male-white female and 1 tan male-tan female pairs (15 clutches) that were included in the parentage analysis and over all reproductive success analyses, but were excluded from comparisons of pair type.

Reproductive Success

Lifetime reproductive success (total number of offspring, WPY and EPY, summed across all years the male was present in the breeding population) did not differ between male morphs, however, there was a trend for tan males to produce more offspring ($F_{1,189} = 3.2184$, $p = 0.07$). This difference could be attributed to our failure to find and analyze additional extra pair offspring of white males outside the study site, differences in parental care or predation. The number of years in the breeding population also did not differ between the male morphs ($t = -1.611$, $df = 251$, $p = 0.11$).

White males achieved a higher proportion of extra-pair young than did tan males ($\chi^2 = 60.607$, $df = 8$, $p < 0.0001$) and EPY were more likely to occur in other white male nests than tan male nests ($F_{1,369} = 55.75$, $p < 0.0001$).

Tan male-white female pairs fledged more offspring (actual reproductive success) in 4 of the 13 years of the study. 1998 ($F_{1,18} = 12.1$, $p = 0.003$) 2001 ($F_{1,21} = 7.99$, $p = 0.01$), 2005 ($F_{1,42} = 5.35$, $p = 0.025$), and 2009 ($F_{1,43} = 9.165$, $p = 0.004$).

The proportion of EPY in nests increased with male experience ($F_{1,377}$ =3.91, p=0.048). Experienced males sought EPC in other nests (χ^2=20.082, df=8, p=0.01) while losing at home. The proportion of EPY also varied with predation, those nests that were partially predated were more likely to contain at least 1 EPY ($F_{1,337}$ =4.2368, p=0.04).

Across all years of the study, the frequency of EPY did not vary in the population (Prob>F) or by pair type (Prob>F). However, proportions of EPY in white male nests varied across years, 1998 ($F_{1,14}$ =4.41, p=0.054), trend in 2002 ($F_{1,11}$ =3.98, p=0.07) and 2006 ($F_{1,34}$ =3.29, p=0.07), 2003 ($F_{1,18}$ =4.7, p=0.044), 2004 ($F_{1,29}$ =6.19, p=0.019), 2005 ($F_{1,37}$ =4.73, p=0.036), 2008 ($F_{1,44}$ =9.01, p=0.004), 2009 ($F_{1,43}$ =14.9, p=0.0004). See Figure 2.

There was no effect of high/low habitat density on the proportion of EPY ($F_{1,378}$ = 2.36, df=1,378, p=0.13) found in nests. Distribution across habitats (bog, pond or forest) did not differ in production of extra-pair young for either pair type [TxW ($F_{2,169}$ =01.2166, p=0.3) WxT ($F_{2,196}$ =0.4363, p=0.65)].

Bateman Gradients

Bateman gradients were calculated to compare the levels of sexual selection between the two male morphs. Comparing white and tan males with relative fitness calculated as a group and separately for each morph, both show an increase in relative fitness with mating success and a measure of sexual selection (Figures 3 -5).

Discussion

Overall, our polymorphic population maintains equal fitness between the two morphs. The alternative male strategies employed by the two morphs of the white-throated sparrow result in the same male fitness. Sexual selection is acting, or has the potential to act, on both males morphs, to varying degrees. Density usually increases the rate of extra-pair offspring by

increasing the availability of neighboring females. The density in our study site has increased significantly over the years, however there has not been a consistent increase in EPY across years. Proportions of EPY didn't differ by habitat, density, or for pair type. Suggesting that there is no benefit of the observed segregation of pairs after a certain density of pairs/hectare is reached. Territory type also does not impact apparent reproductive success. Differential settlement pattern may provide the opportunity for EPC, but doesn't impact overall success.

Male experience did not prevent him from being cuckolded. White males may trade-off seeking EPC in other territories with being unable to prevent them at home. This is a trade off in reproductive success instead of an increase in reproductive success. However, this tactic may be beneficial as a protection against complete loss of reproductive success through predation. Predation also varies with the frequency of EPY in a nest, so the male may also be trading off nest defense in order to seek EPY, limiting the number of offspring he will gain through EPC in neighboring territories.

Relative frequency of EPY doesn't vary across the years within each pair type. No yearly effect on proportion of EPY in either morph nests. However there is variation between years. White male nests had a higher frequency of EPY in their nests in 2003, 2004, 2005, 2008, and 2009. Further work is warranted to explore relationships between EPY production and variation in predation and climate during those years. If predation was high, or there were variations in food supply due to changes in weather, males may have tried to "hedge their bets" by seeking more EPC and spreading offspring in more nests to ensure some survival.

"Bateman gradients" or the sexual selection gradient were calculated for both white and tan males. If we assume that both morphs have equal potential for reproductive success, then both morphs are under sexual selection, however selection is stronger in tan males. If we assume

that tan males are constrained by their genetics and limited to the reproductive potential of their females, tan males are still subject to stronger selection than white males are, however selection on white males has increased slightly and selection on tan males has decreased. Until we more fully understand the genetic mechanisms behind this system, we will not be able to completely understand the forces of selection acting on the males in this system. Our results show an inconclusive opportunity for selection between the two morphs. Both morphs are under some sexual selection, however further work is needed to tease apart the levels of selection acting within this species.

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Tables

Table 1: List of loci used for parentage assignments, including the number of alleles present in the population and Hardy Weinberg equilibrium values. The Dpμ primers have a relatively high frequency of null alleles, however this did not impact our ability to assign parents. Most alleles are significantly different than HW equilibrium, most likely due to the high frequency of null alleles, and the inclusion of some degraded DNA samples that did not properly amplify. PIC (polymorphic information content) values indicate the usefulness of each marker. Values ≥ 0.5 are considered informative.

Locus	Number of Alleles	H_{obs}	H_{exp}	PIC	Frequency of null allele	P value
MME1	31	0.918	0.942	0.939	+0.0128	0.0007
Gf01	31	0.930	0.930	0.925	+0.0134	0.0000299
Gf12	23	0.833	0.761	0.733	-0.0537	<0.0000001
Dpμ01	44	0.845	0.910	0.906	+0.0388	<0.0000001
Dpμ03	39	0.807	0.883	0.874	+0.0445	<0.0000001
Zole_C02	31	0.899	0.897	0.888	-0.0017	0.0000025
Zole_C07	33	0.894	0.942	0.938	+0.0256	0.0000001
Zole_H02	22	0.877	0.864	0.851	-0.0078	0.0113

Figures

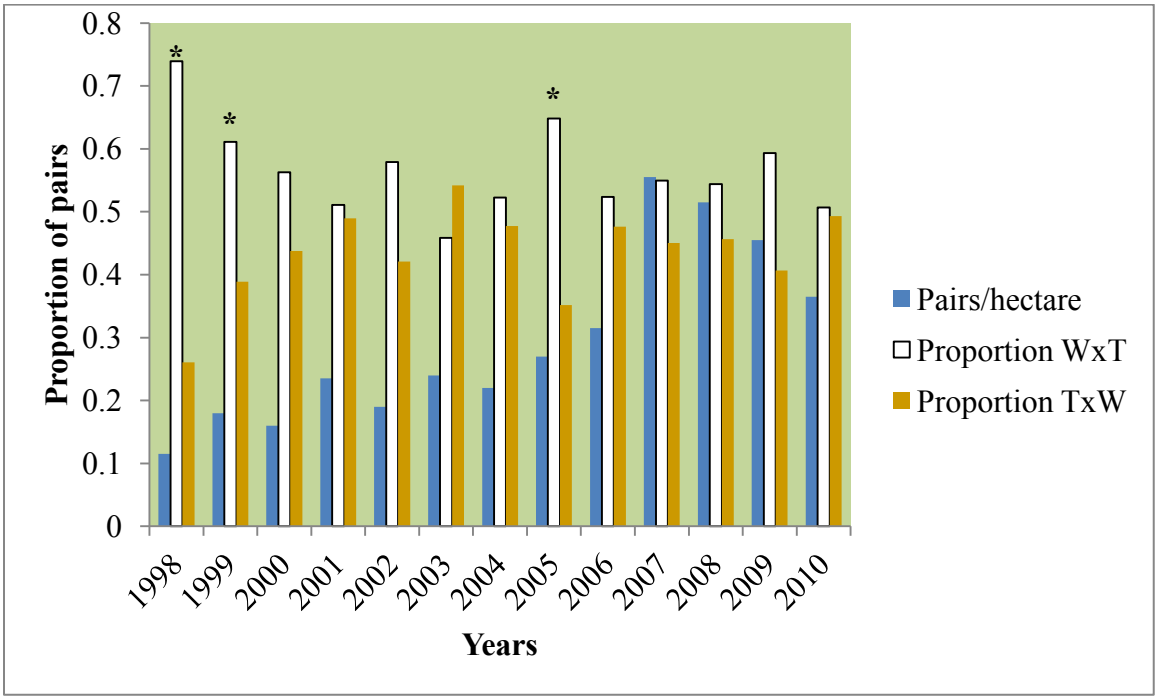

Figure 1: Frequency distribution of pair types across years and the density of all pairs over the years. Years indicated by asterisks show significant differences in the expected proportions of pair types.

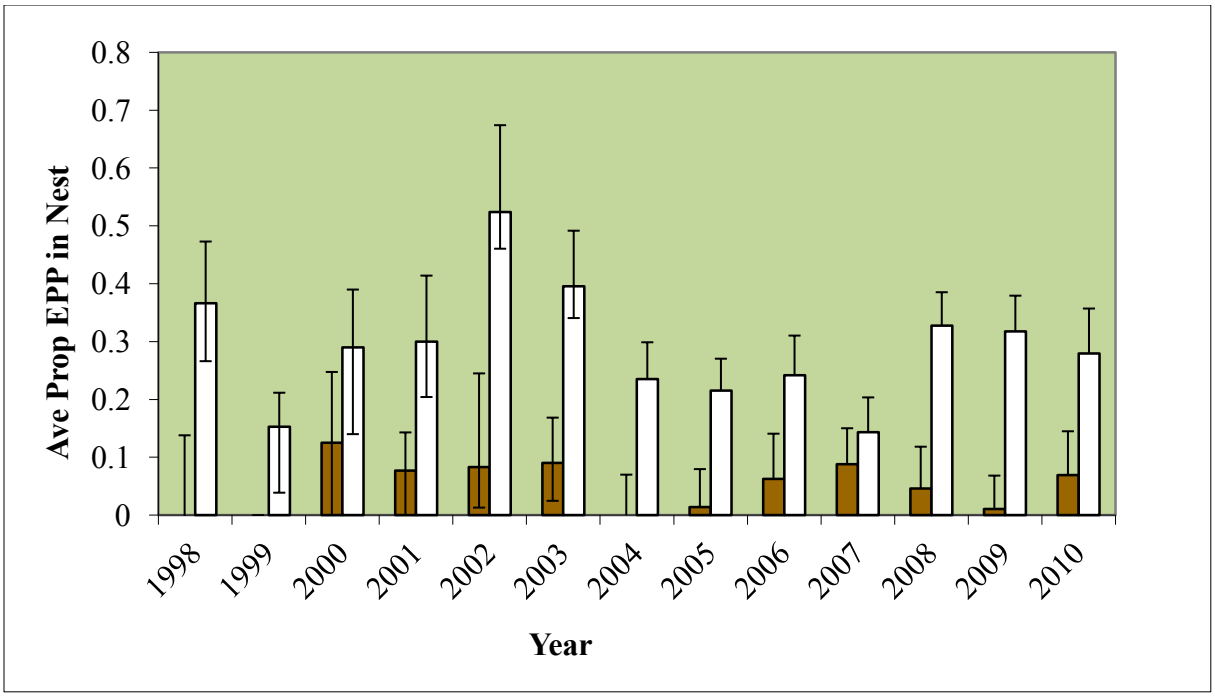

Figure 2: The overall population level frequency of extra-pair young (EPY) in both pair types does not vary during the study, however white males (white bars) do gain more EPY than tan males (tan bars). There was also variation in the frequency of EPY in white male nests in some years. Error bars are standard errors.

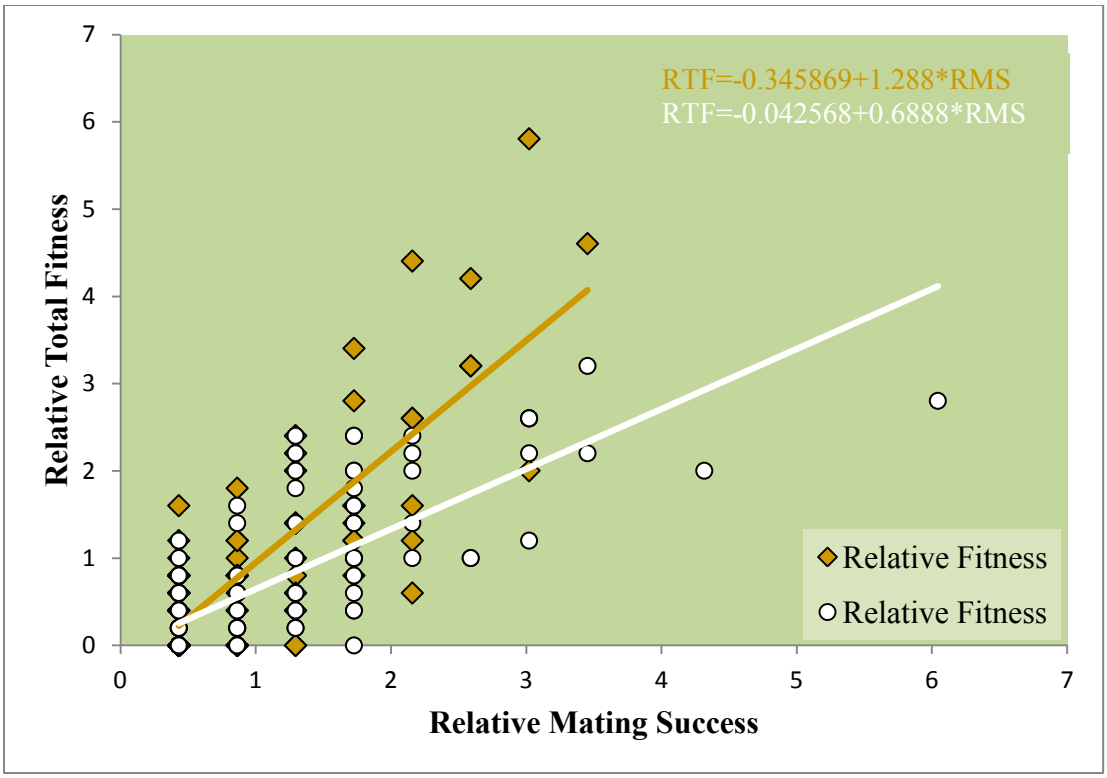

Figure 3: Bateman gradient for total fitness indicating that tan males are under more sexual selection than white males. Tan males are represented by the tan diamonds and white males are represented by the white circles. The strength of selection is indicated by the slope of the regression. Axes are relative measures calculated by dividing each measure by the mean of the whole population.

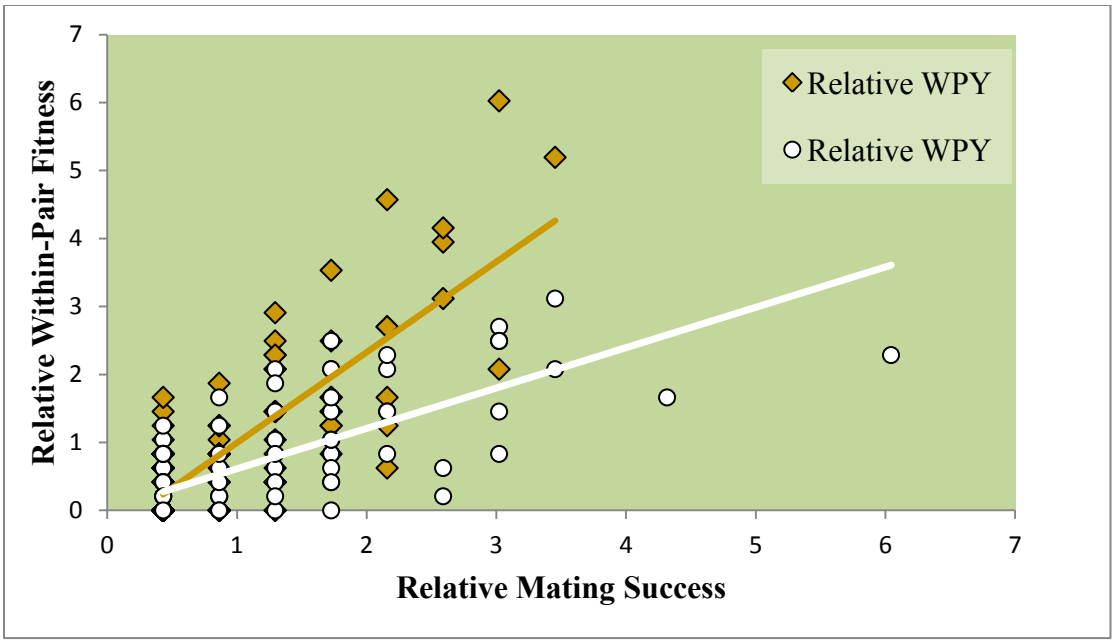

Figure 4: Bateman gradient for within-pair fitness indicating that tan males are under more sexual selection than white males. Tan males are represented by the tan diamonds and white males are represented by the white circles. The strength of selection is indicated by the slope of the repression. The selection that tan males are under is being derived from variance in with-in pair mating success. Axes are relative measures calculated by dividing each measure by the mean of the whole population.

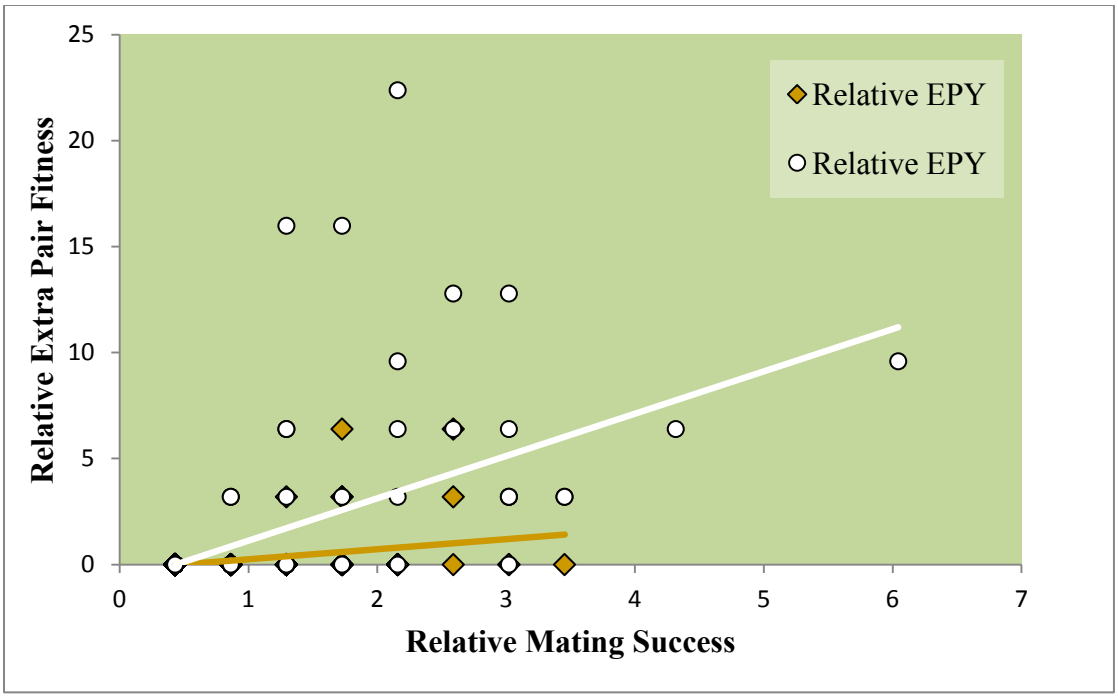

Figure 5: Bateman gradient partitioning out sexual selection from extra-pair young. Tan males are represented by the tan diamonds and white males are represented by the white circles. The regression indicates that tan males are under less sexual selection than white males from the variance in offspring produced by extra-pair matings. Axes are relative measures calculated by dividing each measure by the mean of tan males.

