2016-04-05

Evolutionary Consequences of Recent Secondary Contact Between Myzomela Honeyeaters

Jason Michael Sardell
University of Miami, jsardell@bio.miami.edu

UNIVERSITY OF MIAMI

EVOLUTIONARY CONSEQUENCES OF RECENT SECONDARY CONTACT BETWEEN *MYZOMELA* HONEYEATERS

By

Jason Michael Sardell

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Coral Gables, Florida

May 2016

UNIVERSITY OF MIAMI

A dissertation submitted in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy

EVOLUTIONARY CONSEQUENCES OF RECENT SECONDARY CONTACT BETWEEN *MYZOMELA* HONEYEATERS

Jason Michael Sardell

Approved:

_____ J. Albert C. Uy, Ph.D. Associate Professor of Biology	_____ William Searcy, Ph.D. Professor of Biology
_____ J. David Van Dyken, Ph.D. Assistant Professor of Biology	_____ Guillermo Prado, Ph.D. Dean of the Graduate School

_____
Robb T. Brumfield, Ph.D.
Professor of Biology
Louisiana State University

SARDELL, JASON MICHAEL (Ph.D., Biology)
Evolutionary Consequences of Recent Secondary Contact Between *Myzomela* Honeyeaters (May 2016)

Abstract of a dissertation at the University of Miami.

Dissertation supervised by Associate Professor J. Albert C. Uy.
No. of pages in text. (148)

Secondary contact, the reestablishment of geographic overlap between ranges of closely-related populations that were previously isolated, has important consequences for the evolution of phenotypic biodiversity, as well as for speciation. Yet, opportunities to study evolutionary processes in systems where secondary contact is historically recent are rare, leaving much unknown about this important stage of the speciation process. For my dissertation, I used an integrative approach to conduct the first study of the evolutionary consequences of secondary contact in one such system: the two species of nectivorous *Myzomela* honeyeaters, *M. cardinalis* and *M. tristrami*, which achieved secondary contact on the island of Makira in the Solomon Islands around the turn of the 20th-century. I determined that hybridization between *Myzomela* is ongoing on Makira, in contrast to previous characterizations of this system. Mitochondrial and nuclear DNA sequencing revealed that hybridization is highly asymmetric between these species, with this asymmetric reproductive isolation likely driven by strong cytonuclear incompatibilities. I further determined that hybridization following secondary contact has led to asymmetric introgression, including introgression of melanic plumage-related alleles from *M. tristrami* to *M. cardinalis*. Behavioral experiments to test whether difference in plumage color mediates interspecific interactions in sympatry revealed a potential adaptive role for this introgression. Sympatric species of both populations exhibited biased aggression

toward red *M. cardinalis* mounts, suggesting that melanic plumage may be favored in smaller individuals with hybrid ancestry because it allows them to avoid harmful aggressive interactions. This biased aggression toward *M. cardinalis* was only exhibited by sympatric populations, indicating that it is a consequence of secondary contact. Similarly, secondary contact resulted in rapid asymmetric character displacement in body size between species, likely as a consequence of interference competition. Finally, I leveraged hybridization in this system to demonstrate that a previously-unknown neo-sex chromosome is strongly associated with speciation and plumage divergence in this system. This result provides important evidence in favor of long-standing, but poorly supported theories that genomic sex-linkage is important for the evolution of secondary sexual traits and speciation. Together, these findings provide novel and important insights into the processes by which phenotypic divergence evolves and is maintained in natural populations following secondary contact.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my advisor, Al Uy, for guidance and academic, logistic, and financial support, without which this dissertation would not have been possible. I also wish to thank the remainder of my committee: William Searcy, David Van Dyken, and Robb Brumfield, as well as former members of my committee, Kevin McCracken, William Browne, and Carla Hurt for guidance, advice, and support.

Thank you to Elizabeth Cooper, my coauthor for the work presented in Chapter 5, for critical bioinformatics and laboratory training and support. Additional thanks to Jaime Chaves for assisting with the BEAST analysis presented in Chapter 2, Dan Baldassarre for helpful advice when interpreting my data and drafting manuscripts, and Daven Presgraves for population genetics advice as well as feedback on the manuscript presented in chapter 5. Thanks as well to additional members of the Uy-Searcy labs who provided constructive criticism during this project: Winter Beckles, Sarah Cowles, Jose Hidalgo, Floria Mora-Kepfer Uy, Doug Weidemann, Adrienne Dubois, Gavin Leighton, Karla Rivera-Caceres, Luis Vargas, and Natalia Gutierrez-Pinto.

I wish to thank the Department of Biology at the University of Miami, and in particular, Dr. Athula Wikramanayake and Dr. Alex Wilson, for providing funds for me to attend the 2016 Workshop in Population and Speciation Genomics in Cesky Krumlov. Additional funding for this project was provided by a College of Arts and Sciences Academic Year Dissertation Award, University of Miami College of Arts and Sciences Fellowship, a Society of Systematic Biologists Graduate Student Research Award, the Kushlan Graduate Research Support Fund, the Jay M. Savage Graduate Research Support Fund, the Max and Peggy Kriloff Graduate Student Travel Scholarship, an American

Ornithologists' Union Student Travel Award and a GAFAC Student Travel Award. In addition, thank you to the Center for Computational Science at the University of Miami for computational support and access to the Pegasus cluster.

Sincere thanks to the many people of the Solomon Islands who welcomed me into their communities, and without whom this research would not have been possible. In particular, I would like to thank John and Joyce Murray for welcoming me into their family and providing critically important logistical support. Thank you as well to Jerry Tauni of Kirakira, John Waihuru of Hauta, Kal and Lena Rupen of Pawa Secondary School, Henry Ha'aina of Molehanua, Father Philip Murray of Lata, and the communities of Na Finua, Nasuragina, Fagokoro, Nagasigao, Geta, Namuga, Santa Catalina, Towarodo, Malaupaina, Malaulalo, Maraone, Rama, Naharahau, and Finuamaga for warm hospitality and permission to work on their lands. Many thanks to Vivian Villanueva and Ben for help with acquiring proper visas and permits in Honiara. Thank you to Solomon Islands Ministries of Environment and Education and the Provincial Governments of Makira and Temotu for permission to reside and conduct research in the country. Last, but not least, I want to thank all of my local field assistants, especially Johnson Pepare , Lonsdale Taka, George Wabeasi, Phillip Teo, James Suafuria, and Joseph Waihuru,. Their expertise, intelligence, creativity, and hard work were instrumental for obtaining the data and samples upon which this research was based.

Finally, thank you to my wife Dr. Rebecca Sardell for field assistance, statistical advice, and most of all for the love and support she provided over the past five years.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Chapter 4

Chapter 5

LIST OF TABLES

Chapter 4

Chapter 5

Chapter 1

Introduction

Explaining the processes that govern the origin of phenotypic and genotypic diversity is one of the principal aims of evolutionary biology (Darwin 1859; Coyne and Orr 2004; Price 2008). Although recent attention has been given to the potential for divergence to evolve concurrently with gene flow (Nosil 2008; Michel et al. 2010; Papadopulos et al. 2011), it is generally accepted that periods of geographic isolation between populations play a prominent role in the evolution of biodiversity (Coyne and Price 2000; Coyne and Orr 2004). Specifically, a lack of gene flow allows isolated populations to proceed down different evolutionary trajectories due to selection and/or genetic drift (Dobzhansky 1937; Mayr 1942). This process can lead to the evolution of new species (i.e., "speciation") if populations diverge to the point where they are reproductively isolated. Such reproductive isolation can result either from post-zygotic genetic incompatibilities (e.g., hybrid inviability or infertility) or from pre-zygotic behavioral incompatibilities (e.g., failure to recognized individuals of the other species as potential mates) (Coyne and Orr 2004; Price 2008). The "moment of truth" in determining whether or not two isolated populations have speciated occurs when one or both of their ranges expand resulting in overlapping range distributions (Mayr 1942; Mayr and Diamond 2001). This event, called secondary contact, can have critically important consequences for the continued maintenance of phenotypic and genotypic divergence (Mayr 1942; Brown and Wilson 1956; Harrison 1993; Noor 1999).

If reproductive isolation between allopatric populations is incomplete, then secondary contact between two taxa will result in hybridization, a commonly-observed

phenomenon (Harrison 1993; Mallet 2005; Abbott et al. 2013). Traditionally, gene flow has been perceived as opposing divergence as recombination can result in a genetically admixed population and a breakdown of reproductive barriers (Dobzhansky 1937; Mayr 1942; Rhymer and Simberloff 1996; Taylor et al. 2006). However, phenotypic and genotypic diversity can sometimes be maintained despite hybridization, particularly in the face of strong selection against hybrids in general or certain novel combinations of alleles (Mallet 2007; Nosil 2012). In these- instances, secondary contact and hybridization can promote the evolution of further reproductive isolation via reinforcement (Dobzhansky 1940; Ortiz-Barrientos et al. 2009). Hybridization may also lead to adaptive introgression of certain traits and alleles, which can increase the fitness of the invading taxa via introgression of locally-adapted alleles from the native species, or increase the fitness of the native species by representing a novel source of standing genetic variation (Anderson and Stebbins Jr 1954; Arnold 2004; Seehausen 2004; Rieseberg et al. 2007; Heliconius Genome Consortium 2012; Hedrick 2013). Thus, consequences of hybridization are expected to vary across the genome: neutral alleles will admix freely, while selection will act to oppose introgression of alleles associated with speciation while potentially favoring adaptive introgression of other gene regions (Mallet 2005; Baack and Rieseberg 2007; Butlin 2010; Feder et al. 2012).

Secondary contact can also promote increased phenotypic divergence between species (i.e., “character displacement”). (Brown and Wilson 1956; Grant 1972; Pfennig and Pfennig 2010) This divergence occurs even though adaptation to the local environment and hybridization will both generally promote phenotypic convergence (Grant et al. 2004; Seddon and Tobias 2010; Heliconius Genome Consortium 2012; Stern

2013). Under ecological character displacement, species diverge in phenotype to exploit different resources, as theory predicts that species cannot co-exist if they fill identical ecological niches without one going extinct (Gause 1934; Hardin 1960; Schluter 2001; Dayan and Simberloff 2005). In general, ecological character displacement is commonly framed in the context of niche partitioning, i.e., selection for individuals that are able to better exploit unoccupied ecological niches (Lack 1947; Grant 1972; Fjeldså 1983; Schluter et al. 1985; Dayan et al. 1989; Schluter and McPhail 1992; Dayan and Simberloff 2005). However, ecological character displacement can also result if one species is able to actively exclude the other from high-quality resources through aggressive interactions ("interference competition"), in which case selection will favor the evolution of reduced resource requirements (e.g., smaller body size) in the subordinate species (Whitehead and Walde 1993; Sidorovich et al. 1999; Jaeger et al. 2002; McDonald 2002; Melville 2002; Grether et al. 2013). Character displacement can also be driven by reproductive interactions (Marshall and Cooley 2000; Höbel and Gerhardt 2003; Servedio and Noor 2003; Pfennig and Pfennig 2010). Such reproductive character displacement often , but not always, arises via reinforcement where there is strong selection against hybridization, e.g., due to hybrid inviability or infertility (Dobzhansky 1937; Servedio and Noor 2003). Thus, selection can favor the evolution of phenotypic divergence among traits that are important for mate recognition. Although character displacement has long been recognized as a potential consequence of secondary contact, its general importance for the evolution of phenotypic divergence remains controversial (Grant 1972; Schluter 2001; Dayan and Simberloff 2005; Stuart and Losos 2013).

Because natural secondary contact events are infrequently observed, few studies have examined the evolutionary consequences of secondary contact in systems where such contact occurred historically recently. Systems with human-assisted species introductions have been used to study this critical stage of the speciation process (Rhymer and Simberloff 1996; Mooney and Cleland 2001; Fitzpatrick et al. 2010), but dynamics of these introductions may differ fundamentally from natural colonization events (Strauss et al. 2006), and therefore may provide limited insight into the processes by which species diversity accumulated over evolutionary history. Island species, in particular, have played a prominent role in our understanding of evolutionary processes (Lack 1947; MacArthur and Wilson 1967; Losos and Ricklefs 2009b, a), but only a handful of examples of natural secondary contact have been directly observed between island taxa. For example, in Darwin's finches, secondary contact has resulted in adaptive introgression and ecological character displacement (Schluter et al. 1985; Grant and Grant 2002; Grant and Grant 2009). Sympatric *Ficedula* flycatchers on the Swedish islands of Gotland and Öland exhibit strong post-zygotic reproductive isolation, but character displacement in plumage evolved over 150 and 50 years, respectively (Qvarnström et al. 2010), either due to reinforcement (Svedin et al. 2008) or interference competition (Vallin et al. 2012). In New Zealand stilts, secondary contact has resulted in strong interspecific competition which appears to be driving the native species to extinction, but hybridization has led to extensive introgression from the native to the colonizing species (Steeves et al. 2010). Finally, two species of *Myzomela* honeyeaters on Long Island off New Guinea are believed to have experienced character displacement in size since they both colonized the island following a volcanic eruption in the mid-17th

century (Diamond et al. 1989). Each of these studies suggests that secondary contact can drive rapid evolution on islands, but that its consequences can vary significantly depending on degree of reproductive isolation and ecological competition. Therefore, additional studies of systems where related-species have undergone recent secondary contact will provide important insight into the potential outcomes of this critical event.

Studies of systems where secondary contact resulted in hybridization are also important for the study of evolution because genetic admixture provides unique opportunities to identify genomic regions associated with speciation as well as population divergence in specific traits (Rieseberg and Buerkle 2002; Smith and O'Brien 2005; Lexer et al. 2007; Buerkle and Lexer 2008). Specifically, selection is predicted to oppose introgression of genomic regions that are associated with reproductive isolation, resulting in "peaks" of high genomic differentiation (e.g., as measured by F_{ST}), which will be centered on speciation genes (Turner et al. 2005; Nosil et al. 2009; Nadeau et al. 2012; Nosil and Feder 2012; Renaut et al. 2012; Renaut et al. 2013; but see Cruickshank and Hahn 2014; Burri et al. 2015). Similarly, admixture will break down linkage between alleles associated with specific phenotypes and other loci that diverged between populations due to genetic drift or selection for unrelated traits (Buerkle and Lexer 2008). Therefore, secondary contact can indirectly allow us to test several theoretical predictions regarding the genomics of speciation and divergence. For example, chromosomal rearrangements have been predicted to play an important role in the evolution of phenotypic divergence through the reduction in recombination (Noor et al. 2001; Rieseberg 2001; Kirkpatrick and Barton 2006; Hoffmann and Rieseberg 2008). Indeed, chromosomal inversions have been implicated in speciation in *Drosophila* fruit flies

(Brown et al. 2004), *Anopheles* mosquitoes (Coluzzi et al. 1985) and *Mimulus* monkeyflowers (Lowry and Willis 2010), as well as the formation of "supergenes" associated with the maintenance of distinct plumage phenotypes in Ruffs (Küpper et al. 2016; Lamichhaney et al. 2016) and White-throated Sparrows (Tuttle et al. 2016). Theory also predicts that fusions of autosomes with ancestral chromosomes (i.e., "neo-sex chromsomes") will favor an accumulation of sexually antagonistic traits, such as those important for mate choice (Fisher 1931; Rice 1984, 1987; Kirkpatrick et al. 2004; Van Doorn and Kirkpatrick 2007). However, evidence for a linkage between neo-sex chromosomes or sex chromosome turnover and phenotypic divergence or speciation is currently limited to sticklebacks (Kitano et al. 2009), cichlids (Ser et al. 2010), and tortricid moths (Nguyen et al. 2013). Accordingly, studies of hybridizing taxa can provide important insights into the importance of chromosomal rearrangements in promoting speciation and population divergence, as well as the potential for sex chromosomes to accumulate sexually-antagonistic alleles.

For my dissertation, I studied the evolutionary consequences of secondary contact between two species of nectivorous birds, the Cardinal Honeyeater (*Myzomela cardinalis*) and the Sooty Honeyeater (*Myzomela tristrami*), which achieved secondary contact on Makira in the Solomon Islands around the turn of the 20th century. Mayr and Diamond (2001) first identified this system's excellent potential to provide important insight into this important but poorly understood stage of the speciation process. However, no genetic or behavioral ecology data had previously been collected on the Makira prior to my research. Below, I first describe this system in further detail, and then provide a brief outline of the contents of my four data chapters.

Study System

Myzomela honeyeaters

The genus *Myzomela* is the most species-rich genus of Meliphagidae honeyeaters, with 31 currently-recognized species and several more phenotypically-distinct subspecies distributed throughout Wallacea, New Guinea, Australia, and the Pacific Islands (Rice 1984). *Myzomela* are small, primarily-nectivorous birds that fill an ecological niche similar to that of hummingbirds in the Americas and sunbirds in Africa and tropical Asia (Higgins et al. 2008). In many species within this genus, plumage comprises patches of carotenoid-based red and/or yellow pigmentation alternating with black melanin or white absence of pigments (Koopman 1957). Although the full phylogeny of this genus has not been published, preliminary data suggest that several plumage traits evolved independently multiple times in *Myzomela* (B. Benz, pers. comm.). Furthermore, many species exhibit strong sexual dichromatism, a pattern that is commonly assumed to be indicative of evolution via sexual selection (Barraclough et al. 1995; Kraaijeveld et al. 2011; Seddon et al. 2013). In addition, the dietary-based carotenoid pigments responsible for red plumage have been shown to be an honest signal of mate quality in many birds (Hill 1991; Hill and Montgomerie 1994; Hill 2006). Such an association between signal and mate quality is an important requisite of so-called "good-genes" models of trait evolution via sexual selection (Zahavi 1975; Hamilton and Zuk 1982). Because most closely-related *Myzomela* species are allopatric, it is unclear whether the phenotypic divergence in plumage patterns between species is associated with reproductive isolation in this genus. Relationships between male plumage coloration and female mate choice have been found in some avian species (Baker and Baker 1990; Sætre et al. 1997; Uy et

al. 2009a) but not in others (Moore 1987; Brelsford and Irwin 2009; Baldassarre and Webster 2013).

Makira *Myzomela*

The two species of Myzomela present on the island of Makira, the melanic Sooty Myzomela (*M. tristrami*) and a subspecies of the red-and-black Cardinal Myzomela (*M. cardinalis pulcherrima*, represent an interesting exception to the allopatric distribution of most other species. *M. tristrami* is endemic to Makira and the small satellite islands of Santa Ana and Santa Catalina 7.5 km east of the main island; it is found in all habitats ranging from sea level to the tops of the interior mountain ranges of Makira (900m), although it is historically considered to be more common along the coast and middle altitudes (Dutson 2011). *M. cardinalis pulcherrima* is endemic to lowland Makira (generally within a few hundred meters of the coast, although in 2012 and 2013 I observed individuals along a river bank several kilometers inland), as well as small satellite islands of Ugi and Three Sisters located approximately 8 km and 19 km north of the main island respectively. Additional subspecies of *M. cardinalis* are found on Rennell (approximately 170 km southwest of Makira), the Santa Cruz Islands (approx. 350 km east of Santa Ana), Vanuatu, the Loyalty Islands, and Samoa, with plumage patterns varying between subspecies both in pattern and degree of sexual dichromatism (Higgins et al. 2008). No complete phylogeny of the genus has been published, but preliminary data presented in Chapter 2 suggests that *M. cardinalis* is polyphyletic. The same genetic data supports Mayr and Diamond (2001) hypothesis that *M. c. sanctaecrucis* from Santa Cruz is sister to *M. cardinalis pulcherrima*.

The sympatry of *Myzomela* on Makira is particularly noteworthy as it represents an unusual case of historically recent secondary contact. According to a review of historical collection records (Diamond 2002), *M. tristrami* was the only species of *Myzomela* present on Makira until end of the 19th/beginning of the 20th century. In 1908, A.S. Meek (Rothschild and Hartert 1908) collected no *M. cardinalis* on Makira, but did collect several specimens with intermediate plumage, which Mayr and Diamond (2001) later considered putative hybrids. The Whitney Expedition of 1927 found that *M. cardinalis* had become established on Makira, although *M. tristrami* was still the dominant species in sympatry (Mayr 1945). Twenty-five years later, Cain and Galbraith (1956) observed the opposite pattern, with *M. cardinalis* outnumbering *M. tristrami* in sympatry. Although I observed that *M. cardinalis* was common in Star Harbour in the eastern part of Makira, the species was not observed in this region by the Whitney South Seas Expedition or J. Diamond in the 1970s (Diamond 2002), indicating that *M. cardinalis* has undergone a recent range expansion along the coast of Makira.

Mayr and Diamond (2001) noted evidence of hybridization between the Makira *Myzomela,* but characterized the phenomenon as an example of temporary hybridization following range expansion. Models of mate choice predict that mate choice behavior will vary depending on the pool of potential mates; if conspecifics are rare, then the fitness costs of mating with a heterospecific can be less than the fitness costs of delayed or foregone reproduction associated with searching for conspecific mates, favoring hybridization (Wilson and Hedrick 1982; Real 1990; Wirtz 1999). Diamond (2002) noted that frequency of hybridization between *Myzomela* on Makira appears to have declined since initial secondary contact. Specifically, A.S. Meek collected several putative hybrids

in 1908 prior to the establishment of phenotypically pure *M. cardinalis*, but the Whitney Expedition collected only one putative hybrid in 1927 (Diamond 2002). Furthermore, this latter hybrid was from Santa Anna on the eastern tip of Makira where *M. cardinalis* is currently not established (Mayr 1932; Diamond 2002) . This pattern is consistent with hybridization only occurring when *M. cardinalis* is rare, as during the early stage of colonization or along the forefront of its range expansion, but this hypothesis had not been tested prior to my dissertation (see Chapter 2)

M. cardinalis and *M. tristrami* fill very similar ecological niches in sympatry, and are often found in mixed flocks feeding on flowers in the same tree (Diamond 2002). Furthermore, Three Sisters, Santa Ana, and Santa Catalina are small coral islands with similar vegetation, suggesting that both species have the capacity to thrive in identical habitats. Although *M. cardinalis* has been described as being obligately associated with coconut palms on Makira (Dutson 2001), this claim was not supported by my field observations; I often observed *M. cardinalis* feeding on coconut flowers, but they commonly feed in other flowering trees and nest exclusively in non-palms. The high level of competition for limited food resources (i.e., flowering trees) in sympatry, as well as the high level of interspecific aggression observed in other species of honeyeaters (Ford 1979; Beehler 1980; Ford and Paton 1982; Diamond et al. 1989), suggests that interference competition could potentially have had important evolutionary consequences in this system. Accordingly, the Makira *Myzomela* provide a useful system for testing theories related to ecological character displacement, as well as the consequences of secondary contact for interspecific aggression and signal recognition.

Project Outline

In the following chapters, I present four inter-related studies in which I used an integrative approach to examine the evolutionary consequences of secondary contact between the Makira *Myzomela*. In Chapter 2, I tested hypothesis related to the dynamics of hybridization and introgression between species in which reproductive isolation is not complete. I collected DNA samples from allopatric and sympatric *Myzomela* and sequenced each individual at on mitochondrial and six nuclear markers. By comparing differences in introgression patterns between these classes of markers, I tested which species pairings were involved in hybridization and backcross events, and compared those to predictions of different models of introgression based on observations from similar systems. I also describe unusual patterns of asymmetric phenotypic and genetic introgression which have important consequences for the maintenance of phenotypic divergence in this system. Specifically, melanic plumage alleles from *M. tristrami* have introgressed into the genomic background of *M. cardinalis*, resulting in "cryptic" hybrids which are reproductively isolated from, but phenotypically similar to *M. tristrami*.

In Chapter 3, I present the results of experimental tests for whether plumage divergence between *M. cardinalis* and *M. tristrami* plays an important role in inter-species male-male interactions. Using taxidermy mounts, I simulated territorial intrusions and quantified behavioral responses of each species towards conspecifics, heterospecifics, and unrelated ecological competitors with which they do not compete for mating opportunities. In this manner, I tested the following three hypotheses. First, that aggression will be greater between conspecifics than heterospecifics, as the former will compete more strongly for mating opportunities and resources. Second, that male-male

aggression is driven primarily by competition for mating opportunities, resulting in increased aggression towards heterospecifics among species in which females are more likely to hybridize. Finally, that male-male aggression is driven primarily by competition for resources such as food and territories, resulting in increased aggression toward species which are more likely to dominate resource access.

I also compared body sizes of sympatric male *M. cardinalis* to sympatric male *M. tristrami* and hybrid males (including both phenotypic hybrids and cryptic hybrids – birds that possess *M. cardinalis* mitochondrial haplotypes but melanic plumage typical of *M. tristrami*). Differences in body size are highly correlated with dominance in aggressive contexts between honeyeaters (Ford 1979; Beehler 1980; Ford and Paton 1982; Diamond et al. 1989). With this in mind, I investigated whether patterns of introgression of plumage-related alleles that I describe in Chapter 2 could potentially be explained as adaptive introgression driven by selection to reduce aggression from dominant individuals (Greene et al. 2000; Vallin et al. 2012). Finally, I compared behavioral responses towards territorial intrusions in sympatric populations to those in allopatric populations, to test whether any biases in aggression observed in sympatry are an artifact of ancestral sensory bias or if they resulted from secondary contact.

In Chapter 4, I tested whether secondary contact has had important consequences for body size divergence in the Makira *Myzomela*. Hybridization, as well as shared ecology, should both favor phenotypic convergence in sympatry. In contrast, ecological character displacement will favor increased size divergence in sympatry. If character displacement is driven by niche partitioning, then I predicted that sympatric *M. cardinalis*, the larger species, would have increased size relative to the allopatric source

population, while *M. tristrami*, the smaller species, would have decreased size relative to allopatric populations. If character displacement was driven by interference competition for resources, then I predicted that character displacement would be asymmetric, resulting in reduced body size of *M. tristrami*, the subordinate species, particularly among males. Ecological character displacement remains controversial (Grant 1972; Schluter 2001; Dayan and Simberloff 2005; Stuart and Losos 2013), and this study provides some of the strongest evidence to date that it can evolve over relatively short time scales.

In Chapter 5, I leveraged admixture in this system to identify genomic regions associated with speciation and plumage divergence in the Makira *Myzomela*. I used double-digest restriction-site associated DNA sequencing ("RAD-seq") to generate a genome-wide data set of single-nucleotide polymorphisms ("SNPs"), and mapped these SNPs to available avian reference genomes (Warren et al. 2010) to identify loci that exhibited peaks of elevated genomic differentiation as measured by F_{ST}. I identified evidence of a shared chromosomal rearrangement in both *Myzomela* species involving formation of a previously-unknown neo-sex chromosome, only the second known example from birds (Pala et al. 2012). I tested the hypothesis that neo-sex chromosomes can play an important in speciation by quantifying whether high-F_{ST} SNPs disproportionately map to this region. I also tested the theory that neo-sex chromosomes should favor an accumulation of sexually-antagonistic alleles by performing a genome-wide association study (GWAS) to identify alleles that are highly associated with divergence in plumage color. Finally, I identified SNPs that were perfectly associated with plumage phenotype, mapped them to annotated avian genomes to identify candidate

plumage genes, and tested whether they also disproportionately accumulate on the neo-sex or ancestral sex chromosomes. This chapter represents one of the first studies overall, and the first study in birds, to test fundamental theories of the links between sex chromosome evolution, sexual selection, and speciation.

The final chapter (Chapter 6) summarizes the findings and implications of the results of my dissertation research. Overall, I applied field research techniques, behavioral ecology approaches, and cutting-edge genomic tools to test fundamental hypothesis related to the potential genotypic and phenotypic consequences of recent secondary contact.

Chapter 2

Hybridization following recent secondary contact results in asymmetric genotypic and phenotypic introgression between island species of *Myzomela* honeyeaters[1]

Summary

Hybridization and introgression can have important evolutionary consequences for speciation, especially during early stages of secondary contact when reproductive barriers may be weak. Few studies, however, have quantified dynamics of hybridization and introgression in systems in which recent natural dispersal across a geographic barrier resulted in secondary contact. We investigated patterns of hybridization and introgression between two *Myzomela* honeyeaters (*M. tristrami* and *M. cardinalis*) that recently achieved secondary contact on Makira in the Solomon Islands. Hybridization in this system was hypothesized to be a byproduct of conspecific mate scarcity during early stages of colonization. Our research, however, provides evidence of ongoing hybridization more than a century after secondary contact. Mitochondrial sequencing revealed strongly asymmetric reproductive isolation that is most likely driven by post-zygotic incompatibilities rather than pre-zygotic behavioral barriers. Nuclear introgression was observed from the native species (*M. tristrami*) to the colonizing species (*M. cardinalis*). Nuclear introgression in the reverse direction is almost exclusively limited to birds that are phenotypically *M.tristrami* but possess *M. cardinalis* mitochondrial haplotypes, consistent with introgression of plumage-related alleles into the genomic background of *M. cardinalis*. These results provide unique insight into the

[1] This chapter was published as Sardell, J.M. and J.A.C. Uy. 2016. Hybridization following recent secondary contact results in asymmetric genotypic and phenotypic introgression between island species of *Myzomela* honeyeaters. Evolution 70:257-267.

dynamics and consequences of hybridization and introgression during early stages of secondary contact.

Background

Under the predominant mode of speciation, phenotypic divergence between species first evolves in allopatry (Mayr 1942; Coyne and Price 2000; Coyne and Orr 2004). However, unless such divergence drives either complete pre- or post-zygotic reproductive isolation, any secondary contact will be followed by hybridization (Harrison 1993; Coyne and Orr 2004; Price 2008). Such hybridization, which is relatively common in nature (Mallet 2005), has important consequences for speciation (Anderson 1949; Harrison 1993; Dowling and Secor 1997; Seehausen 2004; Abbott et al. 2013). Hybridization can potentially break down reproductive barriers (Rhymer and Simberloff 1996; Taylor et al. 2006), promote the evolution of reproductive barriers via reinforcement (Dobzhansky 1940; Ortiz-Barrientos et al. 2009), increase fitness of the hybridizing taxa (i.e., adaptive introgression) (Anderson and Stebbins Jr 1954; Arnold 2004; Seehausen 2004; Heliconius Genome Consortium 2012; Hedrick 2013), or increase biodiversity via homoploid hybrid speciation (DeMarais et al. 1992; Gompert et al. 2006; Brelsford et al. 2011; Hermansen et al. 2011). In this manuscript, we investigate the patterns and potential evolutionary consequences of hybridization and introgression between two species of island birds (*Myzomela cardinalis* and *Myzomela tristrami*) that have recently achieved secondary contact.

Consequences of hybridization for speciation may be particularly strong during the early stages of secondary contact, before selection against hybridization can drive the

evolution of strong pre-zygotic reproductive barriers, and when conspecific mating opportunities are often relatively rare for one species due to unequal dispersal (Wilson and Hedrick 1982; Wirtz 1999). This secondary contact generally follows one of two geographic patterns. In the first, changes in climate or other ecological constraints weaken geographic barriers and promote species range expansions that result in secondary contact. When reproductive isolation is incomplete, this secondary contact often results in long-lasting hybrid zones between parapatric species (Harrison 1993; Arnold 1997). In the second pattern, secondary contact results when geographic barriers (e.g., oceans, rivers, mountain ranges) are maintained but do not completely eliminate gene flow resulting from long-distance dispersal (Gill 1970; Grant et al. 1996).

The outcomes of secondary contact resulting from long-distance dispersal across geographic barriers remain relatively understudied, most likely because successful colonization events are rarely observed. Moreover, limited dispersal into the area of sympatry will often cause the consequences of secondary contact events to resolve quickly, both ecologically (e.g., via local extinction or rapid evolution of reproductive isolation) (MacArthur and Wilson 1967; Gill 1970; Mayr and Diamond 2001; Grant and Grant 2014) and on a genomic level (e.g., loss of low-frequency introgressed alleles due to genetic drift). Consequently, most direct knowledge regarding the evolutionary consequences of hybridization following long-distance dispersal has been gleaned from systems where secondary contact resulted from anthropogenic introductions (Rhymer and Simberloff 1996; Mooney and Cleland 2001; Fitzpatrick et al. 2010), which often differ fundamentally from typical natural colonizations (Strauss et al. 2006). A handful of examples of recent secondary contact resulting from natural long-distance dispersal have

been identified, primarily in island taxa, including Galapagos finches (Grant and Grant 2009), New Zealand stilts (Steeves et al. 2010), *Ficedula* flycatchers (Qvarnström et al. 2010), and Norfolk Island white-eyes (Gill 1970). Furthermore, several genetic studies have identified historical episodes of gene flow that followed secondary contact between island species (Caraway et al. 2001; Shaw 2002; Cianchi et al. 2003; Larsen et al. 2010; Nunes et al. 2010; Lamichhaney et al. 2015; Lavretsky et al. 2015), suggesting that secondary contact via long-distance dispersal has had important consequences for speciation. Additional studies of secondary contact events on islands will therefore offer further insights into the potential outcomes of this important stage of the speciation process.

The two species of *Myzomela* honeyeaters on Makira in the Solomon Islands are well-suited for studying the evolutionary consequences of hybridization following secondary contact. These small, primarily-nectivorous birds are members of one of the most species-rich genera of songbirds (Higgins et al. 2008). The all-black, sexually-monochromatic Sooty Myzomela (*Myzomela tristrami*) (Fig. 2.1a) is endemic to all elevations and habitats on Makira, as well as to the satellite islands of Santa Catalina and Santa Ana approximately eight km east of Makira (Fig. 2.1b). An endemic subspecies of the red-and-black, sexually-dichromatic Cardinal Myzomela (*Myzomela cardinalis pulcherrima*) (Fig. 2.1a) is found in coastal and lowland regions of Makira, as well as on the satellite islands of Ugi and Three Sisters approximately eight km and twenty km north of Makira, respectively (Fig. 2.1b) (Diamond 2002; Dutson 2011). The two species are sympatric along the coast of Makira and fill similar ecological niches, often feeding from

the same flowers and behaving aggressively towards each other (Diamond 2002; J. Sardell, pers. obs.).