CHAPTER 3

MORPH RATIO VARIATION IN THE POLYMORPHIC WHITE-THROATED SPARROW

Abstract

Darwin speculated that variation in sex ratios would impact the level of selection acting upon a species. However, could variation in morph ratios also affect sexual selection? Color polymorphisms occur in approximately 3.5% of all avian species, with only 0.9% of Passeriformes having polymorphisms. Species are considered polymorphic when there are two or more color variations that are inherited genetically following a Mendelian distribution within both sexes and are minimally influenced by environmental factors. The existence of the polymorphism is maintained by differing reproductive strategies between the morphs, with each morph having some selective advantages and disadvantages. We have found that morph production does not always follow Mendelian inheritance. Offspring morphs cycle in a frequency-dependent manner with the yearly breeding adult morph ratio. Variation in morph ratio has been identified between years and seasons. This variation in morph ratio may be increasing the strength of sexual selection between the morphs of this species.

Introduction

Factors maintaining genetic variation in natural populations have long been debated, and, as a result, various population genetic models have been proposed. Selectionist theories (or balance theories) suggest that selection is the main factor accounting for polymorphism

(Lewontin 1974, Ayala 1976). Included in this category are various types of selection such as overdominance, spatially or temporally varying environments, epistasis, balance of fitness components, density-dependence, and frequency-dependence (Hartl 1980). Once a genetic polymorphism has evolved in a population, it can be maintained by these mechanisms. Disruptive selection may favor specialization of niches and select for a bi-modal distribution (Skulason and Smith 1995). Genetic diversity may be maintained when heterozygous individuals have a selective advantage over homozygous ones, producing the homozygotes simply by Mendelian genetics. Inbreeding may create homozygous individuals that express deleterious alleles and generally have less genetic diversity than heterozygous individuals allowing them better survive and reproduce (Brown 1997, Hansson and Westerberg 2002). Even when a morph is selected against, polymorphism may be maintained through gene flow from other populations that favor it.

Frequency-dependent selection is believed to be one of the more important mechanisms accounting for the stability of genetic polymorphisms over evolutionary time (Maynard-Smith 1982). Frequency dependent selection provides benefits and costs to an organism, depending on the frequency of its genotype in the population, balancing out the genotypes into a stable equilibrium. This method of selection has been show to be acting on only one species of birds, the ruff *Philomachus pugnax* (Hogan-Warburg 1966, Lank *et al.* 1995). The ruff has genetically controlled alternative mating strategies, independent and sneaker, which are stably maintained.

Species with distinct color morphs are useful for examining these selectionist theories because phenotype indicates genotype, and is likely to have evolved under various types of selection (Roulin 2004). Color polymorphisms occur in approximately 3.5% of all avian species, with only 0.9% of Passeriformes having polymorphisms (Galeotti *et al.* 2003). Species are

considered polymorphic when there are two or more color variations that are inherited genetically following a Mendelian distribution within both sexes and are minimally influenced by environmental factors (Roulin 2004). The existence of the polymorphism is maintained by differing reproductive strategies between the morphs with each morph having some selective advantages and disadvantages (Fisher 1930). Morphs that mate disassortatively reduce the risk of inbreeding by pairing each morph with the opposite morph (Roulin 2004). In white-throated sparrows, this mating strategy is believed to aid in the prevention of offspring that are homozygous for deleterious alleles (Thornycroft 1975). We are examining multiple aspects of the effects of this polymorphism on the white-throated sparrow.

In order to determine which of these is acting on a population, it is useful to determine how gene frequencies change over long periods of time (Sinervo and Lively 1996, Davison and Clarke 2000, Roulin 2004). We have studied a population of the polymorphic white-throated sparrows (*Zonotrichia albicollis*) for over 20 years, providing us with a unique data set on this polymorphic species to analyze long term changes in gene frequency when related to the polymorphism. We analyzed the temporal patterns in gene frequency as well as the effects of possible gene frequency manipulation by reproducing individuals. The polymorphism of our study species is also linked to behavior, so fitness effects of the changing gene frequencies were also analyzed. In addition to morph variation, we also examined biases in sex ratio, and the combination of sex/morph class variation.

White-throated Sparrows (*Zonotrichia albicollis*)

White-throated sparrows are socially monogamous, bi-parental, migratory passerines breeding in northeastern US and Canada. They offer a unique research opportunity since the species exhibits a stable genetic polymorphism caused by a complex rearrangement on the

second chromosome that is correlated with plumage. White morphs (W) are heterozygous ($ZAL2^m$/ZAL2) for the rearrangement while tan (T) morphs are homozygous for no rearrangement (ZAL2/ZAL2) (Thornycroft 1966, 1975). The rearrangement occurs in both sexes which mate disassortatively with respect to the inversion (Lowther 1961). Tan males pair with white females (TxW) and white males pair with tan females (WxT). In addition to plumage differences, each morph displays distinct behavioral phenotypes. White males are more aggressive than their tan counterparts, sing more, and pursue extra-pair copulations (i.e. attempt to mate with females other than their social mate) (Tuttle 2003). Alternatively, tan males tend to settle in isolated areas, are socially monogamous, and spend more time mate guarding and investing in parental care (Knapton and Falls 1983, Tuttle 2003). Visual differences between the morphs are only observable while in breeding plumage, making research on morph differences in nestlings difficult.

Methods

Field Methods

We studied a population of white-throated sparrows breeding near the Cranberry Lake Biological Station (44°15N; 74°78W) in the Adirondack Mountains of upstate New York. This population has been intensely studied since 1988, and therefore offers the ideal opportunity to examine long-term aspects of morph differences within the species. Adults and nestlings are caught and banded, have a unique color combo added for identification, and have blood samples taken. Various morphological measurements are recorded including mass and tarsus. This study focuses on the years 1998 - 2010 and includes morph, sex and growth rate data of 1428 nestlings and 392 clutches from this population.

Molecular Methods

The blood is separated by centrifugation and the hematocrit is stored in lysis buffer at 4°C until molecular analysis occurs. Until recently the only way to determine morph of nestling white-throated sparrows was through karyotyping, thus identifying the presence of the chromosomal rearrangement on chromosome 2. A molecular technique has been developed utilizing PCR amplification of the vasoactive intestinal peptide (VIP) gene that varies at a *Dra1* restriction enzyme site between the morphs, eliminating the need for the sample size limiting karyotyping (Michopoulos *et al.* 2007, Romanov *et al.* 2009) See Figure 6. The accuracy of this method has been checked in two ways. A sample set of 20 visually identified adults, 5 WM, 5 WF, 5 TM and 5 TF were tested and this method was 100% accurate. Additionally, 8 individuals of known karyotypes were checked and also showed 100% accuracy (Figure 7). Finally, we have detected one $ZAL2^m/ZAL2^m$ individual in our population and another from a wintering population, showing that the sensitivity of the protocol with the use of the ABI automated sequencer is enough to identify these individuals without a karyotype. The wintering white female that was identified with this technique was confirmed through karyotyping (Figure 8). We are also able to identify other species through variation in fragment size (Romanov *et al.* 2009).

Statistical Methods

Binomial tests were used to compare observed morph ratios to the expected. We used generalized linear models (GLM) with binomial errors and logit link to analyze brood morph ratio as a function of year and time within the season ("early" vs "late"; i.e. before or after July 1^{st} , with the number of white (W) chicks/brood as the response variable and the total number of nestlings per brood as the denominator. For some analyses we grouped nests according to

whether they were white-biased (WB) or tan-biased (TB). Any unbiased nests were excluded from these analyses. All statistical analyses were conducted in JMP version 9 (SAS, Cary NC) or R (rproject.org).

Results

The overall population morph ratio stays constant across years ($t=1.21$, $N=388$, $p=0.88$) as did the sex ratio ($t=1.11$, $N=388$, $p=0.86$). However, there was frequency-dependent cycling between the morph ratio of the breeding population and the offspring produced (Figure 9). There were also variations in offspring production during individual years between sex (Figure 11), morph (Figure 12) and the four sex/morph classes (TM, WM, WF, TF; Figures 13 and 14).

Morph ratio did not differ between nests with partial predation and those without predation ($t=0.48$, $df = 88$, $p=0.63$). Predation also did not differ between WB and TB nests (Fisher's Exact Test, $p=0.40$). Overall, TxW pairs did not bias offspring towards males or white offspring. WxT pairs did however bias offspring production to WF ($F_{12,102} = 2.62$, $p=0.003$) and WM ($F_{12,184} = 2.16$, $p=0.015$). WxT pairs also biased the proportion of males produced; white males ($t=1.7$, $N= 197$, $p = 0.045$) and tan males ($t=1.75$, $N=196$, $p=0.041$).

TxW pairs produce a greater proportion of white morph offspring in the early clutches than late clutches ($F_{1,35}=4.02$, $p=0.05$), but no differences were found in WxT pairs ($F_{1,30}=0.0809$, $p=0.78$). The reduction in white offspring in later clutches by TxW pairs is driven by increased production of tan males ($F_{1,33}=9.24$, $p=0.005$).

TxW pairs did not vary proportion of males ($F_{2,179} = 0.4657$, $p = 0.63$) or proportion of white offspring ($F_{2,179} = 0.5998$, $p = 0.55$) by territory type. WxT pairs also did not vary the proportion of males by territory type ($F_{2,206} = 0.6857$, $p = 0.69$), however they did bias the proportion of white offspring produced in forest territories ($F_{2,206} = 4.6978$, $p = 0.01$). This

relationship was driven by a trade off in the production of white females ($F_{2,192} = 4.2535$, $p = 0.016$) with a decrease in tan females ($F_{2,189} = 5.845$, $p = 0.0034$) (Figure 10).

There was a variation in the proportion of male nestlings produced over the years of the study $\chi^2 = 48.1$ df = 12, $p < 0.001$, and an interaction with pair type $\chi^2 = 30.25$, df = 12, $p = 0.0026$. The years 1999, 2003, 2005, 2008, and 2009 were all male biased years $p \leq 0.025$.

The proportion of males produced by TxW pairs was male biased in 1998 ($F_{1,14} = 7.94$, $p=0.014$). There was a tan female bias in TxW nests ($F_{1,6} = 7.567$, $p=0.033$) and tan male ($F_{1,9} = 7.396$, $p=0.026$) bias in WxT nests the year 2002. During 1999 there was a trend for TxW pairs to bias towards TF production ($F_{1,18} = 4.25$, $p=0.054$). In 2000, there was a trend for WF production by WxT pairs ($F_{1,18} = 4.308$, $p=0.052$). There was also a trend in 2002 to produce more males in WxT nests ($F_{1,11} = 4.09$, $p=0.06$). During 2003 there was a white bias in ($F_{1,18} = 7.5$, $p=0.012$) in WxT nests. 2010 WxT pairs biased towards males ($F_{1,40} = 6.05$, $p=0.018$), while TxW pairs biased towards white offspring ($F_{1,40} = 6.94$, $p=0.012$). During this year WxT also traded off high production of males by producing fewer white females ($F_{1,40} = 9.0646$, $p = 0.005$) and biasing to TM ($F_{1,40} = 8.51$, $p=0.006$). See Figures 13 and 14 for summary of the tradeoffs between the morph and sex classes.

Discussion

In support of Fisher (1930), the polymorphism is maintained across years at relatively equal proportions. Other species such as the bananaquit (*Coereba flaveola*) have been observed to have cycles in the frequency of the morphs across the range (Wunderle 1981) while in white-throated sparrows we have found cycles between the years of our study. An abundance of WxT pairs cycles alternately with an abundance of white offspring. This cycling ensures that yearly variation within the proportions will be drawn back to an equal proportion of each morph. In this

manner the frequency of each morph is dependent upon the other, in order to maintain their fitness. Additionally, each morph is dependent upon finding a mate of the opposite morph in order to achieve reproductive success and so this cycling ensures there will be mates available.

This also suggests that there is some control that the adults are able to exert over the morphs of their offspring. Individuals who can produce the rare morph in the current population will have their children more likely to find mates in the coming breeding season than those that produce the abundant morph. This is due to the disassortative mating system in this species, causing one morph to almost exclusively mate with the opposite morph. The exact mechanism as to how the birds are assessing the composition of the current population as well as the mechanism for control of the morph of the offspring is not yet known. However, recent work in sex ratio has implied that females may have control over offspring sex, this may be attributed to selective re-absorption of follicles by the female (Pike 2005). Therefore it may also be possible to have morph control by females, however tan females do not have the inversion resulting in only white females possibly being able to manipulate the offspring morph ratio. Thus trends are often present in TxW pairs due to possible female control, while no trend is present in WxT pairs.

The trade-off between producing white offspring and producing sons in TxW pairs suggests that both of these offspring types are expensive to produce and rear. In this pair type, the white female is heterozygous for the sex chromosomes (Z/W) as well as for the morph chromosomes ($ZA2^m/ZA2$). Therefore, the egg she produces will determine both the offspring's sex as well as its morph. In WxT pairs however, the tan female is only heterozygous for the sex chromosomes (Z/W) and it is the white male that is heterozygous for the morph chromosomes ($ZA2^m/ZA2$). The egg that the tan female produces only determines the sex of the offspring, and

the morph is determined by the morph chromosome carried in the sperm that fertilizes the egg. If the female white-throated sparrow is able to selectively produce/reabsorb eggs with particular genotypes, then she could control the sex ratio, and in the case of the white female, the morph ratio, of her offspring.

Due to the tradeoff between producing sons and producing whites in TxW pairs, the female maybe assessing her own condition and determining what type of offspring she should produce. White females will produce either white offspring or sons in order to prevent over exerting herself and reducing the quality of her offspring. Tan females on the other hand, can only control the sex of the offspring, and may assess their condition to decide which sex to produce. Females in good condition, may produce more sons because they know they can provide for their higher needs. Females in poor condition may choose to produce females because they cannot afford the extra energy required to produce high quality males. It is then up to the males sperm to determine the morph of the offspring and if it will be an expensive white or a less costly tan.

Due to the possible female control in the TxW pairs, and their true bi-parental care, they may be better able to adjust their morph ratio to the environment they are in. TxW pairs showed that they had more TB nests in 1999, 2000 and 2003 than the WxT pairs. This could be due to the possibility that there was less food available during these years and so the TxW pairs adjusted to produce fewer of the more expensive white offspring.

Altering the morph ratio of a nest may also affect the fitness of the individual offspring. White offspring were larger when they were in tan biased nests in both pair types, indicating that in order to have larger and presumably more competitive offspring, it is necessary to have more

tan offspring in the nest. This may be due to interactions between chicks in the nest, such as competition and begging for food, behavioral differences which have not yet been determined.

Pairs also seem to adjust their morph ratio according to the time of the breeding season as well. Both pair types produce more whites in their later clutches. This was driven by both pair types producing more WF in the later clutches, as no difference in WM offspring was observed. This could be because WF are the least likely to benefit from the extra time to grow before the winter. Both white and tan males need to grow in order to be able to acquire and defend territories in the next breeding season. Tan females need to build up stores of energy so that they can successfully raise a clutch of young during the next breeding season with little help from their white mate. They must also have enough reserves at the end of the breeding season to make it through migration and through the winter. In contrast to these other types, white females do not have to defend territories, and they receive much parental care for their offspring from their tan mate. This reduces their energy expenditure, allowing them to be more likely to return for subsequent breeding seasons (need stats on return rate here). If parents produced white males or tan males in late clutches, they would not be as large as their competitors and would lose out on territorial disputes, reducing their likelihood of finding a mate and reproducing. Producing tan females in late clutches could cause them to reduce their survival rate due to low stores of energy. The white females benefit the least from the extra time on the natal grounds, and thus are produced at a greater number later in the season. Whites are more aggressive and therefore may benefit from extra time on the natal grounds than tans.

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Figures

Figure 6: Representation of the *Dra I* restriction site within the vasoactive intestinal peptide gene (VIP) used to determine morph in the white-throated sparrow. Tan birds amplify a 285bp fragment. White birds contain a single nucleotide polymorphism (SNP) within the VIP gene that creates the restriction site. The restriction site results in an 89bp fragment and a 190bp fragment.

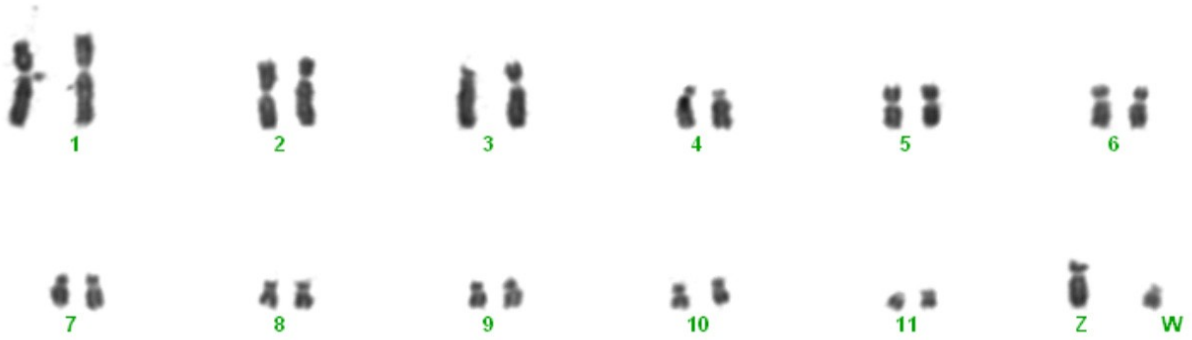

Figure 7: Karyotype of a white morph white-throated sparrow. The rearrangement on the second largest autosome indicated by the shift in centromere (left chromosome of number 2) indicates a white morph. Karyotypes were used to confirm the accuracy of the molecular method.

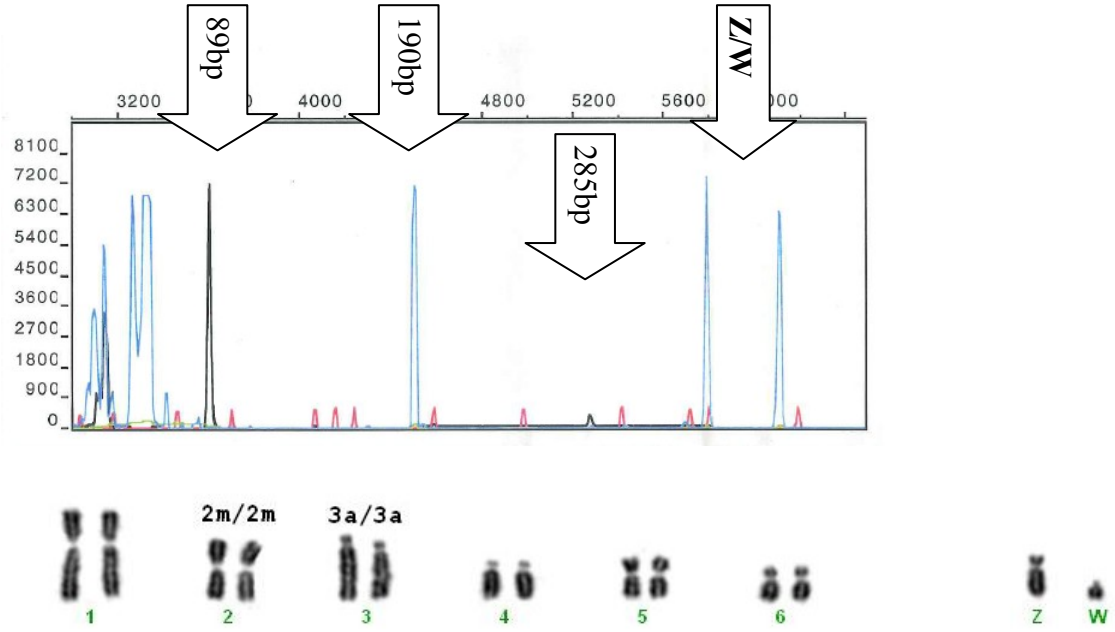

Figure 8: Top: ABI 310 sequencer output of the ZAL2^{m}/ZAL2^{m} female. The lack of a peak at 285bp indicates the absence of the ZAL2 chromosome. The 2 blue peaks at the far right are peaks for the Z and W sex chromosomes amplified using the primers P2 and P8. Bottom: Karyotype of the same individual showing the presence of two copies of ZAL2^{m} and the presence of the Z/W sex chromosomes.