Secondary contact between the Makira *Myzomela* is recent, with *M. cardinalis* successfully colonizing Makira from Ugi and/or Three Sisters during the early-20th century (Mayr and Diamond 2001; Diamond 2002). Ornithologists collected only *M. tristrami* during several trips to Makira in the 19th century (Ramsay 1883). Two of the eight birds that A.S. Meek (Rothschild and Hartert 1908) collected from west Makira in 1908 were later identified by Diamond (2002) as phenotypically-intermediate individuals representing putative hybrids, suggesting that *M. cardinalis* likely had already colonized Makira by the time of Meek's visit. Ernst Mayr and the Whitney South Seas Expedition (Mayr 1945) collected both putative hybrids and *M. cardinalis* from Makira in 1927, although *M. tristrami* was more common in sympatry (Diamond 2002). Finally, Cain and Galbraith (1956) in 1953 and Diamond in the 1970s did not detect any putative hybrids, and found that *M. cardinalis* outnumbered *M. tristrami* in sympatry (Diamond 2002). Based on these collection records, Mayr and Diamond (2001) hypothesized that hybridization in this system only occurs when one species is greatly outnumbered by the other, such as during the earliest stages of secondary contact and along the forefront of *M. cardinalis*'s range expansion around the coast of Makira. In these situations, local scarcity of conspecific mating opportunities among the colonizing species may lead to a relaxation of pre-zygotic isolation (Mayr and Diamond 2001; Diamond 2002; Price 2008), as predicted by models of female mate choice (Wilson and Hedrick 1982; Real 1990; Wirtz 1999). This phenomenon wherein hybridization is most prominent during early stages of secondary contact has been observed in several other systems (Price

2008), including Galapagos finches (Grant and Grant 2014), white-eyes (Gill 1970), woodpeckers (Short 1969), tits (Vaurie 1957), and butterflies (Cianchi et al. 2003). However, because previous expeditions did not explicitly search for hybrid *Myzomela* on Makira, ongoing hybridization in this system may be more common than currently assumed.

We here present the first investigation of the genetic patterns of hybridization and introgression and their potential consequences for speciation in the Makira *Myzomela*. If introgression is persistent and symmetric, then it may eventually lead to the formation of a single panmictic hybrid swarm (as in Larsen et al. 2010; Lavretsky et al. 2015). Alternatively, asymmetric introgression from *M. tristrami* to *M. cardinalis* is predicted under Mayr and Diamond's (2001) hypothesis that hybridization in this system is driven by reduced choosiness (i.e., propensity to hybridize) among female *M. cardinalis* during the early stages of colonization. Asymmetric introgression can also result from asymmetric hybrid viability (Bolnick et al. 2008; Ellison and Burton 2008; Ellison et al. 2008; Werren et al. 2010; Trier et al. 2014), fixed differences in mate choice preferences between populations (Kaneshiro 1976, 1980), or a passive "wave-front" process driven by relative abundance, demography, and dispersal (Currat et al. 2008; Excoffier et al. 2009; Steeves et al. 2010; Rheindt and Edwards 2011). The direction and degree of any asymmetric introgression are important because they influence the potential for adaptive alleles to introgress between species, a mechanism which has been proposed to be an important potential source of genetic variation, particularly in small isolated populations (Anderson and Stebbins Jr 1954; Arnold 2004; Seehausen 2004; Grant et al. 2005; Hedrick 2013). Accordingly, determining the magnitude and direction of introgression in

the Makira *Myzomela* will offer important insight into the potential for hybridization to promote adaptation in this system, as well as providing another example of the potential outcomes of hybridization during the early stages of secondary contact resulting from long-distance dispersal.

Methods

Sampling

We conducted field work at six sites in Makira-Ulawa province, Solomon Islands between May and July in 2012 and 2013. Two sites, Ugi and Three Sisters, contained allopatric populations of *M. cardinalis*; two sites, Santa Catalina and highland Makira, contained allopatric populations of *M. tristrami*; and two sites, North Makira and Star Harbour, contained populations of both species, with the former site representing an older area of sympatry relative to the latter (Fig. 2.1b). At each site, we erected mist-nets in areas where *Myzomela* were observed, often at flowering trees, and collected a blood sample from the brachial vein of each captured individual. Additionally, we collected blood samples from chicks from four observed nests; each nest contained two chicks, and to avoid potential impacts of relatedness bias, our analysis includes genetic sequence data for only one randomly-selected chick from each pair. No adults were captured in the vicinity of any of these nests, so it is unlikely that the parents are included in our dataset. Finally, six samples of *M. cardinalis* and one sample of *M. tristrami* were taken from birds that were incidentally captured in 2008 while mist-netting for an unrelated study at the same localities. In total, samples comprise 121 *Myzomela cardinalis pulcherrima*, 75 *Myzomela tristrami*, and 7 individuals with intermediate plumage. Additionally, during

2012, we collected three blood samples of another *M. cardinalis* subspecies, *M. c. sanctaecrucis*, from Santa Cruz (a.k.a. Nendo) in the Solomon Island province of Temotu, located approximately 370 km east of Makira.

Collected blood was preserved in lysis buffer (Longmire et al. 1997) and stored at -20°C upon return from the field. Genomic DNA was extracted using DNeasy Blood & Tissue Kits (Qiagen) and DNA concentrations were measured using a Qubit 2.0 fluorometer (Life Technologies).

Birds were assigned to species based on plumage phenotypes observed in the field and recorded in photos (Fig. 2.1a). Adult *M. tristrami* are entirely black, while juveniles are black above and gray below with yellow bills. Male *M. cardinalis* possess bright red heads, breasts, and backs, while females are slightly duller with more black on the underparts. Juvenile *M. cardinalis* are highly variable in terms of plumage, ranging from duller versions of male plumage patterns to predominantly gray and olive with patches of red-tinged feathers, but can be distinguished by their yellow nares. Birds possessing abnormal combinations of red and black plumage (e.g., a black body and red back or a black head with patches of red feathers) were classified as phenotypic hybrids (Fig. 2.1a).

Sequencing

We obtained sequence data for one mitochondrial gene and six nuclear markers from each sampled individual. For mitochondrial DNA ("mtDNA"), we used PCR to amplify 1016 bp of NADH dehydrogenase subunit 2 (*ND2*) using the primers H6313 and L5216 and an annealing temperature of 57°C (Sorenson et al. 1999). For nuclear DNA, we amplified the following markers using the primers and protocols cited: Beta-

fibrinogen intron 5 (*FGB*), 411 bp (Kimball et al. 2009); Myoglobin intron 2 (*MYO*), 647 bp (Slade et al. 1993; Heslewood et al. 1998); Rhodopsin intron 1 (*RHO*), 702 bp (Primmer et al. 2002); Transforming growth factor *β*-2 intron 5 (*TGFβ2*), 523 bp (Primmer et al. 2002); Glyceraldehyde 3-phosphate dehydrogenase intron 11 (*GAPDH*), 273 bp (Primmer et al. 2002); and *15246*, 430 bp (Backström et al. 2008a). Successful amplification was confirmed by electrophoresing 3 μl of PCR product in a 1% agarose gel. PCR products were prepared for sequencing using manufacturer's protocols for ExoSAP-IT purification (USB Corp.), BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), and Sephadex purification (Sigma-Aldrich). Sanger sequencing was performed at the Molecular Core Facility of the University of Miami Department of Biology.

Forward and reverse sequence reads were aligned in Sequencher v.4.8 (Gene Codes, Ann Arbor, MI), and all chromatograms were reviewed to visually identify heterozygous sites and errors in base calling. Allelic haplotypes for the nuclear markers were inferred using the PHASE v.2.1 algorithm (Stephens et al. 2001) from DnaSP v.5.10.1 (Librado and Rozas 2009), with the presence of heterozygous indels in certain sequences allowing for the visual determination of specific haplotype sequences from chromatogram data. For most nuclear markers, PHASE was able to assign haplotypes with greater than 95% confidence to more than 98% of all individuals, with nearly all low-confidence assignments representing singleton polymorphisms. However, due to high levels of polymorphism and recombination, PHASE results for *TGFβ2* were characterized by high degrees of uncertainty. Accordingly, we used custom-designed allele-specific primers (Pettersson et al. 2003) to identify allelic phases for nearly all

individuals with ambiguous haplotypes, resulting in the same level of confidence as for the other markers. Haplotype networks for each marker were constructed in TCS (Clement et al. 2000).

Estimation of Divergence Times

Time in allopatry is believed to be important for the evolution of reproductive isolation (Coyne and Orr 2004; Price 2008), so we conducted a phylogenetic analysis to estimate the age of the most recent common ancestor of *M. tristrami* and *M. cardinalis*, as well as the date that the two species colonized Makira and its offshore satellite islands respectively. We constructed a mitochondrial gene tree for 820bp of *ND2* using sequences of two randomly-selected individuals from each of our two allopatric study populations of *M. tristrami* and *M. cardinalis pulcherrima*, three samples of *M. cardinalis sanctaecrucis*, and sequences for other species available on GenBank (Driskell and Christidis 2004; Smith and Filardi 2007; Gardner et al. 2010; Toon et al. 2010; Nyári and Joseph 2013; Andersen et al. 2014). In total, this data set includes 36 sequences (Table 2.1), representing 13 of the 31 currently-recognized species of *Myzomela* (including all Solomon Island taxa), at least three subspecies of *M. cardinalis* (the sampling location of one sequence available on GenBank is not provided in the original study), and the known sister species to this genus, *Sugomel nigrum* (Joseph et al. 2014).

Using MrModeltest (Nylander 2004), we identified GTR+Γ+I as the most appropriate substitution model for Bayesian phylogenetic analyses, based on Akaike Information Criterion (AIC). We then used BEAST v.1.5.4 (Drummond and Rambaut 2007) to perform a Bayesian Markov chain Monte Carlo analysis to construct the

phylogeny and estimate divergence times for the taxa of interest. To obtain a substitution rate prior, we adjusted the molecular substitution rate for avian cytochrome B (*CytB*) (2.1% per million years) (Weir and Schluter 2008) to reflect the relative rate of molecular evolution for *ND2* and *CytB* sequences in the five species of *Myzomela* (*M. cardinalis, M. erythrocephala*, *M. obscura*, *M. rosenbergii*, and *M. sanguinolenta*) for which sequences of both mtDNA genes from the same individual were available on GenBank (Driskell and Christidis 2004). The mean pairwise differences per site for *ND2* between these species is 1.3x that of *CytB* (range: 0.95x-1.56x), resulting in an estimated substitution rate of 2.7% per million years for *ND2*. Analyses were run for 10 million generations applying a Yule tree prior under a relaxed tree framework, sampling every 100 iterations, for a total of 100,000 trees. Adequate convergence of the posterior was confirmed using TRACER v.1.6. After applying a 20% burn-in, the consensus majority-rule tree was constructed in TreeAnnotator v.1.5.4 and used to estimate the mean and 95% confidence interval divergence times for *M. tristrami* and the clade containing *M. cardinalis*, as well as the Makira and Santa Cruz subspecies of *M. cardinalis*.

Confirmation and Quantification of Hybridization and Introgression

Natural selection and genetic drift will facilitate evolution of population-specific alleles, particularly between small isolated populations such as those on oceanic islands which have also undergone one or more founder events associated with island colonization prior to secondary contact (Hedrick 2011). We therefore identified species-specific mitochondrial and nuclear haplotypes as those that are exclusively present in allopatric populations of one species. Presence of such species-specific alleles in

sympatric heterospecifics is considered to be evidence for introgression. This approach does not account for the possibility of introgression of alleles into allopatric populations, conservatively assuming instead that sharing of any alleles in allopatry is due to incomplete lineage sorting rather than past or present gene flow. Moreover, robust sample sizes are important for this approach, as false identification of introgression may occur if alleles that are shared by both species due to incomplete lineage sorting go undetected in allopatric populations of one species. To estimate the potential effect of sample size on our analysis, we used a maximum-likelihood approach to calculate the 95% likelihood-based confidence limit for the true frequency of an allele that was not detected among our allelic sample size (e.g., twice the number of sampled individuals) in each population (Whitlock and Schluter 2015).

Extensive sharing of nuclear haplotypes may result in a failure to detect actual hybrids using our approach. To test our ability to identify hybrids and backcrosses accurately, we used a custom script developed in R (R Core Team 2014) that simulates the outcomes of hybrid pairings based on actual genotypes of allopatric birds. For each simulated hybridization event, alleles for the six nuclear markers were chosen at random from randomly-selected parental genotypes. Simulated offspring were then assigned to the following categories based on the presence of species-specific alleles: (1) hybrid if possessing at least one *M. tristrami*-specific and at least one *M. cardinalis*-specific allele; (2) *M. tristrami* if possessing at least one *M. tristrami*-specific allele but no *M. cardinalis*-specific alleles; (3) *M. cardinalis* if possessing at least one *M. cardinalis*-specific allele but no *M. tristrami*-specific alleles; and (4) ambiguous if possessing no species-specific alleles. 100,000 F_1 and F_2 hybrid simulations were performed for each

of the four possible hybrid pairings of the sampled allopatric populations. A similar approach was then used to backcross each simulated hybrid with randomly chosen individuals from each of the two allopatric parental populations. Simulated backcrosses were assigned to populations based on presence and absence of species-specific alleles as described above.

We used DnaSP to calculate pairwise F_{ST} and Φ_{ST} values between populations for each marker to test for evidence that heterospecific populations are more similar in sympatry than allopatry, as expected under introgression in sympatry. Mean F_{ST} values across all six nuclear markers were computed for each pairwise comparison, and a two-tailed paired t-test with Bonferroni correction was used to test for significant differences between cross-species F_{ST} values for allopatric populations versus populations from within the area of sympatry. Finally, we used STRUCTURE v.2.3.4 (Pritchard et al. 2000) to perform a Bayesian population assignment analysis, based on haplotype data, a model run of 1.5 million iterations, a burn-in of 200,000, and k=2 *a priori* populations.

Results

Sampling and Species Distributions

Myzomela tristrami and *Myzomela cardinalis* continue to coexist on coastal Makira. *M. cardinalis* represented 64% of total captures (30/47) in North Makira and 50% (13/26) of total captures in Star Harbour at sites where both species were observed multiple times. The presence of *M. cardinalis* at Star Harbour indicates that the area of sympatry has expanded recently into the eastern portion of Makira, as the species was absent from Star Harbour when Diamond visited during the 1970s (Diamond 2002). The

current relative species abundances in sympatry are similar to those observed over the past 60 years (Diamond 2002). In most habitats immediately adjacent to the sea (e.g., coastal mangroves and coconuts, beach vegetation), *M. cardinalis* was abundant (i.e., several individuals observed during every visit) but *M. tristrami* was rare (i.e., observed during multiple visits, but never captured during netting and representing less than five percent of all *Myzomela* detected). These areas were not large (approximately 10-200 meters wide), and *M. tristrami* was abundant in similar seaside habitats on Santa Catalina where *M. cardinalis* was absent. *M. cardinalis* was never observed in primary rainforest or isolated secondary habitats (e.g., gardens and villages) in interior Makira, but is common (i.e., reliably observed during every visit) in secondary habitats at 255m in elevation behind the town of Kirakira (North Makira) more than 1.3 km from the coast, and was observed more than 3.5km from the coast in contiguous secondary habitat along the Ravo River. However, a more robust assessment of habitat preferences is needed to quantify whether the species indeed sort ecologically.

Seven putative hybrids were captured on Makira in 2012 and 2013: two in North Makira and five in Star Harbour. These birds possessed intermediate plumage, generally having much more extensive black plumage than typical *M. cardinalis* but with red or reddish patches of carotenoid-based plumage atypical of *M. tristrami* (Fig. 2.1a). No such intermediate birds were observed in allopatric populations, suggesting that they represent true hybrids and not rare color morphs.

One phenotypic hybrid was observed brooding two nearly-fledgling-age chicks in Star Harbour during 2012. Molecular sexing (Han et al. 2009) confirmed that this individual was a female, and both chicks shared mitochondrial haplotypes and at least

one nuclear allele of each of the six sequenced nuclear markers with the female, suggesting that they were indeed its offspring. Both chicks possessed plumage typical of *M. cardinalis* suggesting that they were the product of a backcross between the hybrid and a male *M. cardinalis*. Although anecdotal, this observation demonstrates that this system is not characterized by complete hybrid infertility.

Divergence time estimates

The mitochondrial divergence time analysis dated the most recent common ancestor of *M. cardinalis* and *M. tristrami* at a mean estimate of 5.8 million years (95% C.I: 3.1-9.3 mya) (Fig. 2.2). This estimate is statistically comparable to the 7 million years that has been cited as the average divergence time for avian species pairs exhibiting nearly complete hybrid infertility (Price 2008). The estimated age of the most recent common ancestor of the Makira and Santa Cruz subspecies (i.e., the likely source population for *M. cardinalis*'s colonization of Ugi and Three Sisters (Mayr and Diamond 2001)) was 1.2 million years (95% C.I: 0.24-2.7 mya) (Fig. 2.2), which is within the estimated age of both islands (the Pliocene for Makira, Ugi, and Three Sisters (Coulson and Vedder 1986), and the early Miocene for Santa Cruz (Luyendyk et al. 1974).

Mitochondrial Introgression

Sequencing revealed two highly-diverged clades of species-specific *ND2* haplotypes within the Makira *Myzomela* (Fig. 2.2, Fig. 2.3). Haplotype diversity was much greater among *M. tristrami* than *M. cardinalis* (Table 2.2). All allopatric individuals of both species possessed species-specific mitochondrial haplotypes, as did

all *M. cardinalis* from the area of sympatry (Fig. 2.1c). However, all seven phenotypic hybrids and 22% (7/32) of *M. tristrami* from the area of sympatry possessed mitochondrial haplotypes belonging to the *M. cardinalis* clade, including 33% (5/15) of *M. tristrami* from North Makira and 12% (2/17) from Star Harbour, indicative of mitochondrial introgression from *M. cardinalis* to *M. tristrami.* Finally, two low-frequency mitochondrial haplotypes were present in populations of *M. cardinalis* from Makira and Three Sisters but not from Ugi, suggesting that at least some colonizing individuals originated from the allopatric population on Three Sisters.

Pairwise Φ_{ST} values for *ND2* were consistent with asymmetrical mitochondrial introgression (Table 2.3): Φ_{ST} values for populations of *M. tristrami* from within the area of sympatry versus *M. cardinalis* populations (ranging from 0.61-0.84) were uniformly lower than Φ_{ST} values for allopatric populations (0.97-0.98). Further, pairwise Φ_{ST} values of *M. tristrami* from within the area of sympatry vs. *M. cardinalis* in the older sympatric population, North Makira, (0.62-0.63) were uniformly lower than in Star Harbour (0.84-0.86). In contrast, pairwise Φ_{ST} values for populations of *M. cardinalis* from within the area of sympatry vs. *M. tristrami* populations were essentially equivalent to pairwise Φ_{ST} values for allopatric populations of *M. cardinalis* vs. *M. tristrami*.

Nuclear Introgression

In contrast to mtDNA, none of the haplotype networks for the six nuclear markers showed obvious species-specific haplotype clusters (Fig. 2 4). Indeed, most haplotypes (mean = 76.4% of total sequences, ranging from 42.4% for *TGFβ2* to 98.8% for *FGB*) were shared by at least one allopatric individual of each species. These observed patterns

were consistent either with high levels of incomplete lineage sorting among ancestral populations or extensive historical introgression between species. As with mtDNA, nuclear haplotype diversity was again greater among *M. tristrami* than *M. cardinalis*, consistent with a larger effective population size for the former species. Nuclear markers provided no insight into the source population of the initial *M. cardinalis* colonization event, as all of the *M. cardinalis*-specific haplotypes that were detected in sympatric populations were also found in both allopatric populations of *M. cardinalis* (i.e., Ugi and Three Sisters).

Despite the relatively high frequency of haplotype sharing between species, sufficient inter-specific genetic variation existed across all markers to identify most individuals with hybrid ancestry. 100% (43/43) of allopatric *M. tristrami* and 98% (44/45) of allopatric *M. cardinalis* possessed at least one species-specific nuclear haplotype (Table 2.4). In total, 20% (15/75) of *M. cardinalis* from within the area of sympatry possessed at least one heterospecific nuclear haplotype, including 15% (5/33) from North Makira and 24% (10/42) from Star Harbour. Simulations predicted that *M. tristrami*-specific haplotypes should be present in 68-71% of hybrid backcrosses with *M. cardinalis* (Table 2.5), suggesting that these observations underestimate introgression into *M. cardinalis*.

Nuclear introgression from *M. cardinalis* into *M. tristrami* was restricted almost exclusively to admixed individuals possessing *M. cardinalis* mtDNA. Among sympatric *M. tristrami* possessing conspecific mtDNA haplotypes, only 4% (1/25) possessed an *M. cardinalis*-specific nuclear haplotype (Table 2.4), even though our simulations predicted that 46-65% of hybrid backcrosses with *M. tristrami* should possess at least one *M.*

cardinalis-specific haplotype (Supp. Table 2.5). Among the seven birds that were phenotypically *M. tristrami* but possessed *M. cardinalis* mtDNA haplotypes ("mitochondrial hybrids"), three possessed no heterospecific nuclear haplotypes, three possessed species-specific nuclear haplotypes for both species, and one was classified as genotypically *M. cardinalis* (Table 2.4). Similarly, for the seven "phenotypic hybrids" (birds with intermediate plumage), three were classified as hybrids, two were classified as *M. cardinalis*, and two were classified as *M. tristrami*, based on species-specific nuclear haplotypes (Table 2.4). Simulations predicted that, under this approach, 71-83% of true F_1 hybrids should be identified as genetically hybrid, 13-24% as genetically *M. tristrami*, and only 2-6% as genetically *M. cardinalis* (Supp. Table 2.5).

Maximum likelihood-analysis indicated that sample sizes were likely to be robust enough to minimize the chance of false mischaracterization of alleles as "species-specific." Based on our sample sizes, the 95% confidence interval for the true allele frequency of a nuclear allele that went undetected was between 0.030-0.064 for our populations (Supp. Table 2.6), while the maximum likelihood estimate for each population was zero. As such, any undetected alleles likely represent truly rare variants in the population. Our smallest sample sizes and greatest confidence interval estimates were in the sympatric populations of *M. tristrami* and the allopatric population of *M. tristrami* from Highland Makira. Under our approach for identifying hybrids, failure to detect alleles in the latter population would result in a potential for mischaracterizing shared nuclear alleles as "*M cardinalis*-specific" and, as a result, falsely identifying putative introgression from *M. cardinalis* to *M. tristrami*. However, we note that introgression in

this direction was separately confirmed via mitochondrial sequencing, and in all but one case, nuclear introgression was restricted to known mitochondrial hybrids.

Mean F_{ST} values between populations of sympatric *M. tristrami* and allopatric *M. cardinalis* (ranging from 0.09-0.14) were lower than mean F_{ST} values of allopatric *M. tristrami* vs. allopatric *M. cardinalis* (0.15-0.24) (Supp. Table 2.3), as expected with introgression of *M. cardinalis* alleles into *M. tristrami* in sympatry. Likewise, mean F_{ST} values of populations of sympatric *M. cardinalis* vs. allopatric *M. tristrami* from Highland Makira (0.12-0.13) were marginally lower than mean F_{ST} values of allopatric *M. cardinalis* vs. the same *M. tristrami* (0.14-0.16), consistent with introgression. However, none of these differences were statistically significant (uncorrected P-values: 0.13-0.79) as the observed patterns were not consistent for all six nuclear markers (Supp. Tables 2.7-2.9), which is to be expected if rates of introgression varied across the genome.

The STRUCTURE analysis (Fig. 2.5) also revealed evidence for nuclear introgression in this system. As expected, STRUCTURE identified two species-specific genotypic clusters, with all but one allopatric individual assigned to the conspecific cluster with a posterior probability ("p.p.") >90%, with 91% (29/32) of *M. tristrami* and 96% (44/46) of allopatric *M. cardinalis* assigned with >95% p.p. The exception was an allopatric adult male *M. cardinalis* from Ugi which STRUCTURE assigned with intermediate probability (p.p.: 59% *M. tristrami*, 41% *M. cardinalis*), even though all of its alleles were shared by at least one other allopatric *M. cardinalis*. Still, we could not dismiss the possibility of hybrid ancestry in this individual, as Ugi is only 8 km from

mainland Makira, and there is a published report of vagrant *M. tristrami* from Ugi (Dutson 2001). No *M. tristrami*, however, were sighted during any of our visits to Ugi.

Among sympatric *M. cardinalis*, STRUCTURE assigned nearly all individuals to the correct cluster, but with slightly lower confidence than for allopatric individuals, consistent with low levels of introgression from *M. tristrami*. Although 93% (70/75) of sympatric *M. cardinalis* were assigned to the conspecific cluster with >90% p.p., only 83% (62/75) were assigned with >95% p.p., significantly lower than for allopatric populations (two-tailed Z-test: 2.10, p=0.036) and consistent with backcrossing. Five *M. cardinalis* from the area of sympatry possessed intermediate genetic structure consistent with hybrid ancestry (p.p. of assigning to the conspecific cluster = 68.3-88.6%). Among sympatric *M. tristrami*, 92% (23/25) of individuals with conspecific mitochondrial haplotypes were assigned to the correct cluster with >95% p.p., statistically equivalent to the assignment probabilities for allopatric populations (two-tailed Z-test: 0.18, p=0.857), again consistent with extremely limited nuclear introgression into *M. tristrami* matrilines within the area of sympatry. In contrast, *M. tristrami* possessing heterospecific mitochondrial haplotypes and phenotypic hybrids both varied substantially between individuals in their population assignments (Fig. 2.5), consistent with genetic admixture.

Discussion

Our study represents the first quantification of hybridization and introgression between the two species of *Myzomela* honeyeaters present on Makira in the Solomon Islands, a rare example of recent secondary contact between congeners in an island radiation (Mayr and Diamond 2001). Hybridization between the native *M. tristrami* and

the recent colonizer *M. cardinalis* was previously noted by Mayr and Diamond (2001), who hypothesized that hybrid pairings in this system were limited to the early stages of colonization when conspecific mating opportunities for *M. cardinalis* were scarce (Mayr and Diamond 2001; Diamond 2002; Price 2008). Our findings indicate that hybridization is ongoing more than a century after the identified colonization event, with one phenotypic hybrid captured per every 4.6 *M. tristrami* (i.e., the less common species) captured in sympatry. This ongoing hybridization occurs even though the Makira *Myzomela* shared a most recent common ancestor between 3.1 and 9.3 million years ago. Despite this divergence, our observation of an intermediate-plumage female with near-fledgling age chicks demonstrates that F_1 hybrid sterility is not complete. As a result, hybridization has led to widespread asymmetric introgression in the Makira *Myzomela*, which provides insight into the dynamics of hybridization and its potential consequences for speciation in this system.

Mitochondrial DNA sequencing indicates that all successful F_1 hybrid pairings in this system involve female *M. cardinalis*, with 33% of phenotypically *M. tristrami* individuals from the oldest portion of the area of sympatry (North Makira) possessing *M. cardinalis* mtDNA haplotypes. This asymmetric introgression from the colonizing to the native species is opposite to the pattern predicted by wavefront models of introgression (Currat et al. 2008) and which is observed in nearly all shifting avian hybrid zones (Rheindt and Edwards 2011). In contrast, mitochondrial introgression in this system is influenced predominantly by asymmetric reproductive isolation, i.e., one hybrid pairing is more likely to occur successfully than the reverse. Asymmetric reproductive isolation and mtDNA introgression from *M. cardinalis* to *M. tristrami* is predicted under Mayr and

Diamond's (2001) hypothesis that hybridization in this system was driven by a relaxation of mate choice preferences among colonizing *M. cardinalis* females immediately following secondary contact when conspecific mates were rare. However, if differences in mate choice preferences are primarily responsible for the asymmetric patterns of mtDNA introgression observed in this system, we would expect to observe introgression of *M. cardinalis* nuclear alleles into sympatric *M. tristrami* matrilines, as a consequence of pairings of *M. tristrami* females with all-black mitochondrial hybrid males. Yet only one sympatric *M. tristrami* possessing a conspecific mtDNA haplotype exhibited any degree of hybrid ancestry based on nuclear markers.

Instead, observed patterns of highly asymmetric mtDNA introgression likely result from asymmetric post-zygotic genetic incompatibilities. Incompatibilities between autosomal and mitochondrial genes involved in the OXPHOS pathway have been hypothesized to be an important driver of post-zygotic reproductive isolation between species (Turelli and Moyle 2007; Hill and Johnson 2013), and evidence for a link to asymmetric hybrid viability has been observed in many taxa (Bolnick et al. 2008; Ellison and Burton 2008; Ellison et al. 2008; Trier et al. 2014). Strong post-zygotic reproductive isolation in this system is unsurprising as we estimate that the most recent common ancestor for *M. cardinalis* and *M. tristrami* lived between 3.1-9.3 million years ago, statistically comparable to the 7 million years that has been cited as the average divergence time for species exhibiting nearly complete hybrid infertility (Price 2008). However, direct quantification of the relative viability of offspring from differential parental combinations as well as direct assays of female mate choice preferences are needed to determine the relative importance of pre- and post-zygotic reproductive

isolation to the observed patterns of asymmetric mitochondrial introgression between the Makira *Myzomela*.

Asymmetric hybridization has important consequences for biodiversity in this system. Unlike other known examples of secondary contact on small islands, the Makira *Myzomela* appear to exhibit sufficient reproductive isolation to maintain phenotypic differences between species rather than collapsing into a hybrid swarm (in contrast to Larsen et al. 2010; Steeves et al. 2010; Lavretsky et al. 2015). For example, in contrast to trends immediately following secondary contact, relative species abundances have remained relatively stable since the mid-20th century, and sympatric populations continue to exhibit generally strong species-specific genetic structure. However, reproductive isolation in this system is not sufficient to prevent introgression between species (in contrast to Qvarnström et al. 2010; Warren et al. 2011). Instead, nuclear introgression is occurring from the native species, *M. tristrami*, to the colonizer, *M. cardinalis*. Similar patterns of introgression also characterize several other island systems where long-distance dispersal resulted in secondary contact, including New Zealand stilts (Steeves et al. 2010), Galapagos finches (Grant and Grant 2014), and Mediterranean butterflies (Cianchi et al. 2003). If the colonization patterns on Makira are similar to those directly observed in *Geospiza* (Grant and Grant 2014), then all sympatric *M. cardinalis* should be descendants of a small number of individuals that originally colonized Makira and hybridized with *M. tristrami*, and whose offspring assortatively mated amongst themselves. Indeed, the proportion of *M. cardinalis* possessing heterospecific nuclear alleles is greater in populations that have achieved secondary contact more recently. Based on our simulations (Table 2.5), however, these proportions are significantly less

than those expected under this scenario. Instead, they are more consistent with three backcross events, suggesting one or more of the following: a relatively large founder population of *M. cardinalis*, repeated dispersal of *M. cardinalis* from the satellite islands to Makira, or strong selection for individuals with reduced hybrid ancestry. Therefore, even though reproductive isolation is incomplete, gene flow resulting from secondary contact between these highly diverged island species does not appear to be substantial enough to break down species boundaries. Hybridization does allow, however, for adaptive alleles to potentially introgress into the colonizing species.

The Makira *Myzomela* also exhibit apparent nuclear introgression from the colonizer, *M. cardinalis*, to the native species, *M. tristrami*, in contrast to the pattern observed in nearly all avian hybrid systems (Rheindt and Edwards 2011). However, the rarity of *M. cardinalis* nuclear haplotypes among individuals descended from *M. tristrami* matrilines is consistent with a high degree of reproductive isolation between female *M. tristrami* and the melanic birds that possess *M. cardinalis* mitochondrial haplotypes. Accordingly, it may be more appropriate to characterize gene flow patterns as an introgression of plumage alleles from the native species to the invading one, which matches the observations from other avian systems. Such introgression would be facilitated if black plumage is favored by sexual selection in *M. cardinalis* (e.g., due to sensory bias). Similar patterns of sexual selection driving asymmetric plumage introgression have been documented in hybrid zones between *Manacus* manakins (Brumfield et al. 2001; Stein and Uy 2006) and between subspecies of red-backed fairy-wrens, *Malurus melanocephalus* (Baldassarre and Webster 2013; Baldassarre et al. 2014). However, the relative rarity of mitochondrial hybrids on Makira and the

persistence of presumably-costly carotenoid-based red plumage in allopatric *M. cardinalis* suggest that plumage introgression is unlikely to be driven by shared preferences for black mates. Ecological selection could also favor black plumage on Makira, but an opposite pattern in terms of plumage distribution is found in the chestnut-bellied flycatcher (*Monarcha castaneiventris*) complex, which includes melanic populations on Ugi and Three Sisters and more colorful birds on Makira (Uy et al. 2009b; Uy and Safran 2013). Plumage introgression would also be facilitated if colonization events resulted in relaxed female preference for plumage in *M. cardinalis* females (Kaneshiro 1980), under which introgression would be driven primarily by genetic drift. Alternatively, black plumage among individuals of hybrid ancestry may be favored by disruptive intrasexual selection if it results in decreased aggression that allows otherwise low quality males to hold higher quality territories, as demonstrated in other examples of avian plumage polymorphisms (Greene et al. 2000; Vallin et al. 2012). Finally, the alleles for black plumage may be linked to alleles for genes responsible for non-plumage-related locally-favored adaptations (e.g., disease resistance, metabolism of local food sources, etc.) (Ducrest et al. 2008). Under this hypothesis, plumage has undergone introgression because the recent history of secondary colonization between species has not provided sufficient opportunity for recombination to break down linkage disequilibrium between these alleles. With the advent of affordable next-generation sequencing, it is now possible to examine genome-wide patterns of introgression and identify genomic regions that show signatures of selection. Such follow-up studies on this system will provide further insight into the evolutionary consequences of hybridization and introgression for the maintenance of biodiversity in island systems.