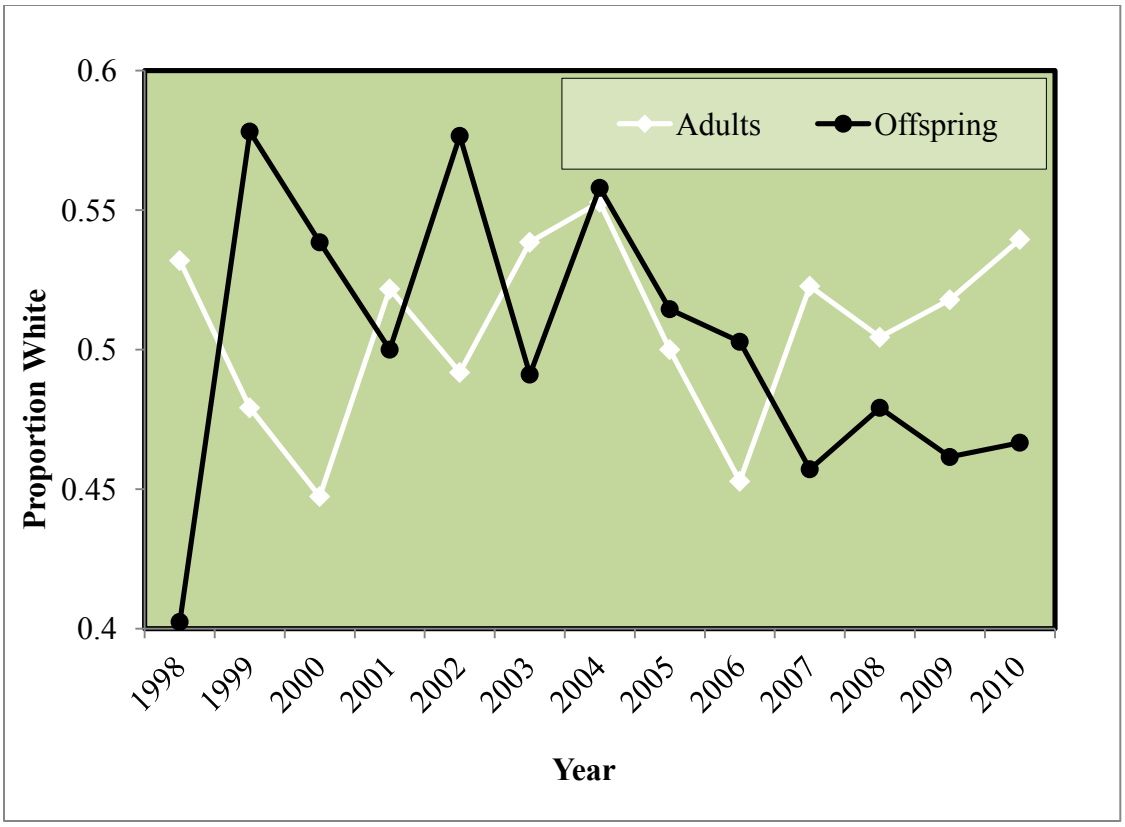

Figure 9: Frequency-dependent cycling of adult and offspring morph ratios.

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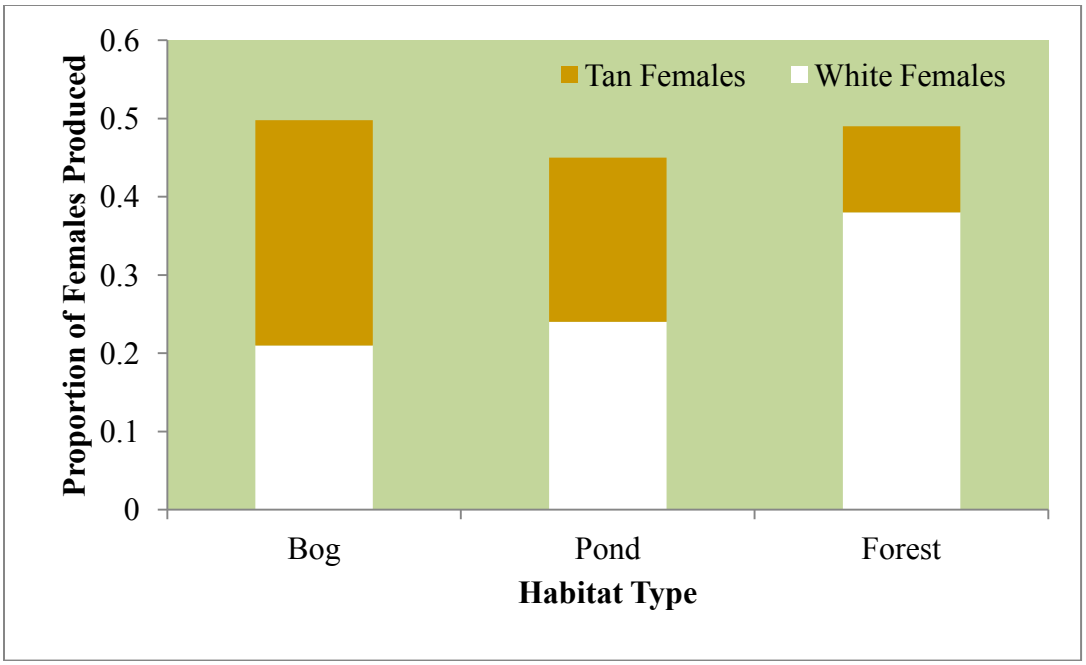

Figure 10: Proportions of female morphs produced by white male-tan female pairs by habitat type. These pairs bias offspring production to white females when their territories are located along the forest edge.

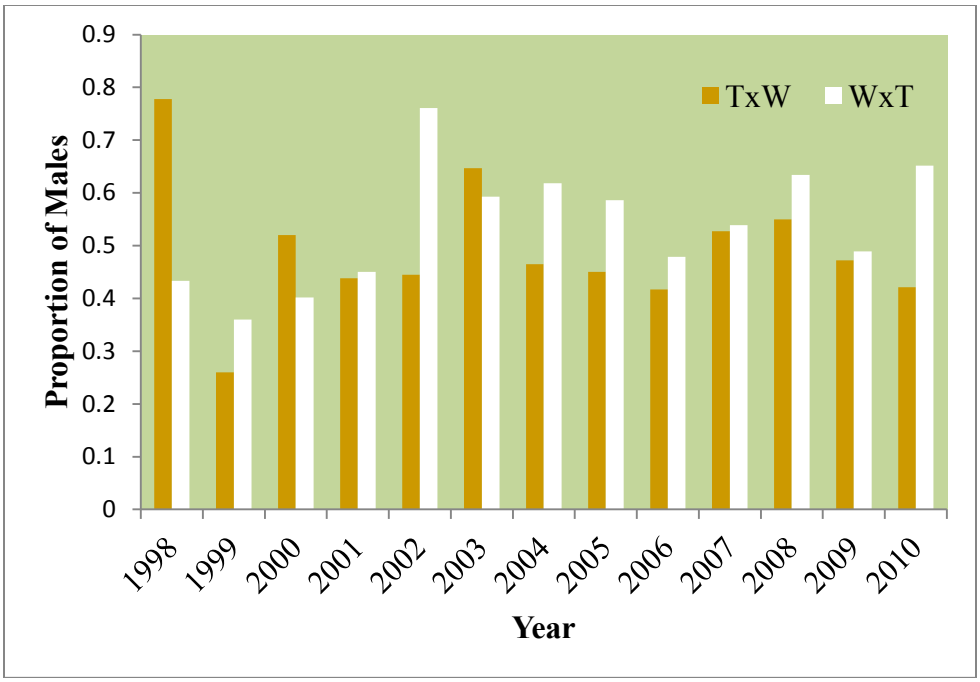

Figure 11: The proportion of male offspring produced by each pair type varied by year.

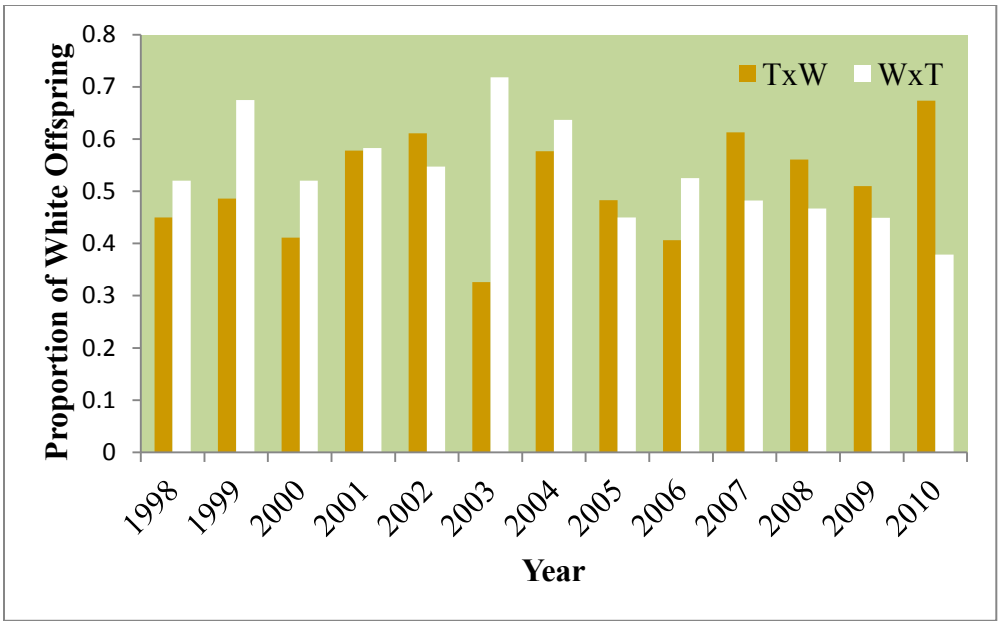

Figure 12: The proportion of white offspring produced by each pair type varied across years.

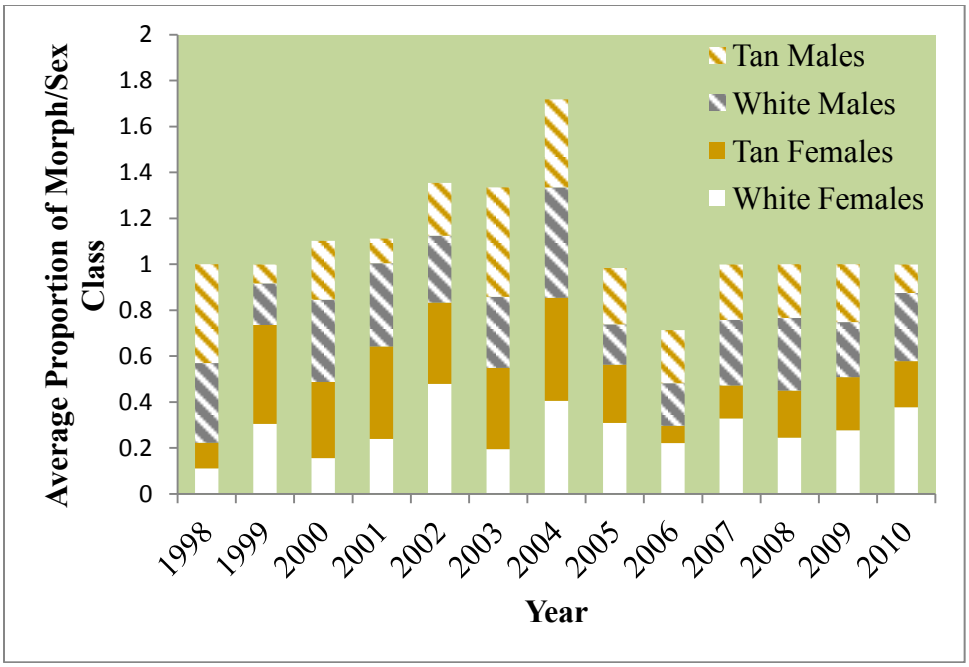

Figure 13: Average proportion of each morph/sex class produced during each year of the study by tan male-white female pairs.

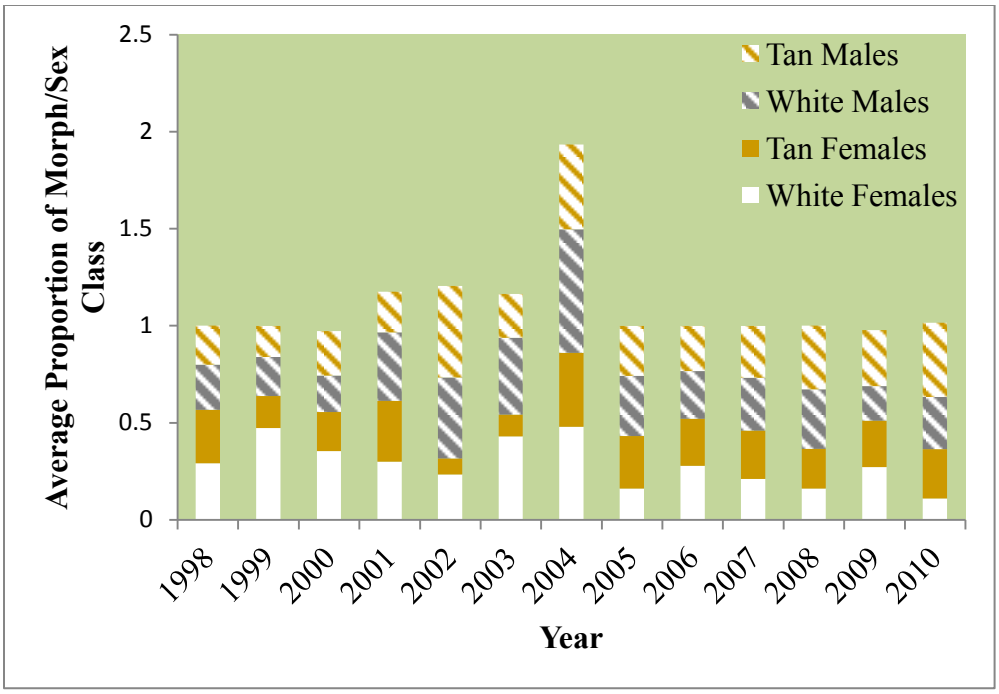

Figure 14: Average proportion of each morph/sex class produced during each year of the study by white male-tan female pairs.

CHAPTER 4

EVIDENCE FOR MULTIPLE MECHANISMS OF SEGREGATION DISTORTION IN THE WHITE-THROATED SPARROW

Abstract

Mendel's first law states that every allele has the same probability of being passed on to each offspring. However, evidence for segregation distortion (SD) is observed in various species, violating this law. SD or drive results in the unequal transfer of genes or chromosomes from one parent to offspring. Inversions appear to occur frequently in species that exhibit some form of SD. Currently, the best known example of autosomal drive occurs in the *t*-locus of the mouse, which is associated with four inversions. White-throated sparrows carry inversions on the second autosome, resulting in plumage (white or tan crown stripes) and behavior differences. Plumage differences within this species between carriers and non-carriers of the inversions provide a marker for SD. Therefore, it is possible to trace the mechanisms of distortion within this species through both sexes. We examine variation in sperm production by white-morph male white-throated sparrows to confirm SD in this species and determine whether distortion varies with spatial and/or temporal environmental variability. We suggest that the presence of transmission distortion allows white-throated sparrows to facultatively adjust morph to a changing environment to maximize reproductive success.

Introduction

Examination of species genetics generally assumes a Mendelian inheritance of alleles, each allele having the same probability (50:50) of being passed on to the next generation. However, various forms of selection will act upon species, resulting in conditions that favor different alleles and changing the frequency of inheritance. Additionally, various mechanisms of transmission ratio distortion (TRD), such as meiotic drive (MD) and segregation distortion (SD) have been proposed to explain an unequal transmission of genes or chromosomes. These conditions result in the general principles of population genetics being violated since the alleles segregate in non-Mendelian fashion. When alleles or chromosomes are found in proportions that differ significantly from Mendelian expectations, it is usually indicative of meiotic drive (Lyttle 1991).

MD is generally used to describe distortion during meiosis in the female line, while SD describes the male counterpart (Purushothaman *et al.* 2008). In many plants and animals male and female meiosis differs in the final number of haploid gametes produced; males resulting in 4 haploid cells, females resulting in 1 haploid cell and 3 polar bodies. These differences enable more opportunity for variation in the female line than in the male line during meiosis, leading to the assumption that females will be more likely to experience a form of TRD than males (Malik 2005).

Sex chromosome drive occurs when either the X or Y (or Z/W in birds and butterflies) are transmitted to the next generation in unequal proportions from the heterozygous parent (male-X/Y or female-Z/W). There is evidence for variation in sex ratio across vertebrate groups (Clutton-Brock 1985, Clutton-Brock and Iason 1986, Frank 1990, Hardy 2002). This variation in sex ratio may allow parents to adapt sex ratio to varying conditions to optimize offspring

fitness and reproductive success. However, understanding the mechanisms that contribute to this variation is complicated. Females may be able to control the sex of the egg ovulated (Pike and Petrie 2003, Pike 2005) and males may be able to vary the production of X or Y sperm produced. However, no evidence of this has been found in white-tailed deer (DeYoung *et al.* 2004).

Numerous sex chromosome drive examples have been found, mainly in *Drosophila*, stalk-eyed flies, and mosquitoes (Jaenike 2001, Tao *et al.* 2001), but there are very few known examples of autosomal drive. One of the most well studied systems for autosomal drive is the mouse *t*-complex (see review; Lyon 2003). Transmission ratio distortion of the *t*-complex can reach levels of 95 - 99% transmission (Bennett 1975, Lyon 2005) of the complex to the next generation. It is thought that the *t*-complex has arisen through multiple inversions and the addition of beneficial alleles, with the complex now having limited recombination between the normal chromosome and the rearranged one (Lyon 2003). The *t*-complex has also been linked to changes in aggression levels in the house mouse (*Mus domesticus*) (Lenington *et al.* 1996). Transmission of the t-locus has also been observed to vary with environment and other genetic variants (Ardlie and Silver 1996). TRD has also been observed in other areas of mouse and human genomes that contain areas of rearrangement (Underkoffler *et al.* 2005). These areas of rearrangement may interfere with the spindle or kinetochore action resulting in unequal segregation. In the white-throated sparrow (*Zonotrichia albicollis*), sequence analysis from within a complex rearrangement on chromosome 2 ($ZAL2^m$, second largest autosome) shows limited recombination of the >1000 genes contained within this autosome and its homolog (ZAL2) (Thomas *et al.* 2008, Huynh *et al.* 2010), suggesting that this species may have another vertebrate example of a non-recombining autosome. Homozygotes for $ZAL2^m$ occur

infrequently (~0.0007%, unpublished data) within this species, preventing/reducing recombination on this chromosome.

This situation may also involve TRD. A case of TRD is only observable when there is a marker for the driven allele so that it can be followed through transmission. Do to this difficulty most of the examples of TRD are found only in model organisms such as *Drosophila* that have been bred for specific traits. In white-throated sparrows, that "marker" could be the white crown plumage, which occurs in heterozygotes ($ZAL2^m$/ ZAL2) of both sexes. Data gathered in our laboratory shows that mated pairs consisting of one white bird (either a male or a female) alter the ratio of white offspring they produce (see Chapter 3 of this dissertation). Therefore, it is possible that different mechanisms of TRD may have evolved in male and female white-throated sparrows. Female meiotic drive is well documented and may similarly occur in white females; however, we propose that white males may also employ some form of segregation distortion. My goal in this study is to confirm segregation distortion in males of this species, and determine whether distortion varies with spatial and/or temporal environmental variability.