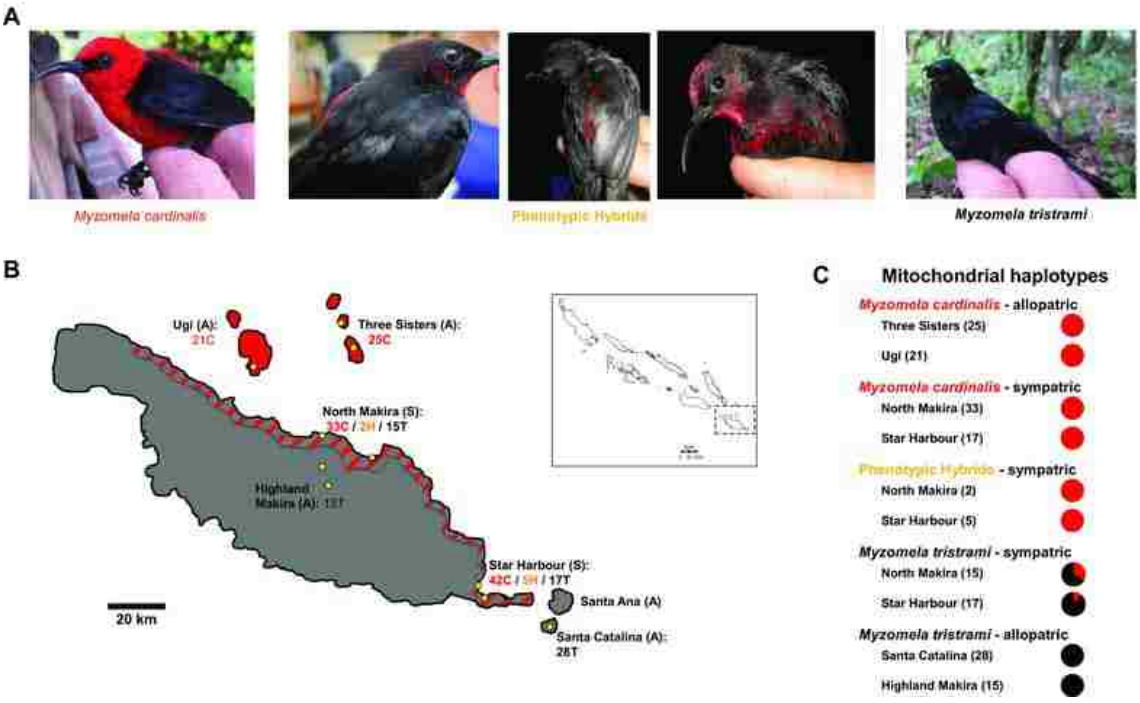


Figure 2.1. (A) Representative examples of plumage for, from left to right: adult male *M. cardinalis*, three putative hybrids from the area of sympatry based on intermediate plumage phenotypes, and adult male *M. tristrami*. (B) Map of Makira and satellite islands showing approximate range of: *M. cardinalis* encompassing Ugi, Three Sisters, and coastal Makira; and *M. tristrami* encompassing Makira, Santa Ana, and Santa Catalina. Sampling localities are shown with sample sizes indicated ("C" = *M. cardinalis*, "H" = phenotypic hybrid, "T" = *M. tristrami* based on plumage phenotypes). Allopatric and sympatric populations are denoted by "A" and "S," respectively. Inset in upper right denotes location of Makira within the Solomon Islands. (C) Proportion of individuals in each population possessing mitochondrial *ND2* haplotypes belonging to two highly diverged clades (see Figure 2.3 for associated haplotype network). All *M. cardinalis* and all phenotypic hybrids possess "red" haplotypes. All allopatric *M. tristrami* possess "black haplotypes." Sympatric *M. tristrami* populations possess both haplotypes, indicative of mtDNA introgression. Numbers after population names represent number of individuals sampled from that population.

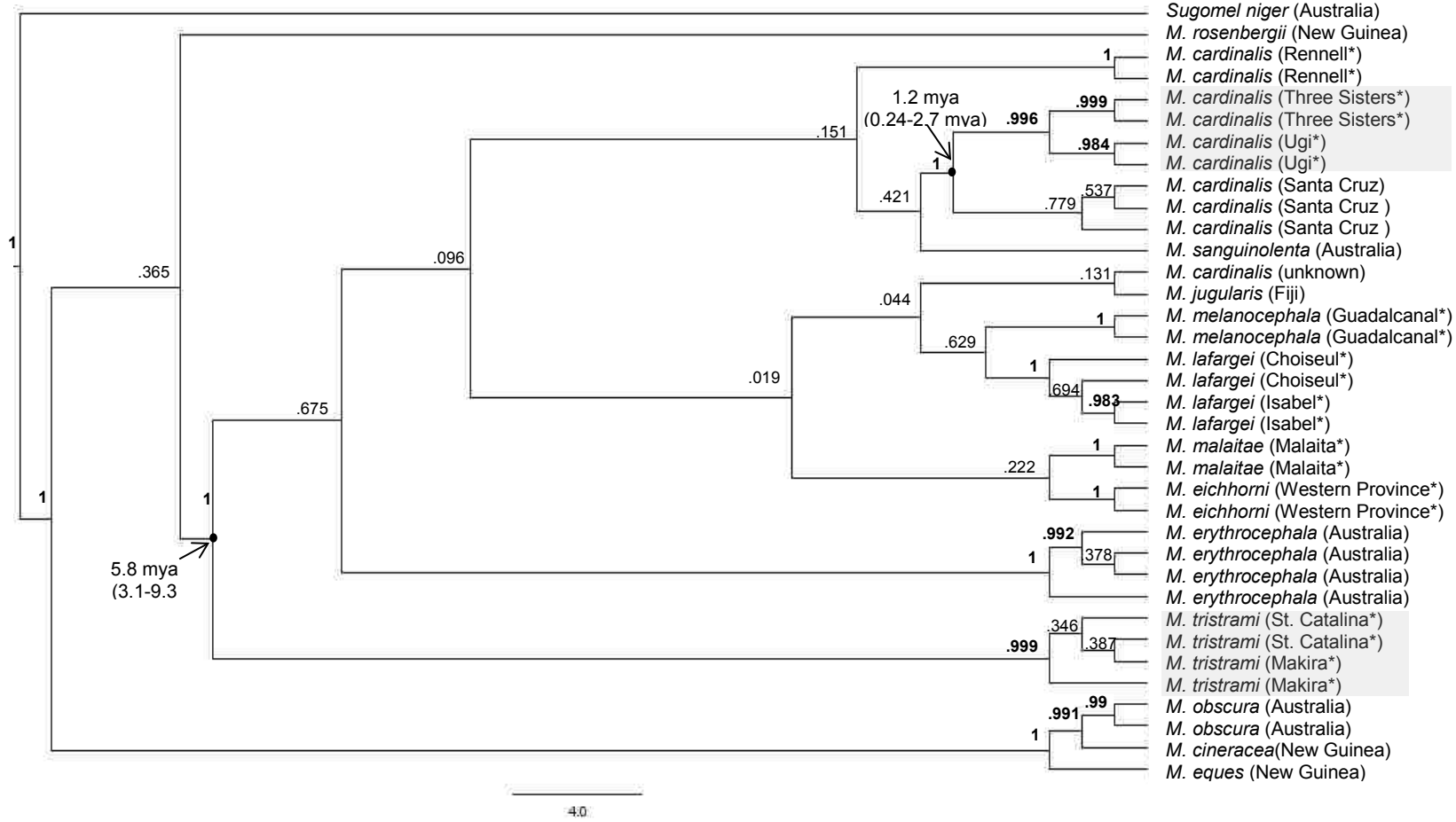


Figure 2.2: Consensus majority Bayesian phylogenetic tree representing evolutionary history of genus *Myzomela* inferred from a single mitochondrial gene (*ND2*). Tree constructed using BEAST v.1.5.4 using randomly-chosen samples collected from allopatric populations in this study and sequences available on GenBank (see Supp. Table 1). Geographic origin of each sample is listed after each species name with asterisks (*) denoting samples from the Solomon Archipelago. Nodes are labeled with Bayesian posterior probabilities. Mean and 95% confidence interval divergence times are noted for two nodes of particular interest for this analysis: the most recent common ancestor of *M. tristrami* and *M. cardinalis*, and the most recent common ancestor of the Ugi and Three Sisters subspecies of *M. cardinalis* and the subspecies of *M. cardinalis* on the Santa Cruz islands. The two taxa that are included in this study are boxed in gray for emphasis.

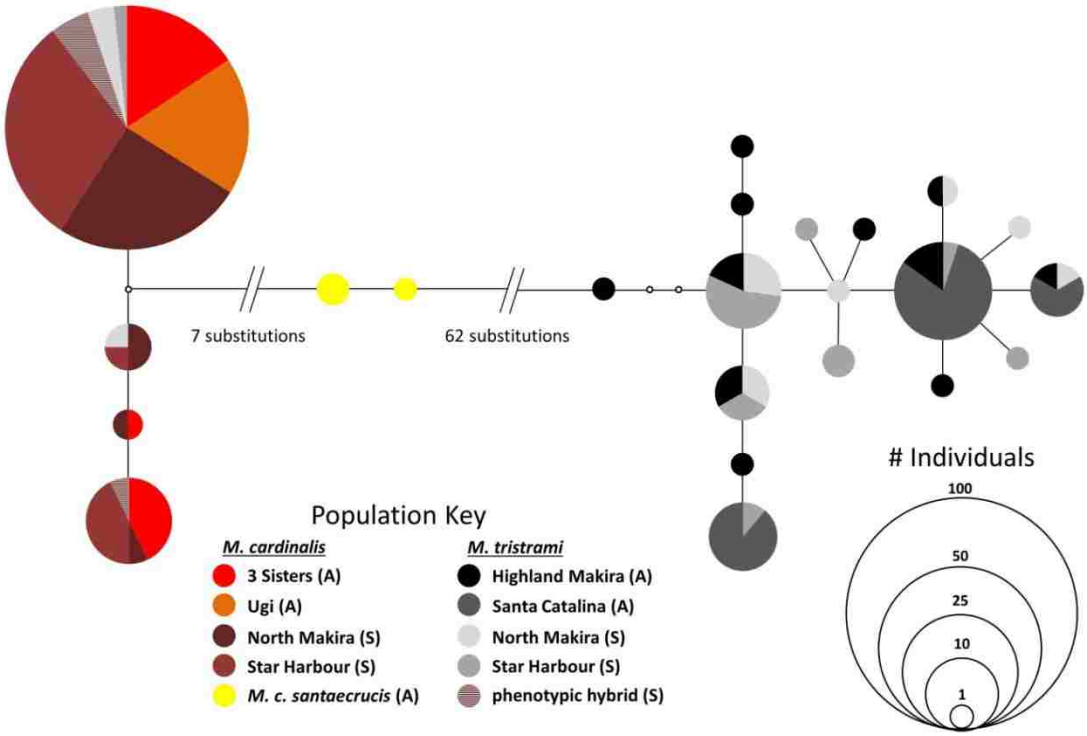


Figure 2.3: Haplotype network for 121 *Myzomela cardinalis*, 75 *M. tristrami*, and 7 phenotypic hybrids sequenced at one mitochondrial marker: NADH dehydrogenase subunit 2 (*ND2*). Each circle represents a haplotype with the size of the circle proportional to the number of haplotypes in the data set. Each line connecting circles represent a single nucleotide change. Small open circles represent inferred haplotypes not represented in the dataset. Colors represent the population sampled, as indicated in the key. Populations labeled "A" are allopatric populations and populations labeled "S" are sympatric populations.

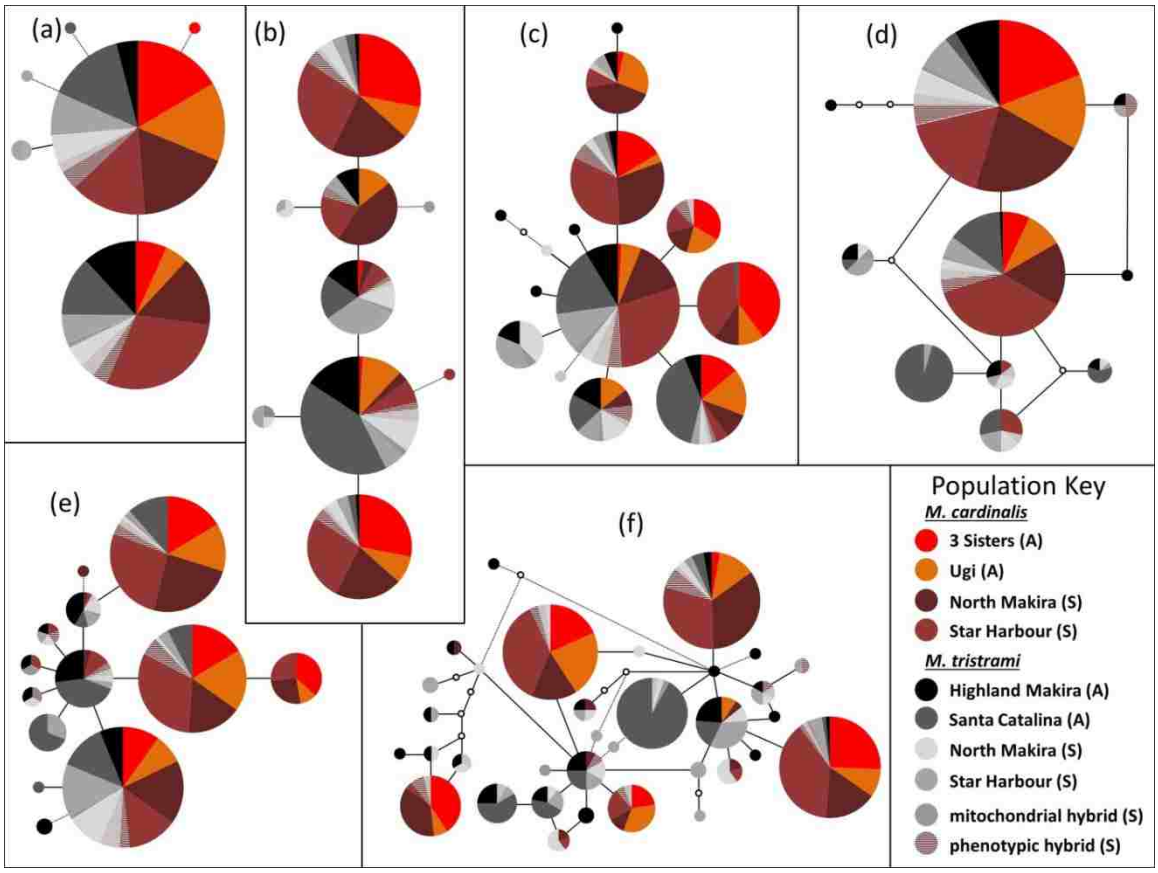


Figure 2.4: Haplotype networks for 121 *Myzomela cardinalis*, 75 *M. tristrami*, and 7 phenotypic hybrids sequenced at six nuclear markers: a) Beta-fibrinogen intron 5 (*FGB*); b) Rhodopsin intron 1 (*RHO*); c) *15246*; d) Glyceraldehyde 3-phosphate dehydrogenase intron 11 (*GAPDH*); e) Myoglobin intron 2 (*MYO*); f) Transforming growth factor β-2 intron 5 (*TGFβ2*). Each circle represents a haplotype with the size of the circle proportional to the number of haplotypes in the data set. Each line connecting circles represent a single nucleotide change. Small open circles represent inferred haplotypes not represented in the dataset. Colors represent the population sampled, as indicated in the key. Populations labeled "A" are allopatric.

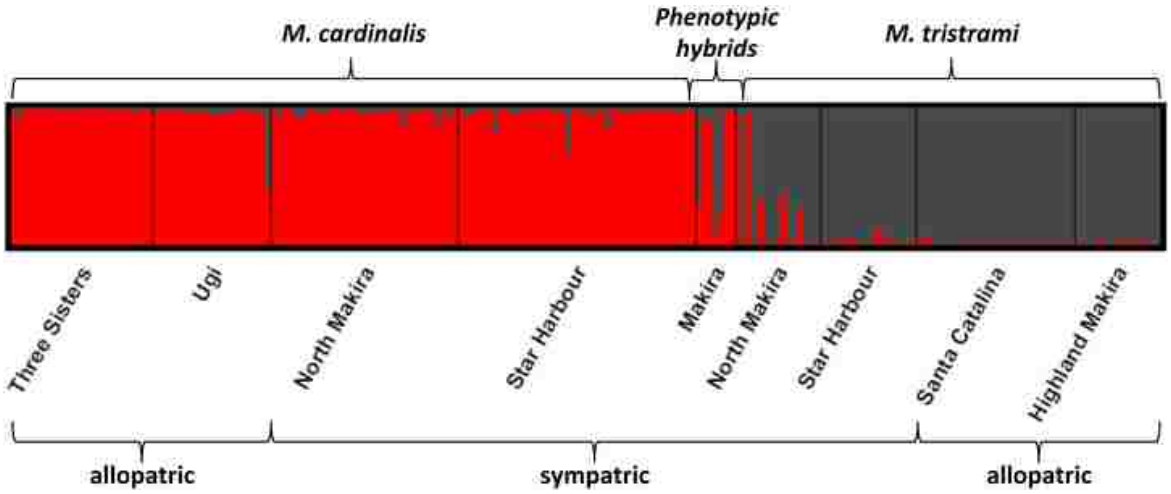


Figure 2.5. STRUCTURE plot based on haplotype data of six nuclear markers for 203 *Myzomela* from Makira-Ulawa province, assuming two population clusters ($k = 2$). *Y*-axis represents Bayesian posterior probability of assignment to clusters representing *M. cardinalis* (red) or *M. tristrami* (dark gray). Plumage phenotype of individuals is indicated above the plot, sampling location is indicated below the plot, with black bars separating locations

Table 2.1: Samples used in phylogenetic analysis

Species	Sampling Location	GenBank Accession #	Museum voucher	Study
M. cardinalis	unknown	AY488292.1	unvouchered	Driskell & Christidis (2004)
M. cardinalis	Rennell, Solomon Islands	DQ469051.1	UWBM58778	Smith & Filardi (2007)
M. cardinalis	Rennell, Solomon Islands	DQ469052.1	UWBM58774	Smith & Filardi (2007)
M. cardinalis	Santa Cruz, Solomon Islands	KU519944	unvouchered*	this study
M. cardinalis	Santa Cruz, Solomon Islands	KU519945	unvouchered*	this study
M. cardinalis	Three Sisters, Solomon Islands	KU519949	unvouchered*	this study
M. cardinalis	Three Sisters, Solomon Islands	KU519870	unvouchered*	this study
M. cardinalis	Ugi, Solomon Islands	KU519927	unvouchered*	this study
M. cardinalis	Ugi, Solomon Islands	KU519750	unvouchered*	this study
M. cineracea	New Guinea	DQ469054.1	UWBM68051	Smith & Filardi (2007)
M. eichhorni	Western Province, Solomon Islands	DQ469053.1	UWBM63036	Smith & Filardi (2007)
M. eichhorni	Western Province, Solomon Islands	DQ469057.1	UWBM63135	Smith & Filardi (2007)
M. eques	New Guinea	DQ469060.1	UWBM68034	Smith & Filardi (2007)
M. erythrocephala	Australia	AY488406.1	MV1198	Driskell & Christidis (2004)
M. erythrocephala	Australia	KC540589.1	ANWC29523	Nyari and Joseph
M. erythrocephala	Australia	KC540590.1	ANWC29639	Nyari and Joseph
M. erythrocephala	Australia	KC540591.1	ANWC29640	Nyari and Joseph
M. jugularis	Fiji	KF970738.1	KUNHM22536	Andersen et al (2014)
M. lafargei	Isabel, Solomon Islands	DQ469055.1	UWBM69655	Smith & Filardi (2007)
M. lafargei	Choiseul, Solomon Islands	DQ469056.1	UWBM63179	Smith & Filardi (2007)
M. lafargei	Isabel, Solomon Islands	DQ469063.1	UWBM60278	Smith & Filardi (2007)
M. lafargei	Choiseul, Solomon Islands	DQ469064.1	UWBM63183	Smith & Filardi (2007)
M. malaitae	Malaita, Solomon Islands	DQ469058.1	UWBM66085	Smith & Filardi (2007)
M. malaitae	Malaita, Solomon Islands	DQ469062.1	UWBM66095	Smith & Filardi (2007)
M. melanocephala	Guadalcanal, Solomon Islands	DQ469059.1	UWBM60359	Smith & Filardi (2007)

M. melanocephala	Guadalcanal, Solomon Islands	DQ469061.1	UWBM60342	Smith & Filardi (2007)
M. obscura	Australia	AY488293.1	ANWCC531	Driskell & Christidis (2004)
M. obscura	Australia	HM230619.1	ANWC33606	Toon, Hughes, and Joseph (2010)
M. rosenbergii	New Guinea	AY488294.1	ANWCE240	Driskell & Christidis (2004)
M. sanguinolenta	Australia	AY488295.1	ANWCC402	Driskell & Christidis (2004)
M. tristrami	Makira, Solomon Islands	KU519835	unvouchered*	this study
M. tristrami	Makira, Solomon Islands	KU519844	unvouchered*	this study
M. tristrami	Santa Catalina, Solomon Islands	KU519811	unvouchered*	this study
M. tristrami	Santa Catalina, Solomon Islands	KU519820	unvouchered*	this study

Museum Abbreviations:
ANWC = Australian National Wildlife Collection
KUNHM = Kansas University Natural History Museum
MV = Museum Victoria
UWBM = University of Washington Burke Museum

* Samples obtained as part of this study were taken from individuals released for a banding study. Photos and feathers were also taken from each individual to verify species indentification.

Table 2.2: Distribution of haplotypes for one mitochondrial and six nuclear markers among allopatric and sympatric populations of *M. cardinalis* and *M. tristrami*.

Marker	Total haplotypes	Total non-singleton haplotypes	Haplotypes shared by allopatric populations of both species	*M. tristrami* haplotypes		*M. cardinalis* haplotypes	
				Shared with sympatric *M. cardinalis* only	Unshared haplotypes	Shared with sympatric *M. tristrami* only	Unshared haplotypes
ND2 (mtDNA)	21	13	0	0	17	2	2
FGB	6	3	2	0	3	0	1
MYO	13	12	3	4	4	0	2
RHO	9	7	4	0	4	1	0
TGFβ2	34	22	3	4	24	3	0
GAPDH	10	8	2	2	6	0	0
15246	14	8	6	0	7	1	0

Table 2.3: Cross-population Φ_{ST} and F_{ST} values for sampled allopatric and sympatric populations of *M. cardinalis* and *M. tristrami*. Values in the top-right above the diagonal are Φ_{ST} for a single mitochondrial gene (*ND2*). Values in the bottom-left below the diagonal are the mean F_{ST} values across six nuclear markers. Values for each individual marker provided in Supplemental Tables 6-8.

		M. cardinalis				*M. tristrami*			
		Allopatric		Sympatric		Sympatric		Allopatric	
		Three Sisters	Ugi	North Makira	Star Harbor	North Makira	Star Harbor	Santa Catalina	Highland Makira
# individuals		25	21	33	42	15	17	28	15
M. cardinalis	Three Sisters	--	0.241	0.082	0.006	0.616	0.848	0.971	0.968
	Ugi	0.020	--	0.074	0.135	0.635	0.862	0.983	0.980
	North Makira	0.045	0.022	--	0.002	0.627	0.857	0.979	0.975
	Star Harbour	0.094	0.079	0.047	--	0.623	0.853	0.975	0.972
M. tristrami	North Makira	0.123	0.093	0.091	0.096	--	0.057	0.265	0.253
	Star Harbour	0.136	0.107	0.101	0.105	0.000	--	0.088	0.050
	Santa Catalina	0.241	0.208	0.231	0.220	0.099	0.134	--	0.076
	Highland Makira	0.164	0.145	0.128	0.120	0.015	0.021	0.141	--

Table 2.4: Percent of sampled *Myzomela* individuals assigned to genotypes based on presence/absence of species-specific alleles for six nuclear markers. Individuals assigned to a given species genotype possess at least one species-specific allele for that species and none for the other. Individuals assigned as "hybrids" possess at least one species-specific allele for each species, and individuals assigned as "ambiguous" possess no species-specific alleles. Populations are specified in the table as allopatric ("A") or sympatric ("S").

Population	Number individuals	Percent of individuals assigned to genotype			
		M. cardinalis	Hybrid	*M. tristrami*	Ambiguous
M. cardinalis					
Three Sisters (A)	25	0.96	0.00	0.00	0.04
Ugi (A)	21	1.00	0.00	0.00	0.00
North Makira (S)	33	0.79	0.06	0.09	0.06
Star Harbour (S)	42	0.71	0.21	0.02	0.05
Phenotypic hybrids (all possess *M. cardinalis* mtDNA haplotypes)					
North Makira (S)	2	0.50	0.00	0.50	0.00
Star Harbour (S)	5	0.20	0.60	0.20	0.00
Mitochondrial hybrids (*M. tristrami* possessing *M. cardinalis* mtDNA haplotypes)					
North Makira (S)	5	0.20	0.60	0.20	0.00
Star Harbour (S)	2	0.00	0.00	1.00	0.00
M. tristrami (possessing conspecific mtDNA haplotypes)					
North Makira (S)	10	0.00	0.10	0.90	0.00
Star Harbour (S)	15	0.00	0.00	1.00	0.00
Santa Catalina (A)	28	0.00	0.00	1.00	0.00
Highland Makira (A)	15	0.00	0.00	1.00	0.00

Table 2.5: Percent of 100,000 simulated F_1, F_2, and backcross pairings that are assigned to specified genotypes based on presence/absence of species-specific alleles for six nuclear markers. Simulations are based on random sampling of observed genotypes from specified *Myzomela* allopatric populations. Genotype assignment is based on the approach described in Table 1.

Simulated Pairing	Percent of individuals assigned to genotype			
	M. cardinalis	Hybrid	*M. tristrami*	Ambiguous
F_1 Hybrids				
Three Sisters x Santa Catalina	0.06	0.81	0.13	0.01
Ugi x Santa Catalina	0.05	0.71	0.23	0.02
Three Sisters x Highland Makira	0.03	0.83	0.13	0.00
Ugi x Highland Makira	0.02	0.73	0.24	0.01
F_2 Hybrids				
Three Sisters x Santa Catalina	0.09	0.70	0.20	0.01
Ugi x Santa Catalina	0.09	0.62	0.27	0.02
Three Sisters x Highland Makira	0.07	0.72	0.20	0.01
Ugi x Highland Makira	0.07	0.64	0.27	0.01
Backcrosses with *M. cardinalis*				
Three Sisters x Santa Catalina	0.31	0.63	0.05	0.01
Ugi x Santa Catalina	0.29	0.58	0.10	0.03
Three Sisters x Highland Makira	0.28	0.66	0.05	0.01
Ugi x Highland Makira	0.26	0.61	0.10	0.03
Backcrosses with *M. tristrami*				
Three Sisters x Santa Catalina	0.01	0.53	0.45	0.01
Ugi x Santa Catalina	0.01	0.45	0.53	0.01
Three Sisters x Highland Makira	0.01	0.54	0.45	0.00
Ugi x Highland Makira	0.01	0.45	0.54	0.00

Table 2.6: 95% likelihood-based confidence limits of the true frequency of a nuclear allele that went undetected in each population, based on number of alleles sampled. Populations are specified in the table as allopatric ("A") or sympatric ("S").

Population	Number of Alleles Sampled	95% Confidence Limit for True Frequency of Undetected Alleles
M. cardinalis		
Three Sisters (A)	50	0.039
Ugi (A)	42	0.046
North Makira (S)	66	0.030
Star Harbour (S)	84	0.024
M. tristrami		
North Makira (S)	30	0.064
Star Harbour (S)	34	0.057
Santa Catalina (A)	54	0.035
Highland Makira (A)	30	0.064

Table 2.7: Cross-population F_{ST} values for sampled allopatric and sympatric populations of *M. cardinalis* and *M. tristrami*. Values in the top-right above the diagonal are for Beta-fibrinogen intron 5 (*FGB*). Values in the bottom-left below the diagonal are for Myoglobin intron 2 (*MYO*).

		M. cardinalis				*M. tristrami*			
		Allopatric		Sympatric		Sympatric		Allopatric	
		Three Sisters	Ugi	North Makira	Star Harbour	North Makira	Star Harbour	Santa Catalina	Highland Makira
M. cardinalis	Three Sisters	--	-0.016	0.031	0.212	0.060	0.058	0.040	0.296
	Ugi	-0.019	--	0.067	0.276	0.106	0.098	0.078	0.366
	North Makira	-0.006	-0.006	--	0.072	-0.018	-0.010	-0.015	0.137
	Star Harbour	-0.012	-0.016	-0.003	--	0.023	0.023	0.054	-0.008
M. tristrami	North Makira	0.150	0.150	0.126	0.154	--	-0.024	-0.023	0.075
	Star Harbour	0.154	0.152	0.144	0.159	-0.004	--	-0.014	0.066
	Santa Catalina	0.059	0.054	0.049	0.055	0.063	0.042	--	0.113
	Highland Makira	0.190	0.189	0.179	0.187	0.029	0.035	0.079	--

Table 2.8: Cross-population F_{ST} values for sampled allopatric and sympatric populations of *M. cardinalis* and *M. tristrami*. Values in the top-right above the diagonal are for Rhodopsin intron 1 (*RHO*). Values in the bottom-left below the diagonal are for Transforming growth factor β-2 intron 5 (*TGFβ2*).

		M. cardinalis				*M. tristrami*			
		Allopatric		Sympatric		Sympatric		Allopatric	
		Three Sisters	Ugi	North Makira	Star Harbour	North Makira	Star Harbour	Santa Catalina	Highland Makira
M. cardinalis	Three Sisters	--	0.031	0.048	0.016	0.147	0.236	0.398	0.266
	Ugi	0.059	--	0.007	0.000	0.091	0.162	0.356	0.183
	North Makira	0.053	0.037	--	0.002	0.170	0.215	0.459	0.269
	Star Harbour	0.066	0.027	0.042	--	0.159	0.224	0.434	0.267
M. tristrami	North Makira	0.068	0.047	0.035	0.094	--	0.023	0.100	0.000
	Star Harbour	0.090	0.089	0.078	0.072	0.042	--	0.227	0.024
	Santa Catalina	0.273	0.251	0.216	0.260	0.140	0.175	--	0.102
	Highland Makira	0.069	0.075	0.051	0.095	-0.010	0.004	0.169	--

Table 2.9: Cross-population F_{ST} values for sampled allopatric and sympatric populations of *M. cardinalis* and *M. tristrami*. Values in the top-right above the diagonal are for Glyceraldehyde 3-phosphate dehydrogenase intron 11 (*GAPDH*). Values in the bottom-left below the diagonal are for nuclear marker *15246*.

		M. cardinalis				*M. tristrami*			
		Allopatric		Sympatric		Sympatric		Allopatric	
		Three Sisters	Ugi	North Makira	Star Harbour	North Makira	Star Harbour	Santa Catalina	Highland Makira
M. cardinalis	Three Sisters	--	0.009	0.029	0.245	0.160	0.124	0.473	0.036
	Ugi	0.056	--	-0.018	0.135	0.104	0.085	0.420	0.027
	North Makira	0.117	0.044	--	0.114	0.095	0.081	0.408	0.031
	Star Harbour	0.036	0.057	0.055	--	0.030	0.059	0.263	0.094
M. tristrami	North Makira	0.151	0.061	0.139	0.114	--	-0.017	0.178	0.021
	Star Harbour	0.151	0.057	0.099	0.091	-0.020	--	0.202	0.004
	Santa Catalina	0.206	0.088	0.267	0.256	0.133	0.175	--	0.290
	Highland Makira	0.129	0.030	0.099	0.086	-0.020	-0.010	0.094	--

Chapter 3

Recently sympatric, hybridizing island birds exhibit unusual patterns of biased male aggressive response towards heterospecifics

Background

Signal divergence between populations can have important evolutionary consequences for interspecies interactions (Lande 1981; West-Eberhard 1983; Panhuis et al. 2001; Ritchie 2007; Price 2008). Much emphasis in this context has been placed on the consequences of signal divergence for female mate choice, assortative mating, and speciation (Boughman 2001; Rundle et al. 2005; Ritchie 2007; Maan and Seehausen 2011; Safran et al. 2013). However, signal divergence can also play an important role in mediating intrasexual (e.g., male-male) competition between species (Grether et al. 2009; Qvarnström et al. 2012), which can arise through competition for mating opportunities between hybridizing taxa or for access to limited resources, such as food sources or breeding territories, between taxa that fill similar ecological niches (West-Eberhard 1983; Grether et al. 2013). Differences in receiver response to conspecific and heterospecific signals can arise in allopatric populations, but secondary contact is expected to play an important role in determining the responses of receivers towards heterospecific signals. For example, interspecific competition in sympatry can result in either increased aggressive response towards heterospecifics (Prescott 1987; Baker 1991; Gil 1997) or increased discrimination in aggression (Lynch and Baker 1991; Kirschel et al. 2009) relative to allopatric populations. Interspecific competition can also drive signal evolution in sympatry, e.g., by selecting for signal convergence (Tobias and Seddon 2009; Prum 2014) or signal divergence (i.e., "agonistic character displacement") (Tynkkynen et al.

2005; Anderson and Grether 2010; Vallin et al. 2012; Grether et al. 2013) between species. Accordingly, additional studies that examine the relationships between signal divergence and interspecies aggression in both allopatric and recently sympatric populations will provide important insight into the interplay of intrasexual selection and signal divergence.

The two species of nectivorous *Myzomela* honeyeaters found on Makira in the Solomon Islands (*Myzomela cardinalis pulcherrima* and *Myzomela tristrami*) represent an excellent system for investigating the consequences of signal divergence for agonistic interactions before and after secondary contact. These species are sympatric in secondary, lowland habitats along coastal Makira, but a review of collection records indicated that secondary contact between the two was recent, with *M. cardinalis* colonizing Makira from the nearby satellite islands of Ugi and/or Three Sisters around the turn of the 20th-century (Mayr and Diamond 2001; Diamond 2002) (Fig 3.1a). The two species are ecologically similar, and often feed in the same flowering trees in sympatry (Diamond 2002), suggesting that competition for resources should play a prominent role in interspecies interactions. In contrast, the species differ notably in plumage color: *M. cardinalis* possesses red carotenoid-based plumage that is brighter and more extensive in males, while both sexes of *M. tristrami* are fully melanic (Fig. 3.1b). Accordingly, plumage color may act as a signal mediating aggressive interactions within and between species in this system.

Several studies have failed to find a link between plumage divergence and aggressive response in birds, with males exhibiting similar levels of aggression toward conspecifics and heterospecifics (Stein and Uy 2006; Flockhart and Wiebe 2009; Greig et

al. 2015). However, in most systems, males display increased aggression towards rivals that more closely resemble themselves, as competition for mates and resources are predicted to be greatest between conspecifics (Mikami et al. 2004; Seehausen and Schluter 2004; van Doorn et al. 2004; Uy et al. 2009a; Uy et al. 2009b). Under this hypothesis, plumage divergence in *Myzomela* will be associated with lower levels of male aggression toward heterospecifics relative to conspecifics. Additionally, if competition for mating opportunities is strong, then this hypothesis predicts that intrasexual selection will favor increased aggression between males that compete most strongly for mates (Irwin et al. 2001; Grant and Grant 2002; Balakrishnan and Sorenson 2006; Uy et al. 2009a). Hybridization between the Makira *Myzomela* is highly asymmetric, with all successful hybridization events involving pairings of red female *M. cardinalis* with black male *M. tristrami* (Sardell and Uy 2016). Thus, reproductive competition should drive male *M. cardinalis* to be more aggressive toward heterospecifics than *M. tristrami*, because the latter faces less competition for conspecific mates. Such asymmetric biases in male agonistic response, where males of one species are more aggressive towards conspecifics while males of the other species are equally aggressive toward both, have been observed in several other systems (Pearson and Rohwer 2000; Dingle et al. 2010; McEntee 2014).