Study Species

White-throated sparrows (WTSP) are socially monogamous, bi-parental, migratory passerines that breed in northeastern North America and Canada. They offer a unique research opportunity since the species exhibits a stable genetic polymorphism caused by a *minimum* of two inversions on the second largest chromosome (Thornycroft 1966, 1975, Thomas *et al.* 2008, Romanov *et al.* 2009). Those birds that are heterozygous ($ZAL2^m$/ZAL2) for the inversions appear as white (W) morphs, having white median crown stripes, while those that are homozygous (ZAL2/ZAL2) without the inversions appear as tan (T) morphs, having tan median crown stripes (See Figures 1 and 2). These morphs occur in both sexes. WTSP have adopted an

unusual mating pattern in which white almost always pairs (> 97% of the time) with tan (*i.e.* disassortative mating; Lowther 1961), resulting in tan male – white female pairs (TxW) and white male – tan female pairs (WxT).

In addition to exhibiting very different plumage, each morph displays distinct behavioral characteristics correlated with the presence or absence of $ZAL2^m$. Genetic and behavioral evidence shows that the two morphs are practicing alternative reproductive strategies (Tuttle 2003). White males are more aggressive than their tan counterparts, sing more, and pursue extra-pair copulations (EPC's), specifically, attempted matings with birds other than their social mates (Tuttle 2003). White males engage in EPC's and gain fitness by fathering offspring in other nests, yet by doing so, they also lose paternity at home (Tuttle 2003). White males tend to settle in areas of high density, increasing encounters with fertile females (Formica *et al.* 2004). By contrast, tan males tend to settle in isolated areas, around ponds (Formica *et al.* 2004), are socially monogamous, and spend more time mate guarding and investing in parental care. This means that overall, TxW pairs invest more heavily in parental care than WxT pairs (Knapton and Falls 1983, Kopachena and Falls 1993). Both pair types attempt for 2 clutches per season with an average of 4 eggs per clutch (range 3-5). Females are solely responsible for incubation and both parents help with the feeding (Falls and Kopachena 1994). In order to maximize reproductive success the pair should produce the sex and morph combination that is most likely to survive and reproduce in the next breeding season.

Morph and sex data for 1425 offspring and 729 nests from the years 1998-2010 have been analyzed for differences in the frequency of each morph offspring produced and between both pair types (See Chapter 3 of this dissertation). The evidence of variation between years, seasons and pair type suggests that there is a mechanism to alter sex and morph in this species.

Unlike tan females (ZAL2/ZAL2), white females (ZAL2/ZAL2^m) carry the rearranged chromosome and as a result, it is possible that they have more control over offspring genotype. However, white males may also be attempting to influence the morph ratio through a bias in sperm production. With this project we investigated the possibility of SD in the white-throated sparrow favoring the production of ZAL2^m sperm in relation to environmental factors.

Methods

The study population was observed at the Cranberry Lake Biological Station (44°15N; 74°78W) in the Adirondack Mountains of upstate New York. This population has been intensely studied since 1988 by Dr. Elaina Tuttle and therefore offers the ideal opportunity to examine long-term aspects of selection cycles. This study includes sperm data from three years of this study 2008 – 2010. Due to the difficulties in collecting and storing sperm we were unable to mirror the years of the morph analysis. All birds in the study site are caught with mist nets and banded with a unique color combination for later identification (Fish and Wildlife permit #22296 to E. M. Tuttle). Plumage and size measurements are taken along with a blood sample (~50-200µl through brachial venipuncture) from each individual. Males also have a sperm sample taken through cloacal manipulation and collected in a micro-capillary tube. Volume is estimated and any contamination is identified. Sperm is then stored in 200 µl of Tyrode's solution (Bavister 1989) and placed in liquid nitrogen (Figure 15). All territories and pairs are also identified through behavioral watches (~80hrs/pair) to determine each pair, type of territory (i.e. high density vs. low density based upon the number of neighbors; > 2 or ≤ 2 neighbors) and the current population size.

Genomic DNA (N=30) was extracted from stored hematocrit using the DNA IQ® System from Promega Co (Madison WI). Gametic DNA (N=28) is also extracted using the DNA IQ®

System, but is treated with a proteinase K digestion (minimum of 2 hours) prior to extraction (DNA IQ® Tissue and Hair kit). Morph is determined by using a PCR amplification and digestion of a *DraI* RFLP (restriction fragment length polymorphism) site on the vasoactive intestinal peptide (VIP) gene (Michopoulos *et al.* 2007) which has been modified and optimized for use on an automated sequencer (see Figure 16) (Romanov *et al.* 2009). "Morph" ratio of the white male sperm was determined by using a proportion of peak height/area from an ABI Prism 310® Genetic Analyzer as an estimate of DNA concentration (Life Technologies, Foster City CA; Figure 17). The peak areas of both white peaks are added together and divided by the tan peak multiplied by the expected 0.5 and then standardized using the average of the genomic peaks (white peaks/tan peak * 0.5)/average genomic peaks. The average of 30 white individual peaks was 0.766 instead of the expected 0.5 due to variation in PCR amplification and fragment analysis from the sequencer. Similar techniques are used to determine the presence of tumor tissue through the identification of reduced suppressor alleles (ABI 2004).

Results

We found a bias in the proportion of "white" sperm produced by males differed significantly from the expected 50:50 ($t=1.84$, $df=27$, $p=0.38$; Figure 18). This variation appears to be dependent upon social habitat. Males that settle in the bog produced more "white" sperm than did males in the lower density habitats of forest and pond ($F_{2,22} = 4.1031$, $p = 0.031$; Figure 19). Males also produced more white sperm when settled in high density territories (*i.e.* with 3 or more neighbors) than in low density territories (2 or fewer neighbors) ($F_{1,24} = 8.39$, $p = 0.0081$; Figure 20). We found no evidence in sperm variation across the breeding season ($F_{1,24} = p = 0.41$) or between sampling years ($F_{2,24} = 1.4769$, $p = 0.25$). However, our sampling was limited by our ability to catch males during specific times of the breeding season and the sample

size should be expanded. Currently, there are no data available on the female aspect of meiotic drive within this species. However, the variation in morph ratios observed would suggest that there is also a female mechanism and should be explored further.

Discussion

TRD alters evolutionary trajectories in unexpected ways. Understanding the mechanisms behind segregation distortion in the white-throated sparrow will illuminate additional mechanisms in the maintenance of polymorphism in this species. Evidence has been found that sexual selection is acting within this species (see Chapter 2), and so segregation distortion may add another level to this selection.

Chromosomal inversions and rearrangements have been associated with various drive systems across multiple groups; from insects (Tao *et al.* 2001), mammals (Lyon 2003), to plants (Jaenike 2001, Malik 2005). Here we have shown that there is also the potential for segregation distortion within the white-throated sparrows. Drive has been associated with differences between chromosome sizes or centromere position in addition to the presence of inversions. Within the white-throated sparrows the complex chromosomal rearrangement involves at least two inversions and a centromere shift. Both of these differences may impact drive within this group. There may also be gene order changes between the two chromosomes, impacting epigentics facilitating the development of drive (Rutkowska and Badyaev 2008).

Of special interest is the observation of transmission distortion in humans. The short arm of human chromosome 6 (HSA6p) has been found to have a skewed transmission in males of European ancestry (Santos *et al.* 2009). This area of HSA6p also has a high rate of linkage disequilibrium as does $ZAL2^{m}$. A gene of interest, SUPT3H, is located within this region. Within white-throated sparrow this gene is located on ZAL2 near the rearrangement, also near an

area of strong linkage disequilibrium (see Chapter 5). SUPT3H is involved in histone acetylation and chromatin remodeling. Further examination of this gene within white-throated sparrows is warranted to determine if this gene influences TRD in this species.

Impacts of centromere binding proteins on drive also have been explored. These proteins can affect the microtubule binding and disrupt the equal segregation of the chromosomes (Rutkowska and Badyaev 2008). At least one centromere binding protein (CENPF) is predicted to be located within the rearrangement in white-throated sparrows. CENPF is located near others genes in the chicken (such as ESRRG) that are located within the rearrangement in white-throated sparrows. Gene function may have been disrupted due to the rearrangements, inhibiting microtubule attachment. Additionally, the shift in centromere location also affects the microtubule attachment, impacting segregation during meiosis (Malik 2005).

Hormone levels have also been implicated in meiotic drive in female birds (Rutkowska and Badyaev 2008). Within white-throated sparrows there have been a variety of studies indicating that hormone levels vary between the morphs and during the seasons (Maney *et al.* 2005, Spinney *et al.* 2006). We have shown that white males can alter the ratio of white and tan sperm they produce based on social habitat. Changes in hormones across years and territories may be the most likely cause of these variations. However, more work is needed to identify the mechanism of distortion within this species. There also may be multiple mechanisms working within this species. Variation in morph ratios in both pair types of this species suggests that both white males and females have an impact on morph production. There may be a male mechanism and a separate female mechanism working in concert in these birds.

Analysis in the white-throated sparrow may illuminate transmission distortions for other species, especially in other avian species that are known to have inversions (since inversions are

commonly associated with drive systems) such as Cardinals (*Cardinalis cardinalis*) and Juncos (*Junco hyemalis*) (Shields 1982).

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Figures:

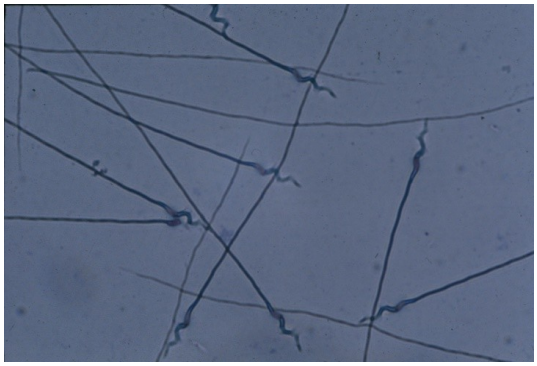

Figure 15: White-throated sparrow (*Zonotrichia albicollis*) sperm.

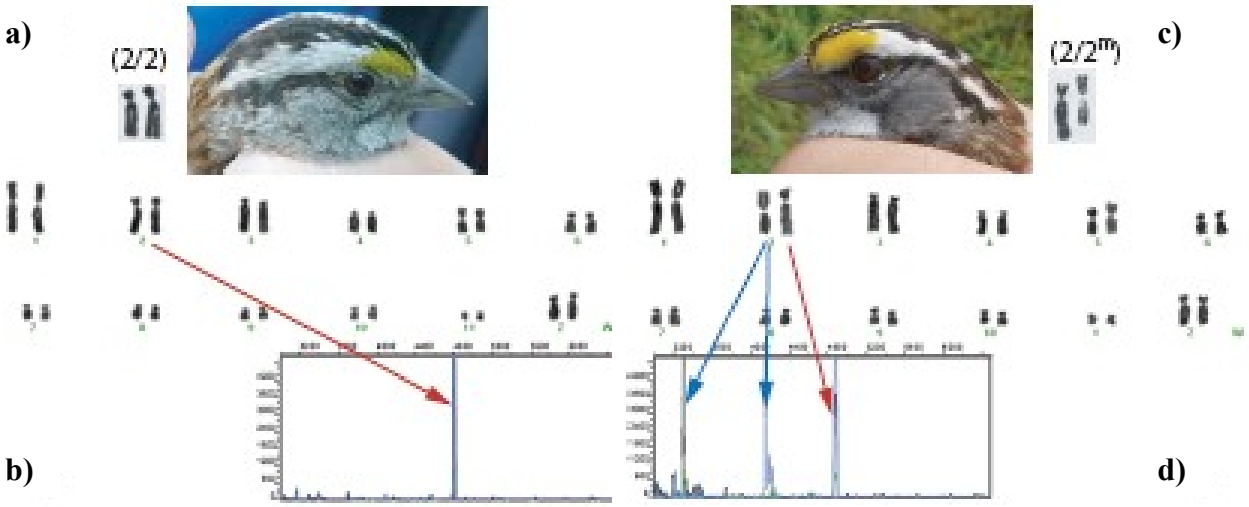

Figure 16**:** Representation of karyotype and molecular sexing for both morphs of the white-throated sparrow. a) Photo and karyotype (chromosome spread) for a tan morph showing both copies of chromosome ZAL2 that lack the rearrangements. b) The ABI Prism 310® output shows a single peak of 284bp for both copies of ZAL2, since there is no restriction site for *DraI*. c) Photo and karyotype for a white morph showing one ZAL2 and one ZAL2^{m}. d) The ABI Prism 310® output shows a single peak of 284bp for ZAL2 and two peaks of 88 and 189bp for the *DraI* digested VIP fragment from ZAL2^{m}.

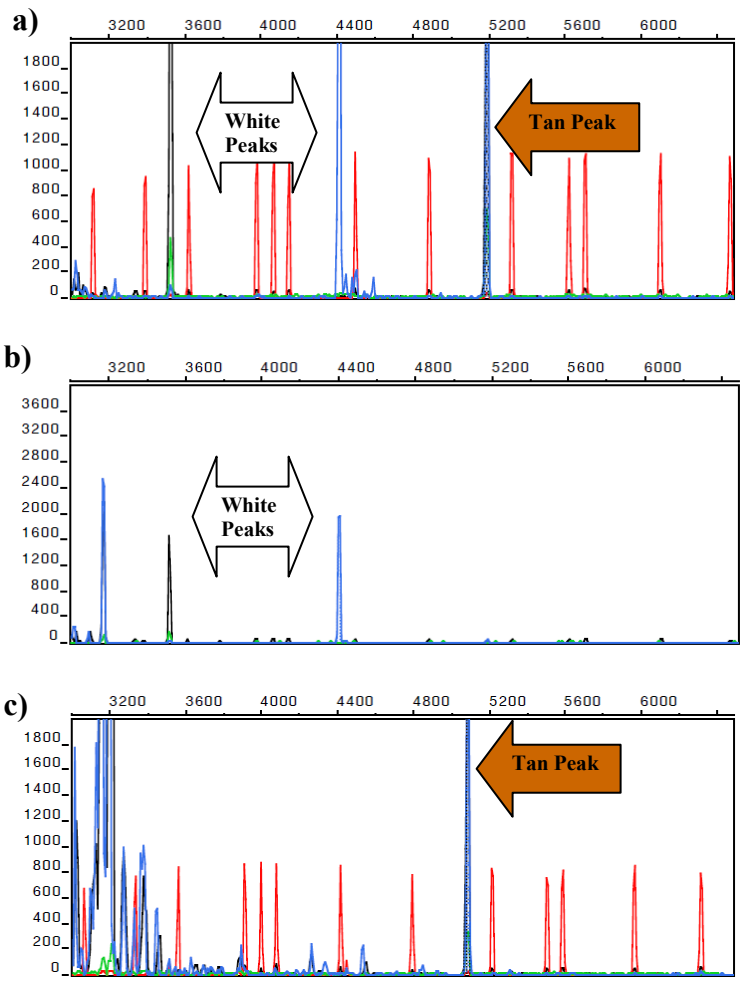

Figure 17: Output from an ABI 310 genetic analyzer. a) The top graph shows an expected 50:50 ratio of white:tan sperm. b) The middle graph shows a 100% bias towards white sperm. c) The bottom graph shows a 0% bias towards white sperm, 100% tan. The size of the respective peaks is used to calculate the proportion of "white" sperm produced by each male.

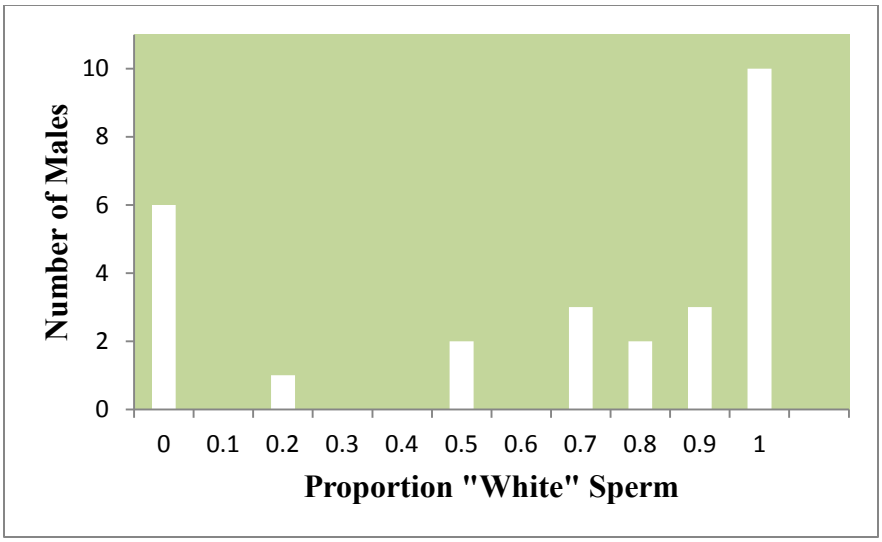

Figure 18: The proportion of "white" " ZAL2^{m} sperm differs significantly from the expected 50:50. t = 1.84, df = 27, p = 0.038.

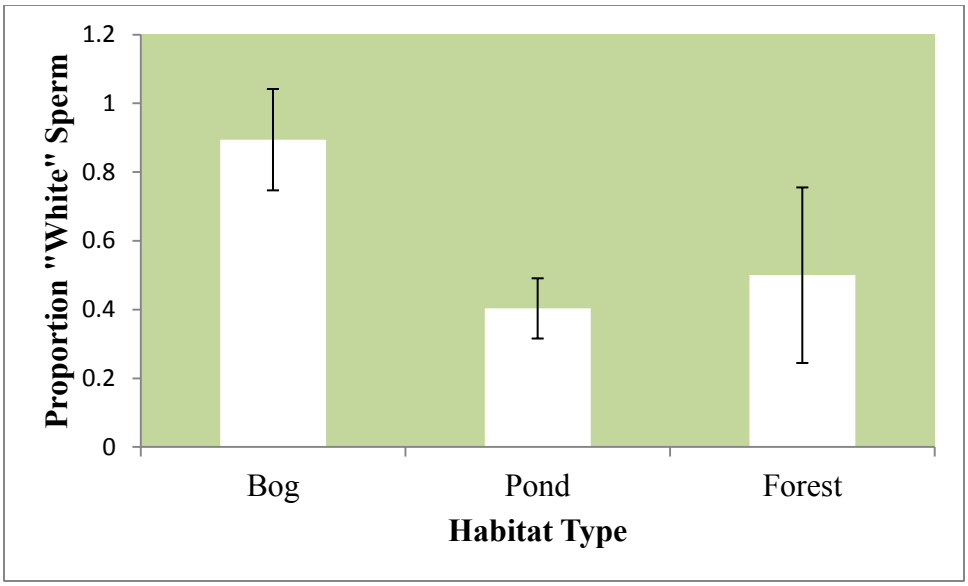

Figure 19: The proportion of "white" ZAL2^{m} sperm produced by white males varies by habitat type. The bog territories are the highest density territories for number of neighbors while the pond territories are the lowest.