Alternatively, aggressive behavior in the Makira *Myzomela* may primarily reflect interspecific competition for resources such as access to flowering trees rather than competition for mates (Qvarnström et al. 2012). In rare cases, such interspecific competition can cause species to exhibit biased aggression toward sympatric heterospecifics. Depending on the system, this bias can reflect increased aggression either

towards a larger, more aggressive species, as in hybridizing cichlids (Dijkstra et al. 2007), or towards a less aggressive, subordinate species, as in reproductively-isolated Australian finches that compete for nesting cavities (Pearce et al. 2011). Biased aggression toward heterospecifics may also result from shared sensory biases towards signals possessed by only one species, as demonstrated in widowbirds (Ninnes and Andersson 2014) and *Sceloporus* lizards (Quinn and Hews 2000; but see Stephenson and Ramírez-Bautista 2012). If sensory biases are important for the evolution and maintenance of plumage signals in *Myzomela*, then we expect allopatric and sympatric populations will exhibit the same biases towards specific signals. Finally, asymmetric hybridization may influence aggressive interactions if receiver responses are genetically determined. In the Makira *Myzomela*, we predict that asymmetric introgression is most likely to result in black "cryptic" hybrids that possess *M. cardinalis* alleles favoring aggression toward red males.

Asymmetric introgression of plumage alleles in this system has resulted in "cryptic hybrids", i.e. birds that possess black plumage typical of *M. tristrami* but *M. cardinalis* mitochondrial haplotypes (Sardell and Uy 2016). Similar introgression of secondary sexual traits between hybridizing species is promoted by interspecific aggression, or lack thereof, in other systems (Greig et al. 2015; While et al. 2015). Accordingly, competition for resources and mating opportunities may also promote phenotypic introgression in the Makira *Myzomela.* In sympatric *Ficedula* flycatchers, selection for signals that elicit reduced aggression from the dominant species drives character displacement for plumage coloration in the subordinate species (Vallin et al. 2012). We hypothesize that introgression of melanic plumage from *M. tristrami* to *M.*

cardinalis may be similarly adaptive in agonistic contexts if two conditions are satisfied. First *M. cardinalis* must be larger than hybrids in sympatry, as size is an important indicator of dominance during agonistic interactions between honeyeaters (Ford 1979; Beehler 1980; Ford and Paton 1982; Diamond et al. 1989). Second, *M. cardinalis* must exhibit reduced aggression toward melanic males compared to males with red plumage.

Using taxidermy mounts, we simulated territorial intrusions to test the hypothesis that divergence between *M. cardinalis* and *M. tristrami* mediates receiver response behavior in this system. By comparing behavioral responses of sympatric males to allopatric males, we also tested whether receiver responses have shifted in response to secondary contact. If so, the known history of this system will provide unique insight into the potential for receiver response to change rapidly as a response to shifting community dynamics (i.e., over the approximately 120 years since *M. cardinalis* colonized Makira). Finally, we tested whether competition between males could have facilitated introgression of visual signals following hybridization in this system, providing important insight into the potential for intrasexual selection to promote adaptive introgression.

Methods

Experimental Stimuli

We created 12 taxidermy mounts to use as experimental stimuli: four male *M. cardinalis* from Kirakira (Makira), four male *M. tristrami* from Kirakira, two male Mottled Flowerpeckers (*Dicaeum tristrami*) from Kirakira, and two male Olive-backed Sunbirds (*Cinnyris jugularis*) from Ugi. These latter two species represent small nectivorous passerines that belong to unrelated families, allowing us to explicitly test for

male responses towards species that compete with *Myzomela* for resources but not mating opportunities. All mounts were created from skins of birds captured in the field during 2013-2014 and humanely euthanized using methods approved by IACUC (University of Miami protocol 15072). Presence of testes was used to confirm that all individuals used for mounts were male. Use of multiple mounts allows us to control for pseudoreplication (Kroodsma et al. 2001). Several previous studies that employed similar experimental setups used four or fewer mounts of each visual stimulus class to successfully test the effect of plumage color on behavioral response (Patten et al. 2004; Uy et al. 2009a; Uy et al. 2009b; Greig et al. 2015).

We used audio stimulus files to entice nearby territorial males to approach within visual proximity of the mount. In contrast to other species of *Myzomela*, *M. cardinalis pulcherrima* and *M. tristrami* are relatively silent, with no consistent territorial song given throughout the day (J.Sardell, unpublished data.). Instead, vocalizations by the Makira *Myzomela* generally fall in three categories: 1) single "chip" notes, most often given by juvenile males, although also given occasionally by adults, 2) harsh "chattering" given primarily during agonistic interactions between individuals, and 3) pre-dawn songs consisting of long bouts of interspersed melodic "chip" and "chatter" syllables, which are only given in the half-hour immediately preceding sunrise. *Myzomela* vocalizations were recorded in the field using a Marantz (Mahwah, NJ) PMD670 digital recorder with a Sennheiser (Old Lyme, CT) shotgun microphone. Because individuals giving pre-dawn songs could not be visually identified to species at the time, recordings of pre-dawn songs were only taken from territories where the identity of the territory-holder had been previously determined. Vocal stimulus files were created using the software program

Raven (Cornell Lab of Ornithology, Ithaca, NY). Calls consisting of either one or more consecutive "chip" notes or "chatter" bouts were isolated from recordings and then repeated with one-to-two second gaps between calls to construct a one-minute series. These series were then combined to create 12 two-minute stimulus files, each consisting of one minute of either repeated "chip" calls or pre-dawn songs followed by one minute of "chatter" calls.

Experimental Design

We repeated experiments for each study population until we had 30 successful trials: 12 with black *M. tristrami* mounts, 12 with red *M. cardinalis* mounts, and 6 with ecological competitors (either *D. tristrami* or *C. jugularis* as noted below). Three trials were conducted with each mount, and each of the 12 vocal stimuli was used for one successful trial with each type of mount.

We conducted experiments either in known *Myozmela* territories or appropriate habitat, avoiding areas within 30m of flowering trees where multiple *Myzomela* had congregated to feed. We placed the mount on an exposed branch 2-5m off the ground and concealed a Radio Shack Mini-Amplifier Speaker (part no. 277-1008) attached to an iPod Nano (Apple Inc.) within 1m of the mount. Each trial lasted eight minutes, comprising two minutes of conspecific vocal stimulus, a two minute intermission, two minutes of the same vocal stimulus, and two minutes of follow-up. A trial was only considered successful if a *Myzomela* approached within 20m of the mount during playback and had an unobstructed view of the mount from its perch. If the first trial elicited no response, we occasionally conducted a second trial, especially if we had previously sighted *Myzomela*

nearby. Unsuccessful trials (i.e. no response to audio stimuli) were ignored for the purposes of evaluating differences in agonistic responses towards conspecific vs. heterospecific visual signals.

All experimental trials were conducted between 15 minutes after sunrise and 10 a.m. by J.M.S., aided by experienced local guides, who hid greater than 10m from the mount and dictated descriptions of behavioral responses into a Philips Voice Tracer 1200 digital recorder. In particular, we noted every time the focal bird flew or vocalized, its position relative to the mount at all times, and any time spent "fighting" with the mount. Focal birds were unbanded, so when more than one bird was present, the bird nearest to the mount was considered the focal bird for purposes of recording behavioral responses. Because birds often responded in pairs, this qualification was most important for experiments that tested responses by *M. tristrami*, a sexually monomorphic species. In contrast, because *M. cardinalis* is sexually dimorphic, we were able to restrict focal observations to responding males only. This experimental limitation hinders cross-species behavioral comparisons, as always focusing on the bird nearest to the mount may overestimate male aggression in *M. tristrami*. However, we note that male *M. cardinalis* consistently were more aggressive than females when both sexes were present during experiments. Responses from juvenile birds were excluded, as both species possess delayed plumage maturation allowing for identification of juveniles.

Study Populations

We conducted experiments in five populations of *Myzomela*, representing allopatric and sympatric populations of both species (Fig. 3.1a). Experiments with

sympatric birds of both species were conducted in heavily-disturbed garden and coconut plantation areas in Kirakira, Makira between 4 August - 25 August 2014. We also separately provide data from 17 additional experimental trials involving *M. cardinalis*, including 15 times where *M. cardinalis* males responded during trials that targeted *M. tristrami* with appropriate vocalizations, and two trials in which incorrect pairings of mount and *M. cardinalis* vocal stimulus were accidentally used. Flowerpecker (*D. tristrami*) mounts were used as controls for both sympatric populations, as this species is much more common than *C. jugularis*, a recent colonizer of Makira (Sardell 2016).

Experiments testing the territorial responses of allopatric *M. cardinalis* were conducted in the village of on Towarodo on Ugi between 5 – 8 Sept. 2014 and 1 – 2 July 2015. Due to equipment failure, only 27 trials were successfully completed (11 with each *Myzomela* mount, and 5 with *C. jugularis*). *C. jugularis* mounts were used to assess behavioral responses to ecological competitors in this population, as this species is common on Ugi, whereas *D. tristrami* is absent.

We initially conducted experimental trials to assess behavioral responses of allopatric *M. tristrami* in interior Makira between 29 Aug – 2 Sept. 2015, but were limited to only 19 trials due to time constraints (15 on ridges near Hauta, elevation 475-700m, and 4 in Na'ara, elevation 50m). Individuals in this population exhibited little aggression toward any mount relative to observations from sympatric individuals, and we suspected that differences in habitat could have been responsible for lower levels of aggression in this population. Notably, *Myzomela* typically dwell in forest canopy, which is higher in primary rainforest in interior Makira than in the secondary vegetation around Kirakira. Accordingly, mounts placed in the forest understory may not have been

perceived as territorial intruders in the former population. Therefore, between 19-23 July 2015, we conducted a full set of 30 trials on allopatric *M. tristrami* on the island of Santa Catalina in secondary habitats (i.e., gardens and coconut plantations) that were similar to those in Kirakira and Ugi. Our main analysis presents the results of trials involving the Santa Catalina population, but we also present in the supplementary materials the results from the interior Makira population, which do not differ qualitatively from experimental data for Santa Catalina.

We observed breeding behavior among *Myzomela* during the testing period in all populations, with the exception of interior Makira. However, many trials in interior Makira elicited responses by pairs of *M. tristrami*, consistent with territorial breeding. Accordingly, we believe that differences in breeding phenology during the time of testing were unlikely to be a major factor driving differences in aggressive behavior between populations.

Analysis of Experimental Results

We used the following metrics to quantify behavioral responses during each trial: (1) time spent physically "attacking" the mount as a percent of total time between the bird's initial response and the end of the trial, (2) percent of remaining time that bird spent within 15 cm of mount, (3) percent of remaining time spent within 2m of mount, (4) percent of remaining time spent between 2-5m of mount, (5) percent of remaining time spent between 5-10m of mount, and (6) percent of remaining time spent between 10-20m. These measures are cumulatively exclusive to reduce systematic correlation, e.g., the last measurement represents the time the bird spent between 10-20m as a percent of

the total time the bird was greater than 10m from the mount after the initial response and before the end of the trial (excluding any time spent within 10m which is represented by measures 1-5). Use of relative rather than absolute measures of time allowed us to minimize the effect of differences in response latency to audio signals between trials. Additionally, we measured (7) closest distance of approach to the mount, (8) total number of calls given during the trial, (9) number of "aggressive flights", defined as any flight in which the focal bird flew directly over or under the mount within 2m, and (10) number of other "non-aggressive flights". Because we expect these measures of behavioral response to be correlated, we used principal component analysis in R (R development team) to collapse them into orthogonal measures of total aggression. Finally, we performed a mixed-model, nested ANOVA on log-transformed PC1 to test for effects of focal species, presence of heterospecifics (i.e., allopatric vs sympatric), and mount type on behavioral aggression, with specific mount nested within mount type. All interactions were tested for significance, and non-significant interactions removed from the model. Residual plots and Shapiro-Wilk normality tests confirmed that log(PC1 + 2) better satisfied the assumptions of data normality than untransformed data ($P = 0.009$ vs. 2.1×10^{-14}). This analytical approach is consistent with several previous studies that experimentally tested response of males toward different vocal and audio stimuli (Grant and Grant 2002; Newman et al. 2006; Uy et al. 2009a; Uy et al. 2009b).

Morphological analysis

Relative size strongly influences the outcomes of agonistic interactions between honeyeaters (Ford 1979; Beehler 1980; Ford and Paton 1982; Diamond et al. 1989). We

used mist-netting to capture 65 adult male *Myzomela* from the area of sympatry on Makira in the field between 2011-2014, including 38 *M. cardinalis*, 22 *M. tristrami*, and 5 hybrids (including both phenotypic and cryptic hybrids). JMS obtained standard morphometric measures from each bird, including mass, bill length (measured from tip to start of nares), bill width at start of nares, bill height at start of nares, tarsus length, unflattened wing chord, and tail length. We then used principal component analysis to collapse the measurements into a single "size" score (per Rising and Somers 1989; Freeman and Jackson 1990). Blood samples were also collected from the brachial vein of each individual, and preserved in lysis buffer (Longmire et al. 1997) until return from the field, upon which DNeasy Blood & Tissue Kits (Qiagen) were used to extract genomic DNA. We used primers and techniques described in Han et al. (2009) to molecularly sex each bird to ensure that only males were included in our dataset. To identify cryptic hybrids, i.e., birds that possessed melanic plumage and *M. cardinalis* mtDNA, we Sanger sequenced each individual at a single mitochondrial marker, NADH dehydrogenase subunit 2 (*ND2*), using the primers H6313 and L5216 (Sorenson et al. 1999), and compared the sequences to species-specific *ND2* haplotypes identified in Sardell and Uy (2016). Finally we used a Mann-Whitney *U* test to determine whether differences in mean male mass between *M. cardinalis*, *M. tristrami*, and hybrids in sympatry were statistically significant.

Results

The first two principal components of behavioral response data from simulated territorial intrusions explain 34.9 percent and 17.0 percent of total variation, respectively.

We applied PC1 as our primary measure of agonistic response towards rivals, because this component is strongly loaded with expected measures of aggression, including time spent attacking or close to the mount, approach distance, and number of "aggressive" flights (Supp. Table S3.1).

In sympatry, males of both *Myzomela* species exhibited biased overall aggression toward red *M. cardinalis* mounts (Fig. 3.2). Furthermore, both species were more likely to attack *M. cardinalis* mounts in sympatry (Supp. Fig. S3.1), albeit not significantly so. The ANOVA for log-transformed PC1 revealed that three factors are significant predictors of agonistic response to territorial intrusions: mount color, presence of heterospecifics (i.e., sympatry vs. allopatry), and the interaction between the two (Table 3.1). Aggression was significantly higher in sympatric populations than allopatric populations. The statistical significance of the interaction between mount color and presence of heterospecifics indicates that sympatry significantly affects the bias for higher aggression toward red plumage signals. Mount ID nested within mount color was not significant, nor were other interaction terms. There was also no support for focal species as a significant predictor of overall level of aggression. However, our experimental protocol may overestimate relative aggression by *M. tristrami* compared to *M. cardinalis*, due to an inability to identify the sex of responding birds in the former species. Furthermore, this experiment only tests aggressive response towards visual stimulus by males that initially responded to audio stimulus. Therefore, we are unable to detect any differences in aggression in which males of one species are more likely to avoid agonistic interactions entirely. Use of untransformed data in an ANOVA did not qualitatively change any of these results (Supp. Table S3.2). Additionally, behavioral

response data from sympatric *M. cardinalis* responding to experiments targeting *M. tristrami* and for allopatric *M. tristrami* from the Makira highlands are qualitatively similar to results from targeted sympatric *M. cardinalis* and allopatric *M. tristrami* from Santa Catalina, respectively, providing further support for these findings (Supp. Figure S3.2).

M. cardinalis males are significantly bigger than *M. tristrami* males in sympatry ($W = 737$, $p = 1.3 \times 10^{-7}$) (Fig. 3.4), suggesting that the former should be dominant in any agonistic interactions between the two species. Hybrids, including cryptic hybrids, are intermediate in size, but differences in mean size from either parental population are not significant (Hybrids vs. *M. cardinalis*: $W = 146$, $p = 0.054$; Hybrids vs. *M. tristrami*: $W = 74$, $p = 0.2572$), likely due to the small sample of adult male hybrids (n=5).