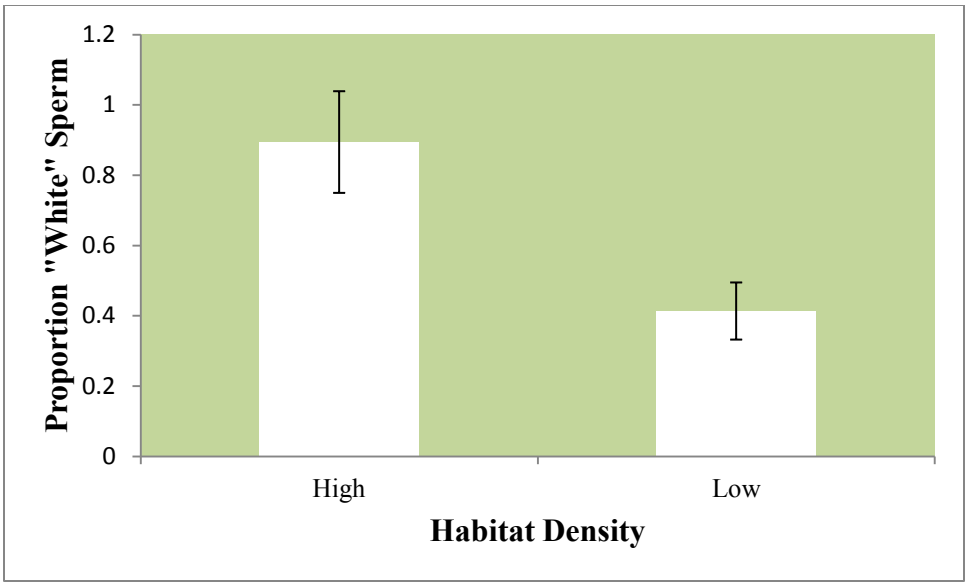

Figure 20: The average production of white male sperm is biased towards “white” ” ZAL2^{m} based upon the social density of their territory. ($F_{2,22} = 4.1031$, $p = 0.03$). Social density is determined by the number of neighbors a male has: High Density > 2 neighbors or Low Density ≤ 2 neighbors.

CHAPTER 5

CANDIDATE GENE MAPPING AND CHROMOSOME EVOLUTION OF THE WHITE-THROATED SPARROW

Abstract

Studies in the field of behavioral genomics have been quickly increasing. Useful model organisms for these studies will have a phenotypic marker for genetic differences. We use a new non-model organism, the white-throated sparrow (*Zonotrichia albicollis*), to examine candidate genes responsible for the differences between the two morphs of this species. White-throated sparrows occur in two morphs that exhibit behavioral and physiological differences between them that are absolutely correlated to a large chromosomal rearrangement on the second largest autosome. Using fluorescent *in situ* hybridization we have mapped candidate genes for the phenotypic and behavioral differences in this species, including VIP, 5HTR1e, and CGA. We have also identified the evolutionary differences between the two versions of chromosome 2 within this species.

Introduction

Understanding genetics is the start to understanding how organisms develop and behave. However, the interactions between genes and groups of genes are very complex, making it difficult to pinpoint those genes that are actually involved in a behavior or trait of interest. Many traits of interest are universal to species. However, currently our model organisms are distantly related to our species of interest or prone to frequent genome changes, making comparisons

difficult. Many vertebrates have a high amount of noncoding DNA and are susceptible to to genome reorganization. These changes make it difficult to identify specific genes and their functions. In order to tease apart the many genes that are involved in different behaviors there needs to be a marker that allows you to track specific genes in organisms. An ideal model organism would have a phenotypic marker to link the specific traits to the genetic basis. Comparative genomics is a useful tool for identifying these behaviors across species, using a model organism to infer relationship in non model organisms. With whole genome sequenced within a variety of organisms, we can apply this knowledge to other groups for comparison.

Chromosomal rearrangements can be characterized as inversions, translocations, deletions and duplications [1, 2]. These can be traced to a variety of factors, including cellular stress [3]. If these rearrangements occur within coding regions phenotypic variation in species that is linked to the genome changes [4]. Inversions have been found to be the most common type of rearrangement in the chicken lineage [5]. Interchromosomal rearrangements are more common in the rodent lineage than other lineages, occurring at a faster rate than in the chicken lineage [5]. Regions where rearrangements occur are not random due to constraints imposed by selection pressures on coding regions and genes that are located in areas of rearrangement may be important in adaptation to changing environmental conditions [6].

Birds are a useful group for the study of genome evolution due to their compact genome size and karyotypes that have been largely conserved through 310 Myr of divergence [7, 8]. This minimalist genome of birds will facilitate the investigation of genome evolution across vertebrates [9]. Comparative chromosome mapping between humans and chickens indicates that chickens have a more similar genome arrangement to humans than do rodents. This difference can be attributed to the higher rate of rearrangements in the rodent lineage compared to the avian

one [7, 10, 11]. The rodent lineage also rearranges three times more frequently than the human [12].

Hughes and Piontkivska [13] reported 34.6% more DNA repeats in humans as compared to the chicken. The compact genome size of birds has eliminated many pseudogenes, intergenic sequences, segmental duplications and retroviral elements [7]. However, despite the conserved and compact genome of birds, they have 20,000-30,000 protein coding genes, similar to the estimates for mammals [14]. These differences make birds an ideal model to use for comparisons to humans. The reduction of repetitive DNA makes identification of genes easier and may also help to reduce the rate of rearrangements. Songbirds specifically are especially important because they learn vocalizations and display complex social behaviors such as courting, monogamy and cultural learning (see review [15]). By examining these species we will be able to identify the genes that are of importance to ecological success [12] such as courting and mating behavior and cultural learning.

White-throated sparrows (*Zonotrichia albicollis*; ZAL) are an ideal species to use for understanding the genetic basis of phenotype. These are small passerines that breed in northern New York and Canada. Since they are currently abundant in the wild, they provide an indication of boreal forest health and so are an ecologically important species. Within this species there are two distinct phenotypes indicated by changes in crown stripe color (white or tan). These phenotypes are absolutely correlated to changes within the second largest autosome (ZAL2) [16-18]. Those individuals that are white are heterozygous for a complex rearrangement within one copy of this chromosome that results in a metacentric (m) configuration ($ZAL2^{m}$) while the other copy lacks the rearrangement and is submetacentric (ZAL2). Tan birds, however, are homozygous for ZAL2. The rearrangement is inherited in a Mendelian fashion and these morphs

mate disassortatively ~97% of the time, maintaining the morphs in equilibrium [19, 20]. Rare cases of homozygous white birds are seen >0.06%, even though occasional white-white pairs are found in nature (E.M. Tuttle, unpublished data). These differences in phenotype provide a visual marker for the changes in genotype within these birds. By examining karyotypes of 46 white-throated sparrows, an additional relationship between the second and third autosome is suggested within this species [18, 21].

In addition to the plumage differences there are also behavioral differences between the morphs. White birds are more aggressive [22, 23], sing more and pursue extra-pair copulations, while the tan birds are monogamous and more parental [24-26]. The morphs also segregate by habitat based on social pressures of neighbors to maximize these differing reproductive strategies. White males settle in neighbor dense areas to increase the opportunity for extra-pair copulations, while the tan males settle in areas with natural borders such as ponds to reduce intrusions by neighbors [27, 28].

Additionally, the chromosomal rearrangement has created a suite of genes that are inherited together (super gene) with limited recombination between the two forms of ZAL2 [29, 30], resulting in a marker to identify the genes responsible for the differences within this species. The rearrangement covers more than 100 Mb of chromosome 2 (almost the entire chromosome) and contains over 1000 genes. Analysis of recombination rates within and without the rearrangement has determined that there are high levels of linkage disequilibrium within the rearrangement and evidence that there is some recombination outside the rearrangement [31]. There are also indications of rearrangements on ZAL3, so there are most likely epistatic interactions between genes on the 2 chromosomes and possibly others.

By knowing that these differences in behavior and phenotype are linked to the chromosomal rearrangement, we have a starting point for identifying genes, or groups of genes that are involved in these behavior and plumage differences. The observed behavioral differences are most strongly evident during the breeding season, suggesting a difference in circulating hormone levels between the morphs. Genes involved in hormone production and reception are therefore of great interest for identifying morph differences. These intraspecific differences provide us with a unique opportunity for comparison of different behavioral aspects and environmental influence within the same species.

A high degree of interchromosomal conservation has been found across at least 30 bird groups through chromosome painting with a few examples of chromosome fission/fusion in some lineages (for reviews see [32, 33]). Passerine chromosomes syntenic to chicken (GGA) chromosome 1 have diverged as 2 macrochromosomes while GGA4 has resulted from a fusion of a macro and microchromosome [34]. However, chromosome synteny does not guarantee a conserved gene order. The number of studies that indicate the presence of gene order changes have increased [35, 36], so inferring gene order from conserved synteny should be done with caution. Microchromosomes have been confirmed to be the ancestral state through comparative mapping with zebrafish [8] while the macrochromosomes have been compared within the turtle lineage and are also ancestral [37]. The chicken karyotype is therefore a good representation of the ancestral karyotype [32]. Due to this high level of conservation of the ancestral karyotype birds are an excellent outgroup for comparison of chromosome evolution. Passerines are the largest group of birds and are highly studied, both in situ and ex situ, examining complex traits such as vocal learning [15], speciation, and a variety of social behaviors. A few important traits for cross species comparison are parental care, sexual behavior and aggression. Understanding

these traits within birds will provide a starting point for understanding these behaviors in more difficult groups to study, including humans.

However, intrachromosomal gene order changes occur more frequently than expected, with inversions being the most common source of the changes [38]. Using comparative genomics we predicted genes located within the white-throated sparrow rearrangement based on the location of the genes within chicken and zebra finch [39, 40]. Here we use BACs (Bacterial artificial chromosomes) and fluorescent *in situ* hybridization (FISH) to map and identify the candidate genes located within the complex chromosomal rearrangement of the polymorphic white-throated sparrow. We are currently working to identify the candidate genes that are responsible for the differences in sexual behavior and parental care between the two morphs, making them an interesting model for identifying the genes responsible for these differences in an organism in a natural setting.

Here we report the results of detailed mapping of ZAL2, the genes identified within the rearrangement, and an analysis of the rearrangement in light of the gene order changes between chicken (*Gallus gallus*; GGA) and zebra finch (*Taeniopygia guttata*; TGU) chromosomes 3, the homolog to ZAL2. Our goal of this study was two-fold; to identify behavioral genes (specifically parental care and monogamy/promiscuity) within the rearrangement that may be linked to the morph differences and to determine the evolutionary history of the rearrangement.

Methods

Sample Collection

Wintering flocks of white-throated sparrows were mist netted on the Indiana State University campus between November and February 2007 – 2011. Captured birds were housed individually on a 16:8 light cycle and supplied with *ad libitum* seed and water. Seed was

supplemented with wax or meal worms, vitamins and fresh greens. Blood was taken through brachial venipuncture and used for molecular analysis. Each bird was sexed using the P2 and P8 primers [41] and morph identified using the modified protocol of Michopoulos *et al.* [42] as in Romanov *et al.* 2009 [21].

We used a non-invasive feather sampling method to collect dividing cells from the birds. Three feathers were pulled from each bird, either from the primary flight feathers or tail to stimulate feather regrowth. Growing feathers were then pulled approximately 17 days later for use in cell culture (see Figure 21). A few tissue samples (eye or trachea) were opportunistically collected from window strikes by David Willard at the Field Museum of Natural History (Bird Division, Chicago, IL). Zebra finch tissue samples were kindly provided by Sarah London (Department of Psychology, University of Chicago, Chicago, IL). All animal use was approved by the Indiana State University IACUC. (Protocol #02-04-2011:EMT/RAG).

Karyotypes

Karyotypes were prepared at the Genetics Division at the San Diego Zoo's Institute for Conservation Research (ICR). Fibroblast cultures were started from 2-3 growing feathers. Briefly, feather shafts were wiped with ethanol, feather pulp was excised from the shaft, and digested for 2 – 5 hours in collagenase at 37 °C with frequent agitation to dissociate the cells. The cells were transferred to a 12.5cm^2 flask with equal parts Minimal Essential Media α (Mediatech Inc, Manassas VA; supplemented with 10% fetal calf serum and 1% pen-strep glutamine) and Fibroblast Growth Media (FGM®-2; Lonza, Walkersville MD) then incubated at 40 °C with 6% CO_2. Once fibroblasts reached confluency they were passed to a 25 cm^2 flask and further expanded for a maximum of 4 passages. The cells were then frozen in media and10% DMSO in liquid nitrogen using a control rate freezer or Cool Cell® (Biocision®,

Larkspur CA). Cell lines for 31 white-throated sparrows have been archived in the Frozen Zoo® (ICR). Fibroblasts from eye or trachea were grown according to Kumamoto 1996 [43].

To harvest metaphase chromosomes from the cells, colcemid was added to the flask at a final concentration of 0.005 μg/ml for a minimum of 4 hours, incubated in 0.067 M KCL hypotonic solution for 6-30 minutes at 37 °C and then fixed with 3:1 methanol-acetic acid. All cell lines were stained with Giemsa, karyotyped to confirm morph and sex class as previously established by molecular methods. Twenty metaphase spreads were counted for each bird to assure they were free of cultural artifact. Image capture and karyotyping was done using CytoVision® software (Leica Biosystems, Germany). Additional slides for FISH were then dehydrated in an ethanol series and stored at -80 °C until use. See Figure 2 for Giemsa stained partial karyotypes.

Probe Identification

Previous work has identified chicken chromosome 3 (GGA3) as analogous to white-throated sparrow chromosome 2 (ZAL2) through chromosome paints [16]. With this information, and the genomes of the model organisms chicken [10] and zebra finch (Warren 2010) we were able to target rearrangement areas and genes of interest for mapping with BACs. Candidate genes were chosen based on function identified in other species or based on location within the chicken karyotype. Previous work has identified the usefulness of cross-species overgo probes and the conserved genome across highly divergent species [44]. The white-throated sparrow BAC library (Bacpac Resources Children's Hospital Oakland Research Institute; CHORI-264) [45] was screened for candidate genes that are potentially located within the rearrangement through a combination of overgo probes and BAC-end sequence (BES) [44, 46]. In order to fully map the rearrangement on ZAL2, we have identified 45 ZAL BACs and 7

TGU BACs of interest in addition to 13 TGU reference markers from previously published data [16] for comparative purposes (See Table 2). TGU markers for the genes CCKAR, BRS3, SYTL4, IRS4 were kindly identified by Christopher Balakrishnan (East Carolina University, unpublished data). Zebra finch clones were obtained either from Clemson University Genomics Institute (TGMCBa) or Arizona Genomics Institute (TG_Ba).

Fluorescent in situ Hybridization

BACs were grown and labeled as in Lear 2001 and 2008 [47, 48]. Briefly, BACs were grown overnight in YT media (Sigma-Aldrich, St. Louis MO) with 12.5µg/ml chloramphenicol and extracted using the Qiagen Midiprep kit (Qiagen, Valencia CA) or ZR BAC DNA Miniprep kit (Zymo Research, Irvine CA). DNA was labeled with SpectrumGreen®, SpectrumRed®, or SpectrumOrange® (Abbot Molecular, Inc. Abbot Park, Illinois) through nick translation. Homologous probes were hybridized to slides for 16 hours (*i.e*. ZAL BACs to ZAL metaphase spreads), heterologous probes and mixed heterologous/homologous probes were hybridized for 92 hours (*i.e.* TGU BACs on ZAL metaphase spreads) using probes of two different colors. Homologous probes were washed at 42 °C and all others were washed at 37 °C, following established stringency. Slides were counterstained with DAPI III (Abbot Molecular Inc, Abbot Park, Illinois) and imaged using a multiphase microscope and fluorescent camera with CytoVision® software (Leica Biosystems, Germany). See Figures 30 and 31 for representations of the hybridization results.

Rearrangement Analysis

Second generation comparative maps of chromosomes 3 and 4 were created for GGA and TGU as in Romanov *et al.* (2011)[46] based upon chicken build 4.0 (GenBank Assembly ID: GCA_000002315.2; RefSeq Assembly ID: GCF_000002315.3) and zebra finch build 3.2.4

(GenBank Assembly ID: GCA_000151805.2; RefSeq Assembly ID: GCF_000151805.1) (See Figures 23-25). These maps were then used for comparison of the chromosomal rearrangement in ZAL. Clones were numbered according to the order of the chicken chromosome (*i.e.* RALGAPA2/TGMCBa0156C22 is #1 since it is the first clone on the distal end of the GGA3 p-arm). See Table 2 for a complete list of clones and the number assignment for the rearrangement analysis. The TGU clone for gene MBOAT2 (TG_Ba0047o03) did not localize in ZAL and so was excluded from the analysis. Additional chromosomes were included in the analysis if a clone that mapped to GGA3 or GGA4 mapped to another TGU or ZAL chromosome.

Using the GRIMM and MGR applications (http://grimm.ucsd.edu/MGR/ [49]) the minimum number of rearrangements between each genome was calculated (*i.e.* rearrangement distance). The GRIMM application was used to infer orientation (or sign) for each gene based upon orientation in GGA. See Table 3 for the unsigned input used in GRIMM and Table 4 for the signed input used in MGR. Once the signed rearrangements were calculated, the gene orders were used to calculate the phylogeny of chromosome rearrangements between each group. Potential ancestors for each node of the tree were also calculated. Previous comparisons of GGA, turkey (*Meleagris galloavo*; MGA) and TGU genomes suggests that all rearrangements can be explained by a series of inversions, the simplest form of genome rearrangement [50]. GRIMM and MGR do however take into account translocations, fissions, and fusions in the algorithm.

With few exceptions of reduced diploid number (*i.e.* stone curlew [51], Falconiformes [52] and Psittaciformes); most birds share a highly conserved karyotype near 2n = 80 [32]. GGA has a diploid number of 78 [53], TGU a diploid number of 80 [54], and ZAL a diploid number of 82 [21]. Bird and mammal lineages diverged 310 million years ago and the first birds appeared

200 mya [7, 55]. Palaeognaths and neognaths split ~ 120 mya with Passeriformes diverging ~100mya from the Galliformes [56] with a minimal number of karyotypic changes between them [34]. Zebra finches diverged ~45 mya with white-throated sparrows diverging ~ 20 my after the zebra finch [57]. Therefore by using GGA and TGU as our comparison groups we have markers at the major nodes if speciation leading to ZAL.

White-throated sparrows follow the standard avian karyotype of a few large macrochromosomes and many small microchromosomes. They have a diploid number of 82, 7-10 pairs of macrochromosomes (8-10 are intermediate in size), 30 pairs of microchromosomes, and 2 sex chromosomes [21]. There is not a clear differentiation between the large and intermediate sizes so there is some variation in nomenclature across studies. We use the standardized numbering system for chromosomes according to size, not including the sex chromosomes. Figure 3 shows our numbering as compared to the numbering of Thornycroft 1975 [18] who numbered the Z chromosome as ZAL4. ZAL chromosomes 2, 4, 4a, 6, and Z were included in the rearrangement analysis. ZAL4, 4a, 6 and Z were included in the rearrangement analysis due to unexpected gene placement of several clones predicted to be on ZAL2 to these chromosomes. In addition, microchromosomes are not thought to be of adaptive value due to the high variation in numbers between species, even though they contain half the gene content of birds [8].

Results

Physical Mapping; Conservation of Passerine Karyotype

We successfully mapped 45 ZAL clones and 19 TGU clones, and 3 duplicate/overlapping clones (we were unable to hybridize one TGU clone to ZAL). GGA2 is all or in part homologous to ZAL1, the clone containing the gene MC4R mapped to ZAL1 just below the

centromere. However more mapping is needed to confirm this homology. See Figures 26 and 27 for map locations of all clones.

Passerine homologs of GGA1 and GGA4 are each represented by two chromosomes (1 macrochromosome and 1 microchromosome) [58]. Contrary to previous predictions of GGA4 being orthologous to ZAL3 [46], preliminary mapping of two genes to ZAL3, monoamine oxidase b (MAOB) and nescient helix loop (NHLH2) indicated that GGA1 is orthologous to ZAL3 (Figure 27). The mapping also supports the karyotypic evidence of another rearrangement within this species (ZAL3/ZAL3^{a}); however more work is needed to map this chromosome in greater detail. ZAL3^{a} shows conserved gene order with GGA1, but there is a shift in the centromere location to the distal end of the p-arm that has resulted in the acrocentric form of ZAL3^{a}. ZAL3, however shares conserved gene order with TGU1 and a submetacentric centromere (see Figure 28 for representation of the changes between these chromosomes). Within other passerines GGA1 has undergone a fission, resulting in a macro and a microchromosome. Further mapping is needed to identify if there is a corresponding microchromosome to ZAL3 and confirm the presence of a fission in the lineage leading to ZAL as has occurred in zebra finch (*i.e.* TGU1 and TGU1a) [59].

Mapping of eight genes located on GGA4 confirms the ancestral karyotype of chromosome 4 as two separate chromosomes within the white-throated sparrow, as found in most birds, but a single chromosome resulting from a fusion in GGA [60]. See Figure 25 (ZAL4 and ZAL4a). For simplicity we have adopted the nomenclature used in the zebra finch and refer to the microchromosome as ZAL4a [59, 61].

We mapped two genes (COL3A1 and MCM6) predicted to be located on GGA7 and TGU7 (Figure 27). Both of these genes mapped to a small/intermediate submetacentric

chromosome that we predict to be ZAL7-10. Due to the small size we are unable to determine the exact chromosome number since all within this size range are approximately the same length. Further work is needed to identify the exact location.

Physical Mapping; ZAL2

Clone CH264-305i10 hybridizes two signals on ZAL2^{m}, one at the telomere of the q arm and another within the p arm just above the centromere. This signifies the breakpoint for this inversion in both arms of the chromosome. BES has indicated that there is a noncoding intergenic region opposite the TAF1B gene, suggesting that the breakpoint may occur within the non-coding region; further sequence work is needed to confirm this. Within zebra finch there is also a faint second signal on TGU3, perhaps identifying an additional rearrangement within this species that has not been identified yet due to the repetitive nature of the intergenic region.

Clone CH264-226A16 was predicted to have two hybridization points within chicken and zebra finch, but only maps to one location within ZAL. To confirm the double signal we attempted to hybridize to TGU metaphase spreads, however this clone failed to map. This clone is also located close to the break point region and may have a high degree of sequence variation between the two species preventing the hybridization.

One gene order change of particular interest is the orientation of LIN28b and GRIK2. These genes are located in conserved order in GGA3, TGU3 and ZAL2^{m}; however they are separated by the centromere on ZAL2. This change of gene order suggests that the rearrangements within ZAL2 are more derived than those of ZAL2^{m}.

A gene of special interest to the differences within the white-throated sparrow is proopiomelanocortin (POMC). POMC is the precursor to the melanocortin system, which has a wide range of influence, including color differences, differences in sexual behavior and

aggression [62]. We were unable to identify a clone that contained this gene with either overgo probes or BES. It is, however, predicted to be near other genes that are present within the rearrangement of ZAL2, based upon location within GGA and TGU. Preliminary work has identified significant differences in sequence between the two morphs (E.M. Tuttle, unpublished data) which would make it difficult to use cross species overgo probes or BES for identification. Clone CH264-069M12 is predicted to be near POMC and did indeed map near a breakpoint within the p arm of $ZAL2^{m}$. We were able to identify clones for this gene in GGA and MGA, however both of these failed to hybridize, mostly likely due to the sequence divergence at this gene.

Zebra finch clone TG_Ba0047o03 (MBOAT2) failed to hybridize in white-throated sparrows. This clone is also predicted to be very close to the breakpoint area so there may be too much sequence difference between the species to allow for hybridization.

Evidence of Interchromosomal Rearrangements

Clone CH264-003L20 unexpectedly mapped to ZAL6 (Figure 27) instead of ZAL2 as predicted based on its overgo probe alignment with the chicken and zebra finch genomes. This may be due to an incorrect identification of the gene located within this clone or errors in the sequence assembly. However, this clone is located near a breakpoint within the rearrangement and thus may have been translocated during one of the inversions.

NOL10 (clone CH264-342B23) also mapped to an unexpected location, ZALZ (Figure 27). This clone was predicted to map to ZAL2, near the breakpoint and so may have been translocated during one of the rearrangements in the area. We attempted to confirm our clone identification by mapping onto zebra finch metaphase spreads. However the clone failed to

hybridize, suggesting that there may have been several changes in this area between these two closely related species preventing the signal.

Interestingly, clone CH264-006D10 which was predicted to map to GGA4 and TGU4a mapped to ZAL2 just above the centromere. We also confirmed this clone with zebra finch metaphases where it indeed mapped to TGU3 (Figure 31). This clone was chosen through alignment of BES to the chicken and zebra finch genome, which is still being updated frequently as it is sequenced at a deeper coverage. Either there are repetitive elements within this clone that resulted in misalignment or it is currently mislocated in the zebra finch genome. Since we were able to hybridize this clone to TGU3 it is most likely an error in placement in the genome. We may find that there will be other unexpected placements as the genomes are continually updated.

In other species interchromosomal rearrangements are associated with areas that contain pseudogenes and other repetitive elements [7, 8]. The areas surrounding the clones CH264-305i10, CH264-342B23, and CH003L20 should be examined for these sequence elements to identify the causes of the rearrangements.

Genome Rearrangement Analysis

Our predictive rearrangement scenario suggests that ZAL2^{m} has fewer rearrangements than ZAL2 (Figure 32). There are eleven rearrangements between ZAL2 and ZAL2^{m}. There are 18 rearrangements between them and the most recent predicted common ancestor between ZAL, TGU and GGA. See Table 5 for the gene order of the two predicted ancestors in this phylogeny.

Discussion

White-throated sparrows show major pericentric rearrangements of chromosomes 2 and 3 - both of which provide much insight into the function and evolution of structural differences in genes and chromosomes. Both ZAL2 and ZAL2^{m} show a high degree of rearrangement between

themselves, and with other avian species (i.e. chicken and zebra finch). It was originally hypothesized that ZAL2^{m} was the derived arrangement and the ZAL2 was ancestral [18], primarily because ZAL2 is more prevalent in natural populations. However, based on our analyses here, ZAL2^{m} is actually more closely related to chicken than previously thought. The rearrangement on ZAL3 still needs further examination, but indicates the presence of a second complicated rearrangement. Based upon the frequency of inheritance, ZAL3 is also involved to some degree with the morph differences observed within this species [21, 46].

We previously predicted that the rearrangement arose before the radiation of the *Zonotrichia* [21]. However, the current study emphasizes how complex the rearrangement is suggesting the divergence between them most likely started much earlier than we previously predicted. Passerines represent the largest radiation of birds, occupying a wide range of environments and displaying extreme behavioral diversity. The subfamily Emberizinae diversified and colonized the New World ~24 mya, during a time of significant environmental changes [57]. The timing of diversification during environmental changes may have facilitated genome reorganization as a method of creating gene suites to survive the changing environment.

This paper provides an excellent starting point for sequencing the genes identified within the chromosome rearrangement and correlating the variation to environmental and social factors. We will continue mapping efforts to identify the relationship of the rearrangement on ZAL3 to ZAL2 and anchor the assembled genome. Although resolution by FISH did not always allow determination of gene order, this will be possible with the genome sequence. We expect to see even more microinversions present within the white-throated sparrow genome than is currently evident from our mapping efforts and will provide even more clues to this unique species.

Völker *et al.* previously identified a high degree of intrachromosomal rearrangements between chicken and zebra finch whole genomes [61].

Comparisons between chicken and turkey karyotypes show a high degree of conservation between them [63], with physical mapping suggesting 20-27 intrachromosomal inversions between them [64]. Turkey and chicken diverged 20-40 mya [65] a similar divergence time between white-throated sparrows and zebra finch. Therefore we would predict a minimum of 27 rearrangements between them due to the reduced generation time of the smaller birds. A comparison of willow warbler loci with loci on GGA3 and TGU3 predicts a minimum of two inversions between these groups [66]. Additionally, microrearrangements in gene order are being found more frequently than previously thought. As technology advances and we add more markers for mapping non model organisms we will identify more changes [38, 67, 68].

The locations of several genes of interest were identified through our BAC based mapping efforts. Bone morphogenic protein 2 (BMP2) and 5 (BMP5) are located within the rearrangement. BMP2 has been implicated in differences in beak size between species of Darwin's finches [69], and perhaps is also responsible for the variation in beak size between the two morphs of white-throated sparrows.

Clone CH264-32G21 contains the gene CGA (glycoprotein hormones, alpha polypeptide) and the serotonin 1 e receptor gene (5HT1e). Each of these genes may be responsible for some of the behavior differences observed between the morphs. There are four human glycoprotein hormones; chorionic gonadotropin (CG), luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH) which are important sexual hormones. The serotonin system is a complex and incompletely understood system that is also associated with

sexual behavior in humans. Both of these genes provide an interesting avenue to further explore with sequencing.

Genes located on GGA4 have been implicated in behavior differences such as sexual behavior and aggression in mice and may be responsible for some of the differences in white-throated sparrows [70] These genes are BRS3, CCKAR, IRS4 and SYTL4. We successfully mapped these genes within the white-throated sparrow to ZAL4 or ZAL4a. Even though these genes are not located within the rearrangement, we did find that at least part of GGA4/TGU4a has been translocated to ZAL2. The clone CH264-006D10 which is located near BRS3 in GGA and IRS4 in TGU maps to ZAL2 in the p-arm above the centromere, instead of the predicted location of ZAL4. Hence, regulatory genes may have been linked within the rearrangement. Interestingly we also mapped this clone to TGU3 instead of the predicted TGU4a based upon BES sequence. Closer examination of any regulatory genes that may be located on TGU3 should be examined.

Genes within some warbler lineages that are located on TGU4a have become sex linked, such as SYTL4. However, no linkage is evident in other passerine lineages [71]. There is evidence of multiple rearrangements on 4a between chicken and zebra finch so there may be other sex linked genes within passerines that have not been identified yet. Some genes that are not sex linked, but have been implicated in sex differentiation are located close to the clone CH264-006D10 such as SOX3 and HTR2c and may be located within the rearrangement. Additionally, we have evidence of a translocation between ZAL2 and the Z; genes from the Z may have become linked with the rearrangement. Instead of sex linked traits, we may have sex differentiation traits that have become linked to morph.

Areas where clones failed to hybridize are most likely due to sequence divergence and highlight areas to begin looking for the differences between passerines and the morphs of the white-throated sparrow. There are reduced recombination rates in macrochromosomes as compared with a very high rate within microchromosomes, due to the relationship of obligate crossing over during meiosis to chromosome size. Recombination between the micro and macrochromosomes is also prevented by the nuclear organization of birds [72]. During mitosis there is a segregation of the macrochromosomes from the microchromosomes within the nucleus. The locations of the chromosomes may facilitate or prevent translocations due to proximity within the nucleus. These locations may also provide a structural influence on gene regulation through close association of genes and regulatory factors. The extreme variation in the rates of recombination between chromosome sizes may explain why there has not yet been degeneration between the larger chromosomes ZAL2/ZAL2^{m}.

This research provides an excellent starting point for further analysis of the genes identified within the chromosome rearrangement and for correlating the genomic variation to environmental, health, and social factors. We will continue mapping efforts to identify the relationship of the rearrangement on ZAL3 to ZAL2 and anchor the assembled genome. We expect to see even more microinversions present within the white-throated sparrow genome than is currently evident from our mapping efforts and will provide even more clues to this unique species. Additionally this data will be used to anchor the genome to the established karyotype for further analysis [6]

Voss *et al.* predicts that GGA3 is ancestral (*i.e.* ZAL2) through comparison with *Ambystoma* and *Xenopus* [73] due to conserved gene synteny and so may be comparable to conserved gene blocks within the human genome (HSA). Additionally, human and chicken

chromosome 4 (HSA4 and GGA4) are largely conserved [32, 74] across groups, providing a model for examining the genes located within these blocks. Approximately 78% of mammalian imprinted genes appear to cluster on the highly conserved chicken macrochromosomes 1, 2, 3, and 5. Less than half of the predicted 100-200 imprinted genes have been identified [75]. The white-throated sparrow will potentially provide a model for identifying additional genes that are imprinted in mammals.

The high level of rearrangement (both inter and intrachromosomal) found in our study follows the recent finding by Völker *et al.* of high levels of rearrangement between the chicken and zebra finch genome [61]. Polymorphic karyotypes in birds have been observed in several other species including zebra finch [76], rufous-collared sparrow [77], junco [78], cardinal and white-crowned sparrow [79]. These species show a variety of rearrangements, however obvious phenotypic differences have not been noted to the extent they are in the white-throated sparrow. There may be some variation within these groups and further work is warranted. Rearrangements may play a role in speciation; examining the other species with similar rearrangements, specifically those in the *Zonotrichia* clade, may elucidate the evolution of these groups.

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Table 2: Complete list of BACs used for mapping analysis. Gene names are listed according to the chicken genome build. Clone number (corresponding to either CHORI, Arizona or Clemson BAC library resources), predicted gene function, gene number for the genome rearrangement analysis and the location within the white-throated sparrow genome. Zebra finch clones are in italics. Clone identified by overgo probe hybridization are indicated by an asterisk. Zebra finch clones used by Thomas *et al* 2008 [16] are indicated by ‡. The two clones that represent 2 different locations within GGA are underlined.

Gene Symbol	Clone Name	Gene Name	Function	Map #	Location
RALGAPA2	*TGMCBa0156C22‡*	Ral GTPase activating protein, alpha subunit 2	Catalytic subunit of the heterodimeric RalGAP2 complex	1	ZAL2
LBH	*Ba0094A01‡*	limb bud and heart development homolog (mouse)	Transcriptional activator which may act in mitogen-activated protein kinase signaling pathway	2	ZAL2
MIR1641	CH264-377H05	microRNA mir-1641	post-transcriptional regulation of gene expression	3	ZAL2
NW_001471668	CH264-052H21	Non-coding region		4	ZAL2
BMP2	CH264-040C07*	Bone morphogenic protein 2	Associated with spermatogenesis, bone and cartilage formation w/ FOXL2 upregulates follistatin (sex determination, development)	5	ZAL2
DUSP10	*Ba0077A01‡*	dual specificity phosphatase 10	response to stress	6	ZAL2
TGFB2	*TG_Ba0056A01‡*	transforming growth factor, beta 2	Involved in the regulation of cellular processes, including cell division, differentiation, motility, adhesion and death	7	ZAL2
ESRRG	CH264-039P02*	Estrogen-related receptor gamma	Estrogen-related receptor gamma and transcription factor	8	ZAL2
MEMO1	CH264-451M05	mediator of cell motility 1	May control cell migration by relaying extracellular chemotactic signals to the microtubule cytoskeleton	9	ZAL2
HNRNPU	CH264-005K17*	heterogeneous nuclear ribonucleoprotein U	associated with pre-mRNAs in the nucleus and appear to influence pre-mRNA processing and other aspects of mRNA metabolism and transport	10	ZAL2
FMN2	CH264-003F02*	formin 2	Maintenance of mitotic spindle	11	ZAL2
RYR2	CH264-031F24*	ryanodine receptor 2	Required for cellular calcium ion homeostasis. Required for embryonic heart development	12	ZAL2
T	*TG_Ba0300O04‡*	T, brachyury homolog (mouse)	Involved in the transcriptional regulation of genes required for mesoderm formation and differentiation.	13	ZAL2
FNDC1	CH264-025I01*	fibronectin type III domain containing 1	May be an activator of G protein signaling (By similarity)	14	ZAL2
SF3B5	CH264-048N03*	splicing factor 3b, subunit 5, 10kDa		15	ZAL2

BM439915	*TG_Ba0013A01‡*	cDNA clone pgr1n.pk001.j9 5' similar to no significant hits (pLog(P) 4), mRNA sequence		16	ZAL2
ESR1	CH264-217L17	estrogen receptor 1	essential for sexual development and reproductive function, but also play a role in other tissues such as bone	17	ZAL2
VIP	CH264-001G10*	Vasoactive intestinal peptide	diverse biological actions including promotion of neuronal survival, regulation of glycogen metabolism, stimulation of prolactin release from the pituitary	18	ZAL2
SNX9	CH264-019C13*	sorting nexin 9	May be involved in several stages of intracellular trafficking	19	ZAL2
SCAF8	CH264-046M03*	SR-related CTD-associated factor 8	May play a role in mRNA processing	20	ZAL2
ECHDC1	*TGMCBa0021H11‡*	enoyl CoA hydratase domain containing 1		21	ZAL2
TRMT11	*TG_Ba0352K14‡*	tRNA methyltransferase 11 homolog	Catalytic subunit of an S-adenosyl-L-methionine-dependent tRNA methyltransferase complex (By similarity)	22	ZAL2
DSE	CH264-041N10	dermatan sulfate epimerase	Converts D-glucuronic acid to L-iduronic acid (IdoUA) residues	23	ZAL2
HDAC2	CH264-007F22*	histone deacetylase 2	plays an important role in transcriptional regulation, cell cycle progression and developmental events	24	ZAL2
FYN	CH264-075P04*	oncogene related to SRC, FGR, YES	implicated in the control of cell growth	25	ZAL2
FIG4	CH264-020A10*	FIG4 homolog, SAC1 lipid phosphatase domain containing (S. cerevisiae)	Plays a role in the biogenesis of endosome carrier vesicles (ECV) / multivesicular bodies (MVB) transport intermediates from early endosomes	26	ZAL2
AKD1	CH264-003L20*	chromosome 6 open reading frame 199		27	ZAL6
PDSS2	CH264-226A16	prenyl (decaprenyl) diphosphate synthase, subunit 2	Supplies decaprenyl diphosphate, the precursor for the side chain of the isoprenoid quinones ubiquinone-10	28	ZAL2
CN228284	*TGMCBa0120H07‡*	RJB052F05.ab1 RJtestis *Gallus gallus* cDNA 5-, mRNA sequence		29	ZAL2
PREP	CH264-177L19	prolyl endopeptidase	Cleaves peptide bonds on the C-terminal side of prolyl residues within peptides that are up to approximately 30 amino acids long	30	ZAL2
LIN28B	CH264-148O09*	lin-28 homolog B (C. elegans)	Acts as a suppressor of microRNA (miRNA)	31	ZAL2

			biogenesis		
NW_003763720	CH264-377H05	genomic scaffold, Gallus_gallus-4.0	non-coding region	32	ZAL2
GRIK2	CH264-017G05*	glutamate receptor, ionotropic, kainate 2	May be involved in the transmission of light information from the retina to the hypothalamus	33	ZAL2
ZNF292	*Ba0064A01‡*	zinc finger protein 292	May be involved in transcriptional regulation	34	ZAL2
CGA	CH264-32G21	glycoprotein hormones, alpha polypeptide (clone also includes the serotonin 1e receptor)	four human glycoprotein hormones are chorionic gonadotropin (CG), luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH)	35	ZAL2
ME1	CH264-056J24*	malic enzyme 1	encodes a cytosolic, NADP-dependent enzyme that generates NADPH for fatty acid biosynthesis	36	ZAL2
ELOVL4	*TG_Ba0071A01‡*	ELOVL fatty acid elongase 4	Condensing enzyme that elongates saturated and monounsaturated very long chain fatty acids	37	ZAL2
EEF1A1	CH264-021M23*	eukaryotic translation elongation factor 1 alpha 1	responsible for the enzymatic delivery of aminoacyl tRNAs to the ribosome	38	ZAL2
DST	CH264-128K03	Dystonin	Cytoskeletal linker protein	39	ZAL2
BMP5	CH264-003C04*	bone morphogenetic protein 5	Induces cartilage and bone formation	40	ZAL2
FAM83B	CH264-334H09	family with sequence similarity 83, member B	Open reading frame	41	ZAL2
MBOAT2	*TG_Ba0047o03*	membrane bound O-acyltransferase domain containing 2		NA	NA
TAF1B	CH264-305I10	TATA box binding protein (TBP)-associated factor, RNA polymerase I, B	involved in the assembly of the PIC (preinitiation complex) during RNA polymerase I-dependent transcription	42	ZAL2
NOL10	CH264-342B23	nucleolar protein 10		43	ZALZ
NW_001471673	*TG_Ba0055A01‡*	genomic scaffold, Gallus_gallus-4.0	[non-coding region]	44	ZAL2
RAB10	CH264-069M12	member RAS oncogene family	May be involved in vesicular trafficking and neurotransmitter release	45	ZAL2
FAM167A	Ch264-017J19*	family with sequence similarity 167, member A		46	ZAL2
SUPT3H	CH264-165F06	suppressor of Ty 3 homolog (S. cerevisiae)	Probable transcriptional activator	47	ZAL2
GPR116	CH264-226A16	G protein-coupled receptor 116		48	ZAL2
LOC422179	CH264-006D10	uncharacterized LOC422179		49	ZAL2
BRS3	*TGAC-371L12a* *TGAC-339F17a*	bombesin-like receptor	Sexually dimorphic	50	ZAL4a
SYTL4	*TGAC-126N08a*	synaptotagmin-like 4	Sexually dimorphic intra-cellular signaling protein	51	ZAL4a
IRS4	*TGAC-94D10b*	insulin receptor substrate 2-B-like	Sexually dimorphic intra-cellular signaling	52	ZAL4a

	TGAC-35E15b		protein		
CUL4B	CH264-020M20*	Culin 4B	Important for DNA replication	53	ZAL4a
SMAD1	CH264-019P04*	SMAD family member 1	neural crest differentiation, midbrain development, positive regulation of dendrite morphogenesis	54	ZAL4
SNX25	CH264-062N08*	sorting nexin 25		55	ZAL4
NAAA	CH264-001C24*	N-acylethanolamine acid amidase		56	ZAL4
PGM2	CH264-006C05*	phosphoglucomutase 2		57	ZAL4
CCKAR	*TGAC-204M21b* *TGAC-308H24a*	Cholecystokinin A receptor	neuroactive ligand-receptor interaction, feeding behavior, satiety, forebrain development, neuron migration, release of β-endorphin and dopamine, sexual behavior	58	ZAL4
MC4R	CH264-004A01*	melanocortin 4 receptor	Implicated in obesity in humans, associated with sexual activity	NA	ZAL1
NHLH2	CH264-002i12*	Nescient helix loop	HPA axis, sexual behavior, reproduction longevity, sperm development	NA	ZAL3
MAOB	CH264-013L24*	Monoamine oxidase B	excitability, activity, exploration, aggression	NA	ZAL3
MCM6	CH264-34E23*	Mini chromosome maintenance 6	Essential for genome replication	NA	ZAL7-10
COL3A1	CH264-002i01*	Collagen type III α 1	Codes for connective tissue	NA	ZAL7-10

Figure 21: Representation of the feathers used to establish white-throated sparrow fibroblast cell lines.

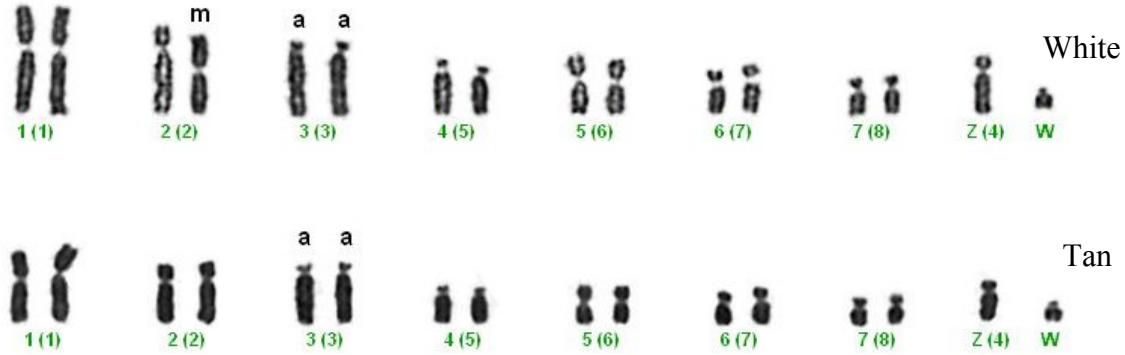

Figure 22: Karyotype representing the first 7 autosomal pairs and the ZW sex chromosomes of the white-throated sparrow. A white morph karyotype is shown on the top with ZAL2/ZAL2^{m} and the acrocentric arrangement of ZAL3 ($3^{a}/3^{a}$). The bottom karyotype represents a tan morph ZAL2/ZAL2 also with the acrocentric arrangement of ZAL3 ($3^{a}/3^{a}$). The numbers in parentheses indicate the numbers used by Thornycroft [17, 18].

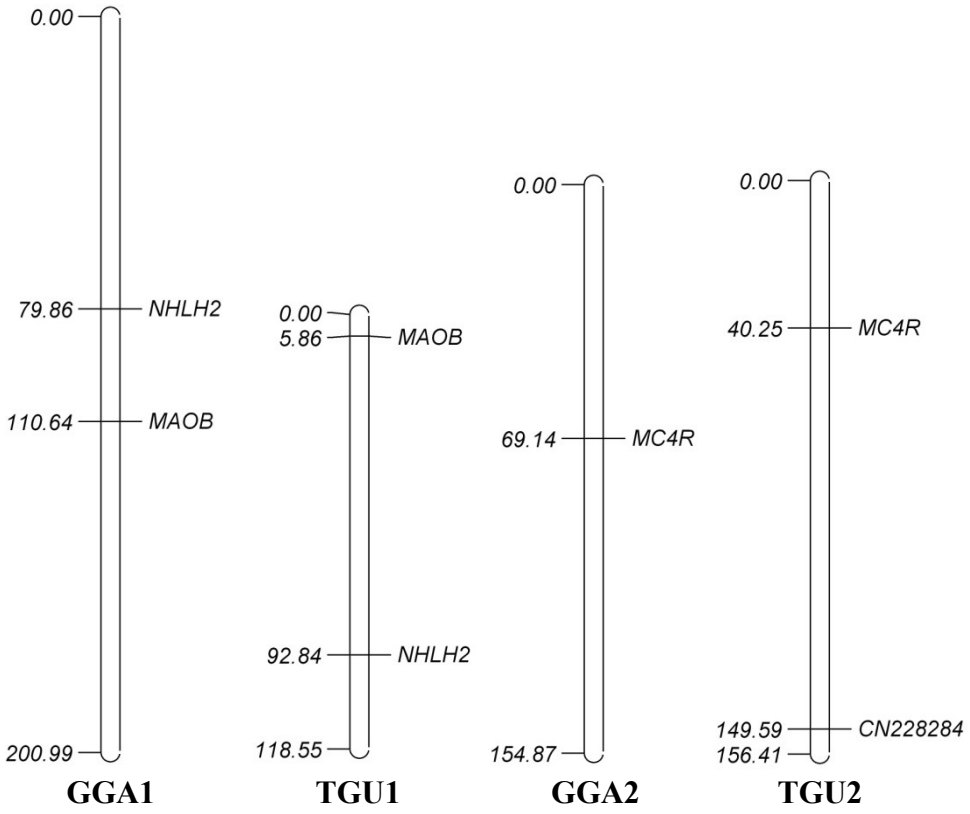

Figure 23: The second-generation chicken-white-throated sparrow and zebra finch-white-throated sparrow comparative cytogenetic maps of chromosomes 1-4 based on sparrow BAC assignments. Chicken chromosomes are designated as GGA and zebra finch chromosomes are designated as TGU. Locations are listed in megabases.

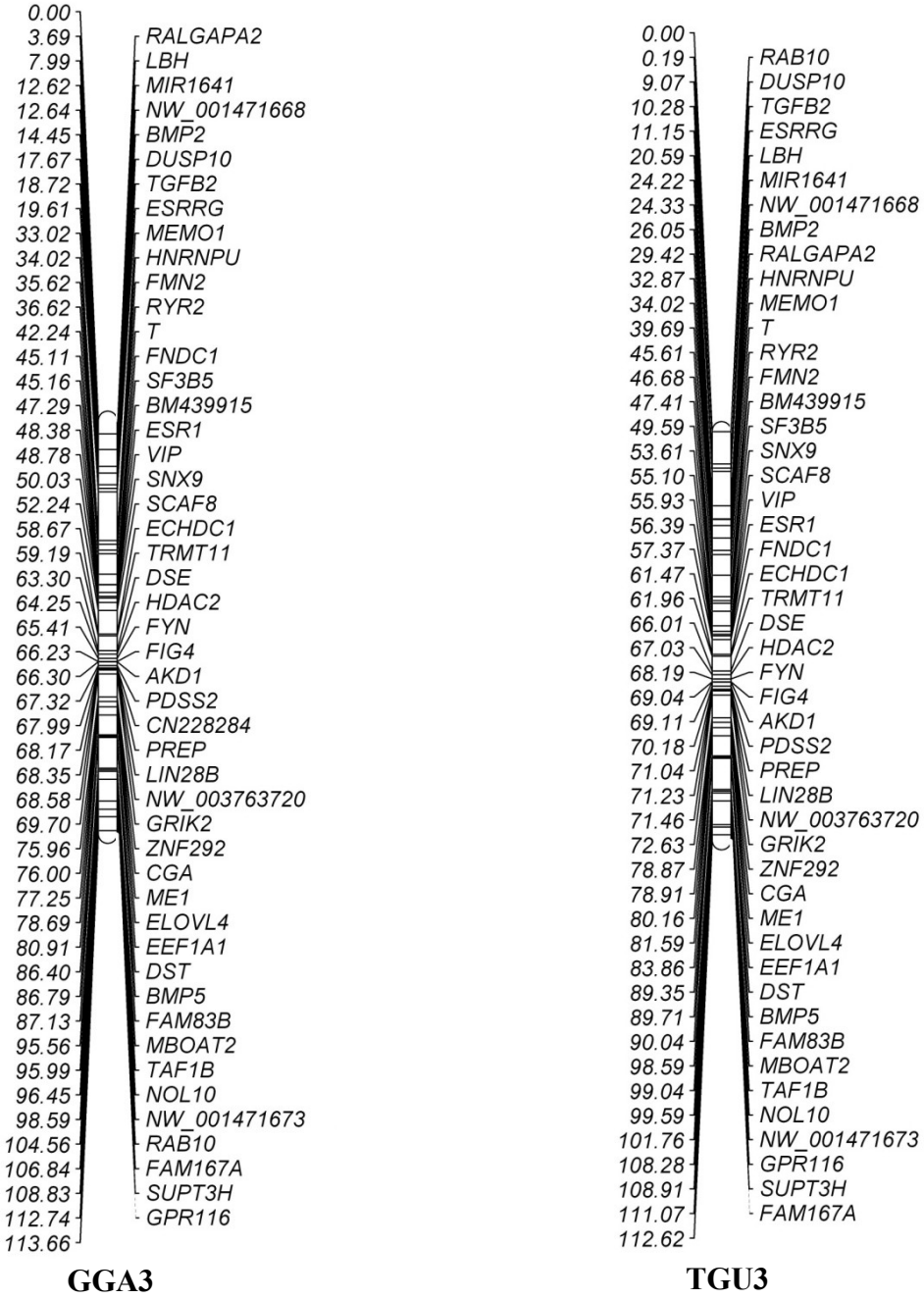

Figure 24: The second-generation chicken-white-throated sparrow and zebra finch-white-throated sparrow comparative cytogenetic maps of chromosome 3 based on sparrow BAC assignments. Chicken chromosomes are designated as GGA and zebra finch chromosomes are designated as TGU. Locations are listed in megabases.

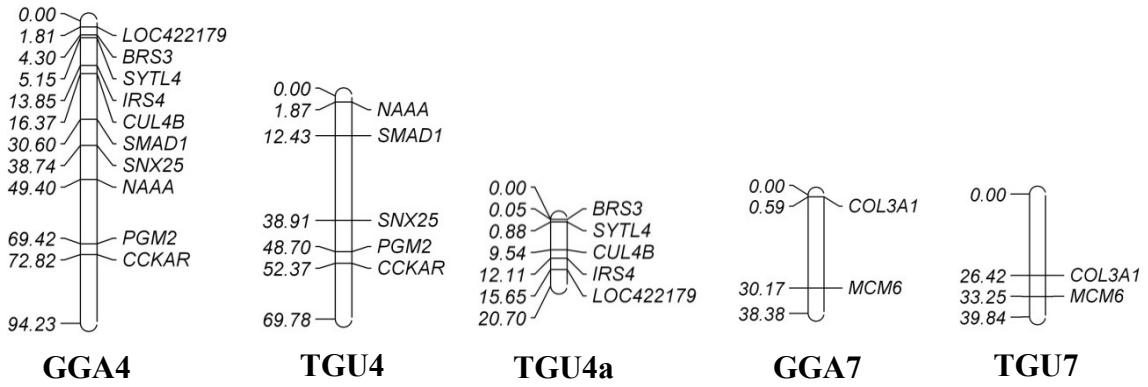

Figure 25: The second-generation chicken-white-throated sparrow and zebra finch-white-throated sparrow comparative cytogenetic maps of chromosomes 4 and 7 based on sparrow BAC assignments. Chicken chromosomes are designated as GGA and zebra finch chromosomes are designated as TGU. Locations are listed in megabases.

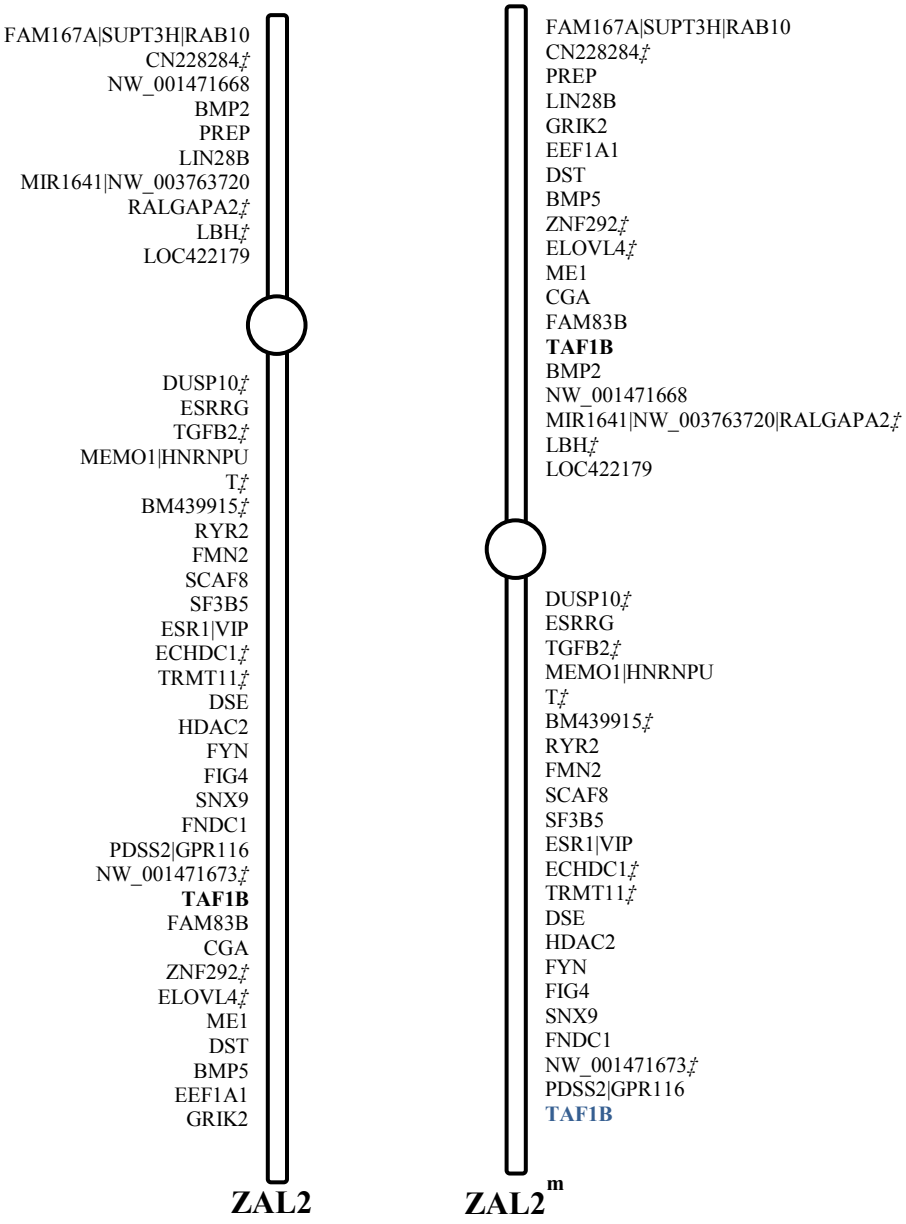

Figure 26: Mapped locations of the 64 BACs used in this study. TAF1B is the breakpoint clone and occurs in two locations on ZAL2^{m}. Genes marked with ‡ indicate zebra finch clones that were used for comparison to previously published mapping [16]. The blue highlighted portion was eliminated from the rearrangement analysis to avoid an unequal number of genes.

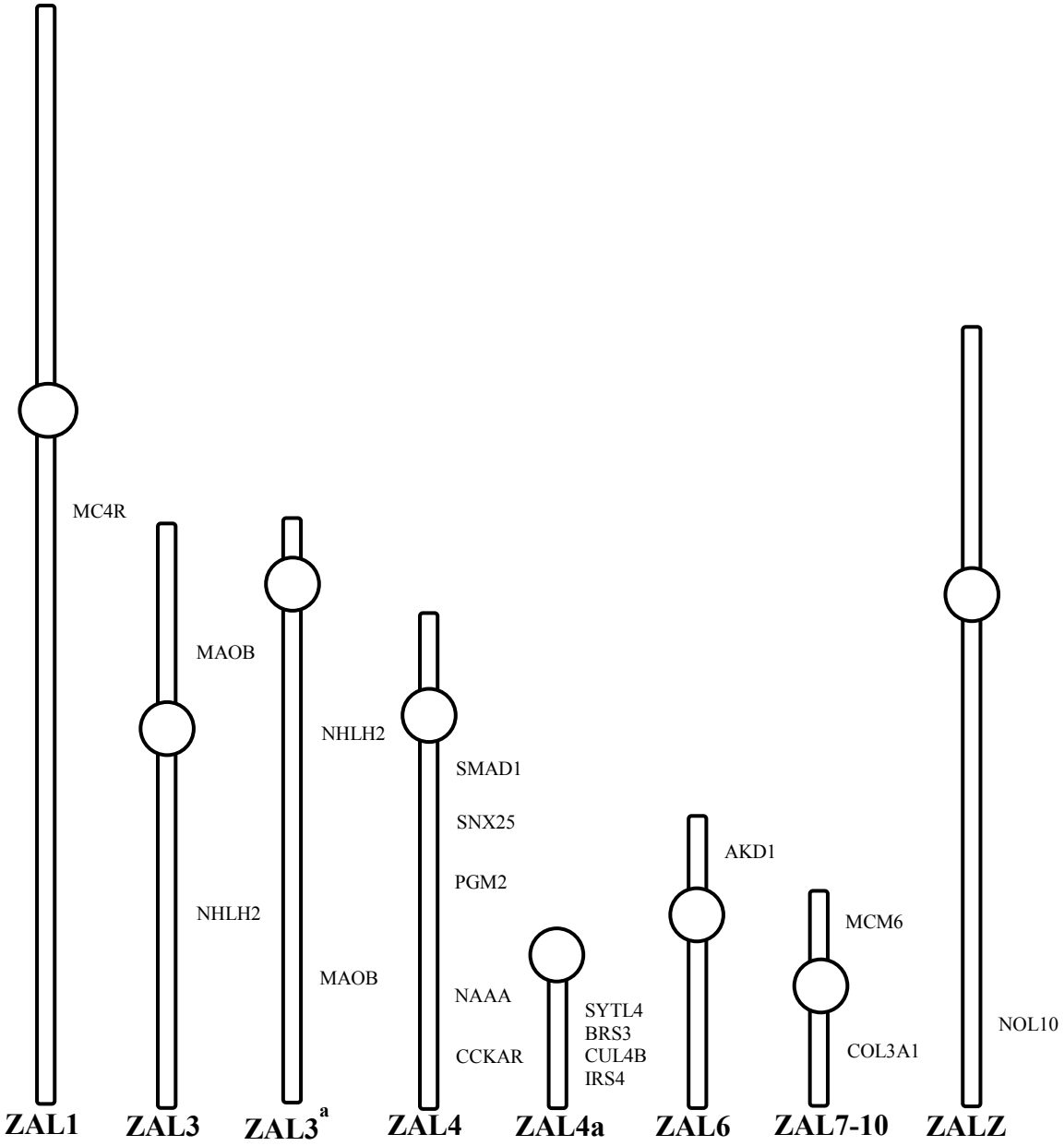

Figure 27: Additional map locations of clones used in this study that did not localize within the chromosomal rearrangement on ZAL2.

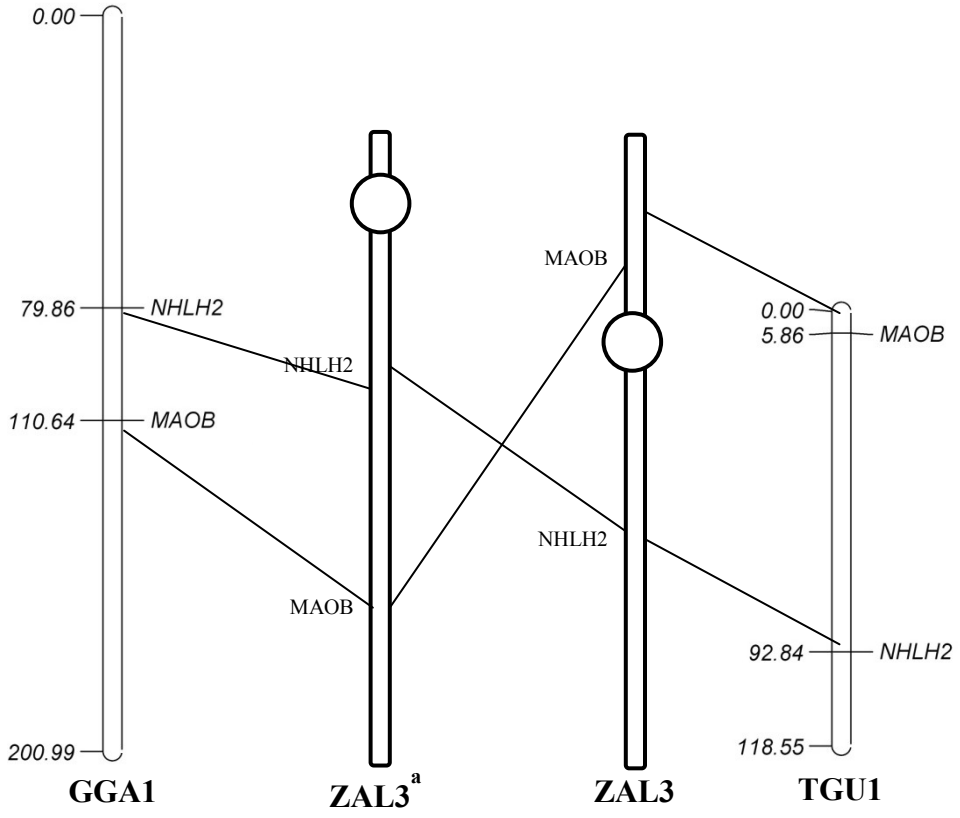

Figure 28: Representation of the changes in gene order and chromosome arrangement between chicken (GGA), zebra finch (TGU) and white-throated sparrow (ZAL).

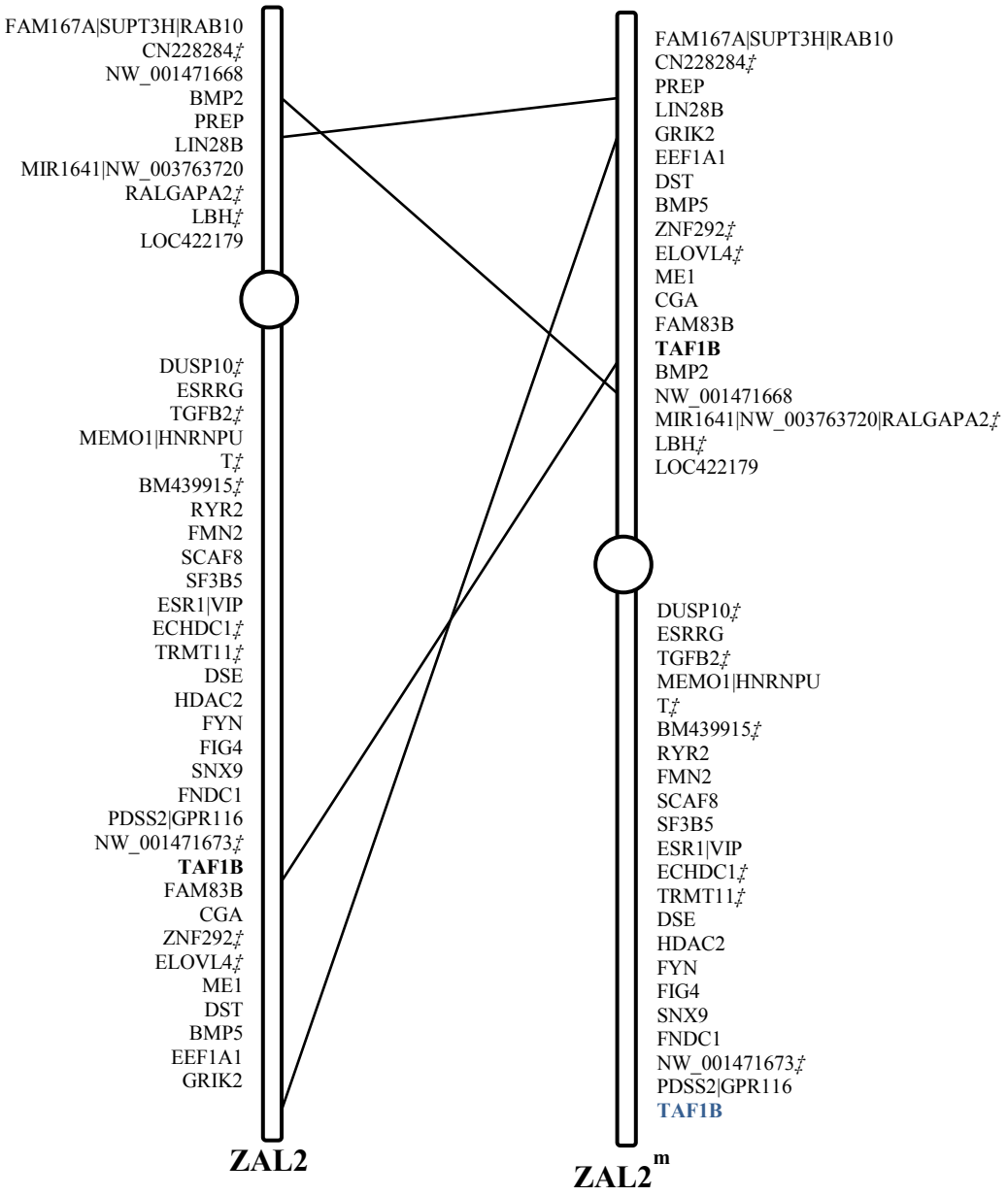

Figure 29: Diagram showing a simplified rearrangement differences between ZAL2 and ZAL2^{m}. TAF1B represents one of the breakpoints in this rearrangement. Zebra finch anchor loci from a previous study are indicated by ‡ [16].

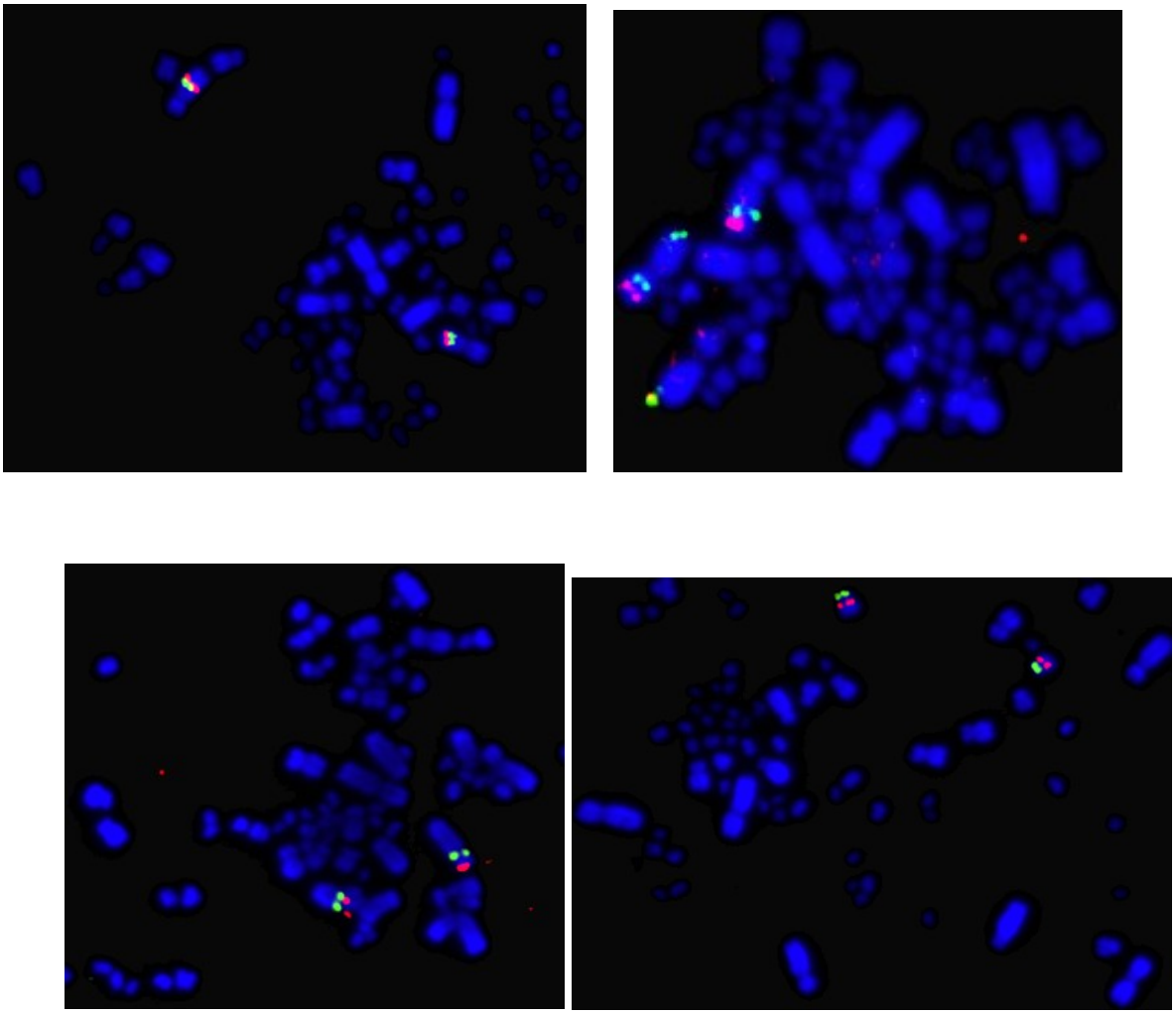

Figure 30: Images of FISH mapping in white-throated sparrows. The top left image is HDAC2 (red) and SF3B5 (green). The top right image shows the breakpoint clone CH264-305i10/TAF1B (green) and GRIK2 (red). The breakpoint is indicated by the presence of two hybridization points on ZAL2^{m}. Bottom left image is BMP2 (red) and ESSRG (green). The bottom right hand picture shows genes MCM6 (green) and COL3A1 (red) that map to ZAL7-10.

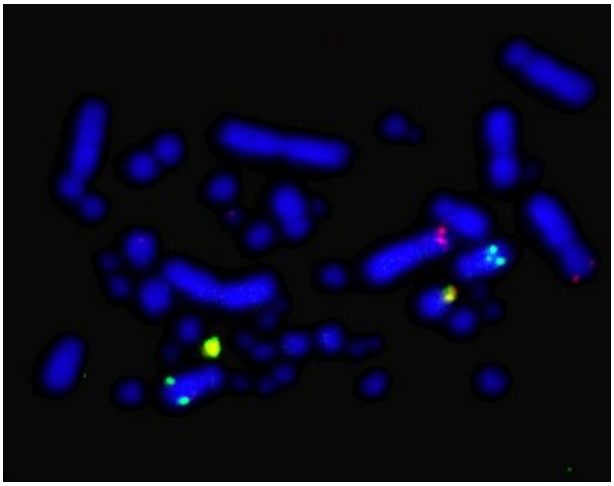

Figure 31: White-throated sparrow BAC clones mapped to zebra finch metaphase chromosomes to confirm locations. Gene CCKAR (green) maps to TGU4 and LOC422179 (red) maps to TGU3. LOC422179 was predicted to be on TGU4a and so represents either an error in the current sequence or misidentification of the clone. It is most likely a translocation within passerines since this clone also maps to ZAL2.

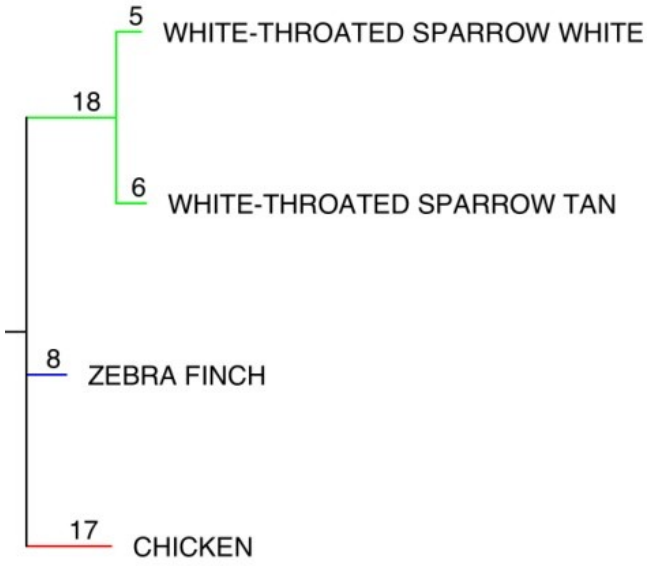

Figure 32: Preliminary rearrangement reconstruction between chicken, zebra finch, and each morph of the white-throated sparrow. The numbers represent the number of reversals between each lineage.

Table 3: Unsigned data input for the GRIMM program [49] used to calculate the orientation/strand location of the genes in zebra finch and white-throated sparrow. Genes were numbered according to location in chicken and used to estimate the gene orientation in the other lineages.

Chicken	
GGA3	-1 -2 -3 -4 -5 6 -7 8 9 10 -11 -12 13 -14 -15 16 17 18 19 20 21 -22-23 24 25 -26 27 28 -29 30 -31 32 -33 -34 35 36 37 38 39 40 -41 42 -4344 45 -46 -47 -48 $
GGA4	49 50 51 52 53 -54 -55 -56 57 58$
Zebra Finch	
TGU2	29 $
TGU3	6 8 3 4 5 10 9 13 12 11 16 15 19 20 18 17 14 21 22 23 24 25 26 27 28 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 48 47 46 $
TGU4	55 53 54 56 57 $
TGU4a	51 50 52 49 $
White-throated sparrow Tan	
ZAL2	46 47 45 29 4 5 30 31 3 32 1 2 49 6 8 7 9 10 13 16 12 11 20 15 17 18 21 22 23 24 25 26 19 14 28 48 44 42 41 35 34 37 36 39 40 38 33 $
ZAL4	51 50 52 $
ZAL4a	54 53 56 55 57 $
ZAL6	43 $
ZALz	27 $
White-throated sparrow White	
$ZAL2^{m}$	46 47 45 29 30 31 33 38 39 40 34 37 36 35 41 42 5 4 3 32 1 2 49 6 8 7 9 10 13 16 12 11 20 15 17 18 21 22 23 24 25 26 19 14 44 28 48 $
ZAL4	51 50 52 $
ZAL4a	54 53 56 55 57 $
ZAL6	43 $
ZALz	27 $

Table 4: Signed data input obtained from the GRIMM program and then used for phylogenetic rearrangement analysis using the MGR program [49].

Chicken	
GGA3	-1 -2 -3 -4 -5 6 -7 8 9 10 -11 -12 13 -14 -15 16 17 18 19 20 21 -22-23 24 25 -26 27 28 -29 30 -31 32 -33 -34 35 36 37 38 39 40 -41 42 -43 44 45 -46 -47 -48 $
GGA4	49 50 51 52 53 -54 -55 -56 57 $
Zebra Finch	
TGU2	29 $
TGU3	45 6 -7 8 -2 -3 -4 5 -1 -10 -9 -13 12 11 -16 -15 19 20 -18 -17 -14 21-22 -23 24 25 -26 27 28 30 -31 -32 -33 -34 35 36 37 38 39 40 -41 42 -43 44 48 47 -46 $
TGU4	56 54 55 57 58 $
TGU4a	50 51 -53 -52 -49 $
White-throated Sparrow Tan	
ZAL2	46 -47 45 -29 -4 5 30 -31 3 32 -1 -2 49 6 -8 7 9 10 -13 16 12 11 -20 -15 17 18 21 -22 -23 24 25 -26 -19 -14 -28 48 -44 -42 41 -35 34 -37 -36 39 40 -38 33 $
ZAL4	-54 -53 56 55 57 $
ZAL4a	51 50 -52 58 $
ZAL6	27 $
ZALz	43 $
White-throated Sparrow White	
$ZAL2^{m}$	46 -47 45 -29 30 -31 -33 38 39 40 34 -37 -36 -35 -41 42 -5 4 3 32 -1 -2 49 6 -8 7 9 10 -13 16 12 11 -20 -15 17 18 21 -22 -23 24 25 -26 -19 -14 44 -28 48 $
ZAL4	-54 -53 56 55 57 $
ZAL4a	51 50 -52 58 $
ZAL6	27 $
ZALz	43 $

Table 5: Predicted gene order of the ancestors for the phylogenetic tree calculated by the MRG program. Ancestor 5 is the predicted ancestor for the node between the two rearrangements of white-throated sparrow chromosomes. Ancestor 6 is the predicted ancestor at the branch between chicken, zebra finch and white-throated sparrow.

Ancestor 5	
	46 -47 45 -29 30 -31 -5 4 3 32 -1 -2 49 6 -8 7 9 10 -13 16 12 11 -20 -15 17 18 21 -22 -23 24 25 -26 -19 -14 -28 48 -44 -42 41 35 36 37 -34 -40 -39 -38 33 $
	43 $
	27 $
	51 50 -52 58 $
	-54 -53 56 55 57 $
Ancestor 6	
	-1 -2 -3 -4 5 46 -47 -48 $
	-28 -27 26 -25 -24 23 22 -21 -18 -17 -14 -15 19 20 16 -11 -12 13 -10 -9 -8 7 -6 -45 -44 43 -42 41 -40 -39 -38 -37 -36 -35 34 33 32 31 -30 29 $
	56 -51 -50 55 57 58 $
	-54 -53 -52 -49 $

CONCLUDING REMARKS

I have examined several levels of selection acting in the white-throated sparrow. In my first chapter I have reviewed how climate impacts bird communities and the measures that are useful for ecological studies. Work by others on this project has shown that climate differentially affects the morphs of the white-throated sparrow. Changing climate can impact selection at all levels of this species. My work has shown that selection is acting within this species at multiple levels.

Sexual selection is acting within both morphs of this species. White males pursue extra-pair paternity, however, unexpectedly, tan males also achieve some (27.5% vs 4.6% extra-pair young). Also unexpectedly, sexual selection appears to be stronger in the monogamous, duller tan male. This may possibly be due to differences in mate quality. The tan males are the preferred male morph by both female morphs, these males may then have preferential choice of higher quality females, increasing their reproductive success.

Variation in morph ratio may also be increasing the strength of sexual selection. Morph ratio varies in a frequency-dependent manner. There also appears to be trade-offs between sex and morph. Pairs may be facultatively adjusting offspring production to maximize reproductive success.

A mechanism that may be acting within this species is segregation distortion. Segregation distortion occurs within white males of this species, and appears to be correlated with differences in social environment. White males are able to bias sperm production for white