Discussion

We found that sympatric populations of both species exhibit aggression biases toward red *M. cardinalis* mounts over melanic *M. tristrami* mounts. Aggression biases among *M. cardinalis* towards conspecifics was expected, as competition for mates and resources are often expected to be greatest between males of the same species (Mikami et al. 2004; Seehausen and Schluter 2004; Uy et al. 2009a). In contrast, aggression biases toward red *M. cardinalis* mounts by *M. tristrami* represents an unusual example of heterospecifics eliciting a stronger response than conspecifics. These species differ most notably in plumage, suggesting that coloration acts as an important signal mediating agonistic contests between sympatric *Myzomela* on Makira. Several previous studies similarly interpreted aggressive response towards taxidermy mounts of different birds

species as a measure of response to plumage signal variation (McDonald et al. 2001; Patten et al. 2004; Stein and Uy 2006; Ligon and Hill 2009; Uy et al. 2009a). Still, we cannot eliminate the possibility that other traits which differ between mounts, such as size, are more important than plumage in determining receiver response toward simulated territorial intrusions.

Aggression Biases in Terms of Reproductive and Ecological Competition

Observed patterns of behavioral responses in the Makira *Myzomela* are opposite to those predicted by reproductive competition. *M. cardinalis* females hybridize with *M. tristrami* males, but *M. tristrami* females never successfully hybridize with *M. cardinalis* males (Sardell and Uy 2016). Accordingly, *M. tristrami* compete much more strongly with conspecifics than heterospecifics for mating opportunities, which should favor increased aggression toward conspecifics, while *M. cardinalis* should show elevated aggression toward *M. tristrami* males with whom they compete for conspecific mating opportunities. Instead, *M. tristrami* males exhibit elevated aggression towards *M. cardinalis* while *M. cardinalis* males are more likely to ignore *M. tristrami*. The similar levels of aggression exhibited by *Myzomela* males toward *M tristrami* and unrelated ecological competitors further suggests that competition for mates is less important than ecological competition for limited resources such as high-quality food sources or breeding territories in influencing male aggressive response in this system. These results indicate that, although male response towards signals sometimes reflects reproductive isolation in birds (Baker and Baker 1990; Baker 1991; Patten et al. 2004), this

relationship does not hold in all species (see also Seddon and Tobias 2010; Greig et al. 2015).

While rare, biased aggression towards heterospecifics has been experimentally observed in at least two other systems. Blue cichlid fish exhibit aggression biases toward larger, dominant red males, but only in hybridizing populations (Dijkstra et al. 2007). This pattern is similar to that observed in the Makira *Myzomela* , where aggressive response is greater towards the larger, more dominant *M. cardinalis* (Diamond 2002) relative to *M. tristrami* and smaller ecological competitors, which represent less of a threat in terms of resource competition. In contrast, long-tailed finches (*Poephila acuticauda*) exhibit preferential aggressive towards subordinate Gouldian finches (*Erythrura gouldiae*) with which they compete for limited nesting cavities but not mates, and do not differ in size (Pearce et al. 2011). Sexual dimorphism may also play a role in promoting aggression biases in this system. Because plumage patterns differ between *M. cardinalis* males and females, they can act as a signal that allows receivers to reliably evaluate whether an intruder is a rival male. In contrast, *M. tristrami* is sexually monomorphic, so an intruding bird possessing melanic plumage may represent either a rival male or a potential mate.

M. cardinalis are significantly larger than *M. tristrami*, which strongly suggests that the former species should be dominant during any interspecies agonistic interactions (Ford 1979; Beehler 1980; Ford and Paton 1982; Diamond et al. 1989), yet species was not a significant predictor of aggressive response during our experiments. However, we caution against interpreting these results to conclude that both species are equally aggressive. First, biases in our experimental protocol due to differences in sexual

dimorphism will lead us to overestimate aggression of sexually monomorphic *M. tristrami* relative to *M. cardinalis.* Furthermore, our experiment only tested for differences in response among males that responded positively to audio stimulus. Therefore we cannot detect differences in aggression that manifest as a decreased likelihood of responding to audio stimulus. Accordingly, our data do not refute the hypothesis that aggression biases towards *M. cardinalis* in this system reflect increased aggressive response toward more aggressive males.

Aggression Biases May Drive Plumage Introgression

We hypothesize that aggression biases toward *M. cardinalis* in sympatry drive adaptive introgression of melanic plumage in this system (Sardell and Uy 2016). Because individuals with hybrid ancestry are intermediate in size between *M. cardinalis* and *M. tristrami*, selection should favor signals that help these individuals avoid costly agonistic interactions with larger, dominant *M. cardinalis*. In the Makira *Myzomela*, selection for avoidance favors melanic plumage in smaller individuals, such as those with hybrid ancestry, with red carotenoid-based plumage continuing to potentially act as a signal of dominance in larger *M. cardinalis* males. Avoidance of interspecific aggression similarly drives character displacement in plumage in *Ficedula* flycatchers (Vallin et al. 2012), and delayed plumage maturation in *Passerina* buntings (Greene et al. 2000). In both of these systems, plumage patterns that elicit reduced aggressive response enable subordinate individuals to exploit high quality. Moreover, asymmetric behavioral responses also drive introgression of plumage signals in manakins (Stein and Uy 2006) and fairy-wrens (Greig

et al. 2015) due to female mate choice preferences. However, this is the first study that links avoidance of interspecific aggression with adaptive introgression of plumage traits.

By favoring introgression of plumage traits between *Myzomela*, intrasexual selection in the Makira *Myzomela* impedes the ability of the same traits to act as honest signals of reproductive compatibility between individuals. Specifically, genetic data indicate that post-zygotic incompatibilities result not only in absolute reproductive isolation between females with *M. tristrami* mitochondrial haplotypes and *M. cardinalis* males, but also in a lack of backcrossing between the females and individuals with hybrid ancestry, including cryptic hybrids (Sardell and Uy 2016). Accordingly, female *M. tristrami* that use melanic plumage to recognize potential mates risk mating with cryptic hybrids with which they are reproductively incompatible, thereby favoring opposing evolutionary trajectories of signal response in males and females.

Comparisons of Aggression in Sympatric and Allopatric Populations

Our results indicate that biased aggression of sympatric *M. tristrami* toward *M. cardinalis* is a result of sympatry, with shifts in behavioral responses towards heterospecific occurring sometime during the approximately 120 years since secondary contact. Statistically significant biases were not detected in either of the two allopatric populations of *M. tristrami* that we tested, and observed attacks in these populations all involved black mounts (n=1 for each population). Accordingly, we reject the hypothesis that biased aggression towards *M. cardinalis* is due to ancestral sensory bias (e.g., preferential aggression toward red carotenoid-based plumage). Environmentally-mediated differences in signal conspicuousness may alternatively be responsible for the

behavioral differences between populations. However, experiments in Kirakira, Ugi, and Santa Catalina were all conducted in similar types of habitats and light profiles do not differ significantly between these three sites (J.A.C. Uy, unpublished data). Although allopatric *M. cardinalis* from Ugi only attacked conspecifics (Supp. Fig. S3.1), there was no statistically significant evidence for biased aggression toward heterospecifics in this population, in contrast to sympatric populations. Sympatry may favor increased aggressiveness if secondary contact results in increased overall competition. Alternatively, aggression is correlated with dispersal ability in other birds (Duckworth and Badyaev 2007), and a link between dispersal ability and aggression could have pleiotropically selected for increased aggression during colonization of Makira by *M. cardinalis*.

Hybridization can also potentially result in adaptive introgression of alleles associated with receiver responses, but this does not appear to drive aggression biases in the Makira *Myzomela*. We successfully captured four *M. tristrami* males that exhibited strong aggressive response to simulated territorial intrusions, including three that were aggressive towards *M. cardinalis* mounts. Of these four males, only one, which exhibited aggression toward *M. cardinalis*, possessed hybrid ancestry based on a mismatch between plumage phenotype and mitochondrial haplotype. These preliminary results indicate that biased aggression towards *M. cardinalis* among *M. tristrami* is not predominantly driven by introgression of associated alleles from *M. cardinalis*.

The differences in aggressive response between allopatric and sympatric populations of *Myzomela* indicate that population-level shifts in receiver response can occur over short time scales. If receiver response is genetically controlled, then these

shifts may represent strong selection for increased aggression towards *M. cardinalis* in sympatric populations (Johnstone 1997). Alternatively, aggression biases in this system may represent learned behavior based on individuals' prior life experiences, as associative learning has been shown to influence male response during agonistic interactions in other species of birds (Richards 1979; Hansen and Slagsvold 2003; Jankowski et al. 2010). Further research into the ontogeny of aggressive response behavior in this system is needed to determine which of these mechanisms is responsible for the behavioral shift that followed secondary contact in this system

Conclusions

Signal divergence is commonly assumed to promote increased discrimination between species and decreased frequency of interspecific aggression between males, but the results of this study demonstrate that signal divergence can sometimes lead to elevated male aggression towards heterospecifics relative to conspecifics. Moreover, we have shown that receiver response to these signals is a highly labile trait that can shift rapidly following secondary contact. Simultaneously, aggressive interactions between males can favor the introgression of signals, even if such signal introgression is maladaptive for signal use in the context of mate choice. Combined, these results provide novel and important insight into the potential evolutionary consequences of intrasexual selection for population divergence and speciation in hybridizing taxa.

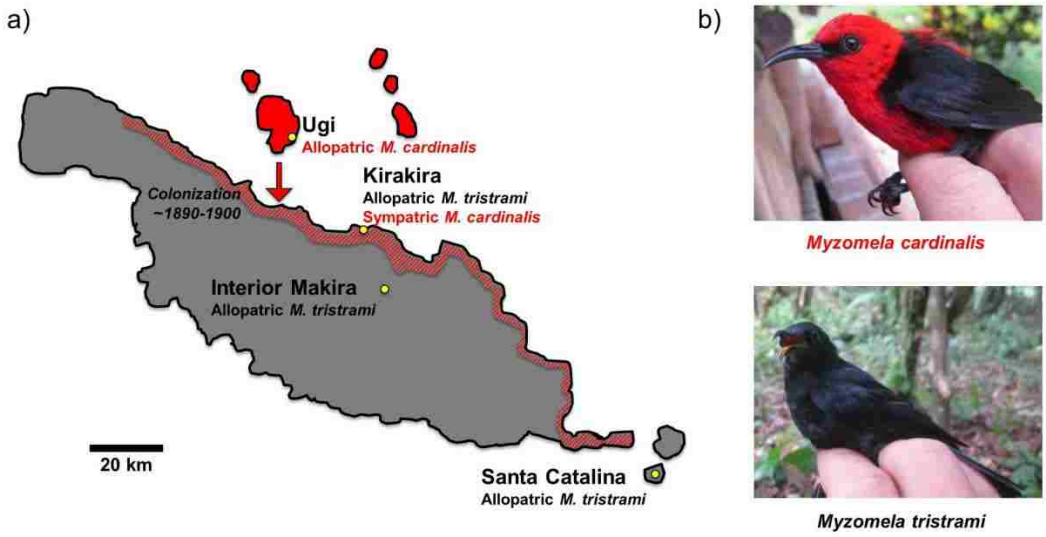


Figure 3.1: (A) Map of Makira and satellite islands showing approximate range of *M. cardinalis* and *M. tristrami*. Study populations are denoted by yellow dots. (B) Typical plumage of adult male *M. cardinalis* (top) and adult male *M. tristrami* (bottom).

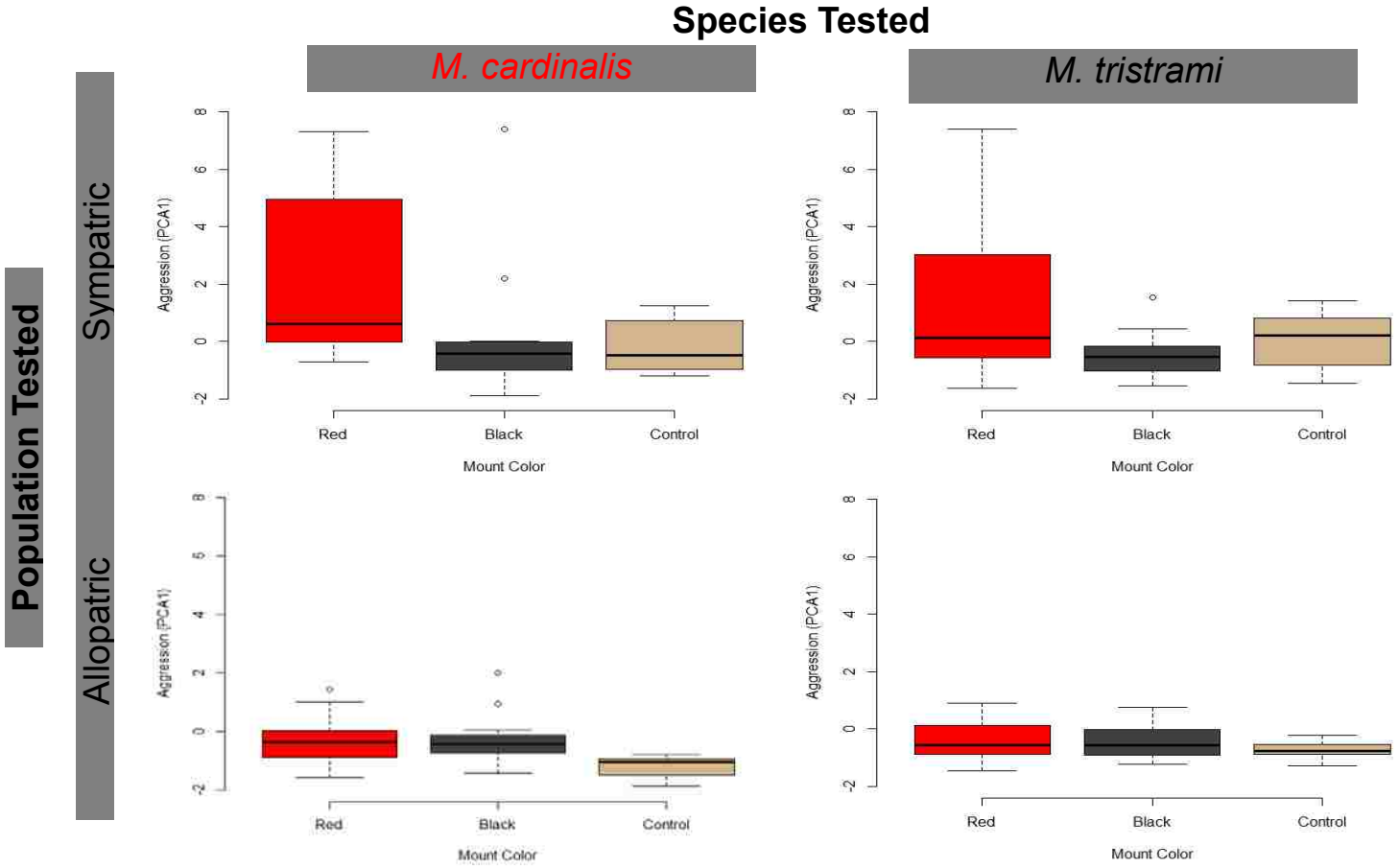


Figure 3.2: Aggressive response by *Myzomela* towards taxidermy mounts of red *M. cardinalis* males, black *M. tristrami* males, and unrelated ecological competitors. Graphs represent different populations tested: sympatric *M. cardinalis* from Kirakira (upper left), sympatric *M. tristrami* from Kirakira (upper right), allopatric *M. cardinalis* from Ugi (lower left), allopatric *M. tristrami* from Santa Catalina (lower right). Positive principal component values indicate more intensive response. Box plots represent 25th and 75th percentile of aggression scores towards each mount with mean aggression represented by solid black line.

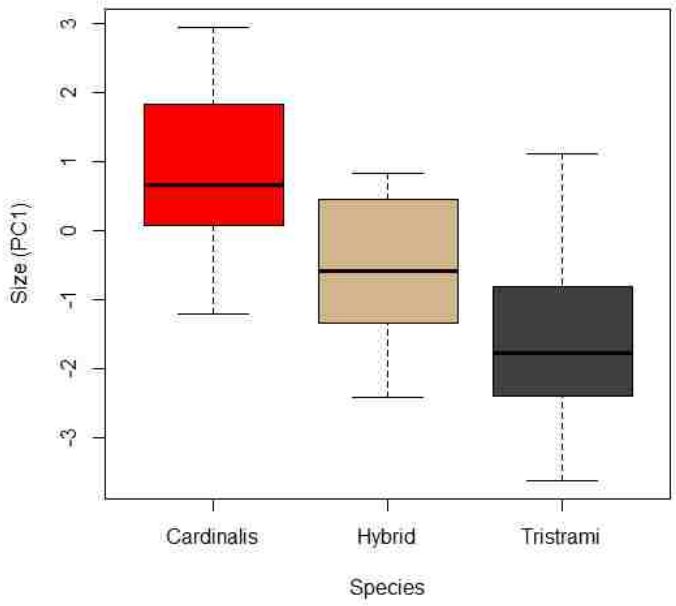


Figure 3.3: Distributions of adult male sizes in sympatric populations of *Myzomela* on Makira. Based on sample sizes of 38 *M. cardinalis*, 22 *M. tristrami*, and 5 hybrids (including phenotypic hybrids and "cryptic hybrids", i.e., black birds possessing *M. cardinalis* mitochondrial haplotypes). Box plots represent 25th and 75th percentile of aggression scores towards each mount with mean aggression represented by solid black line.

Table 3.1: ANOVA of aggressive response (log-transformed PC1) of *Myzomela* males towards simulated territorial intrusion using different color mounts. All nonsignificant interactions, including mount ID nested within mount color were removed from the model. Results of Tukey's HSD test presented for pair-wise comparisons of mount color.

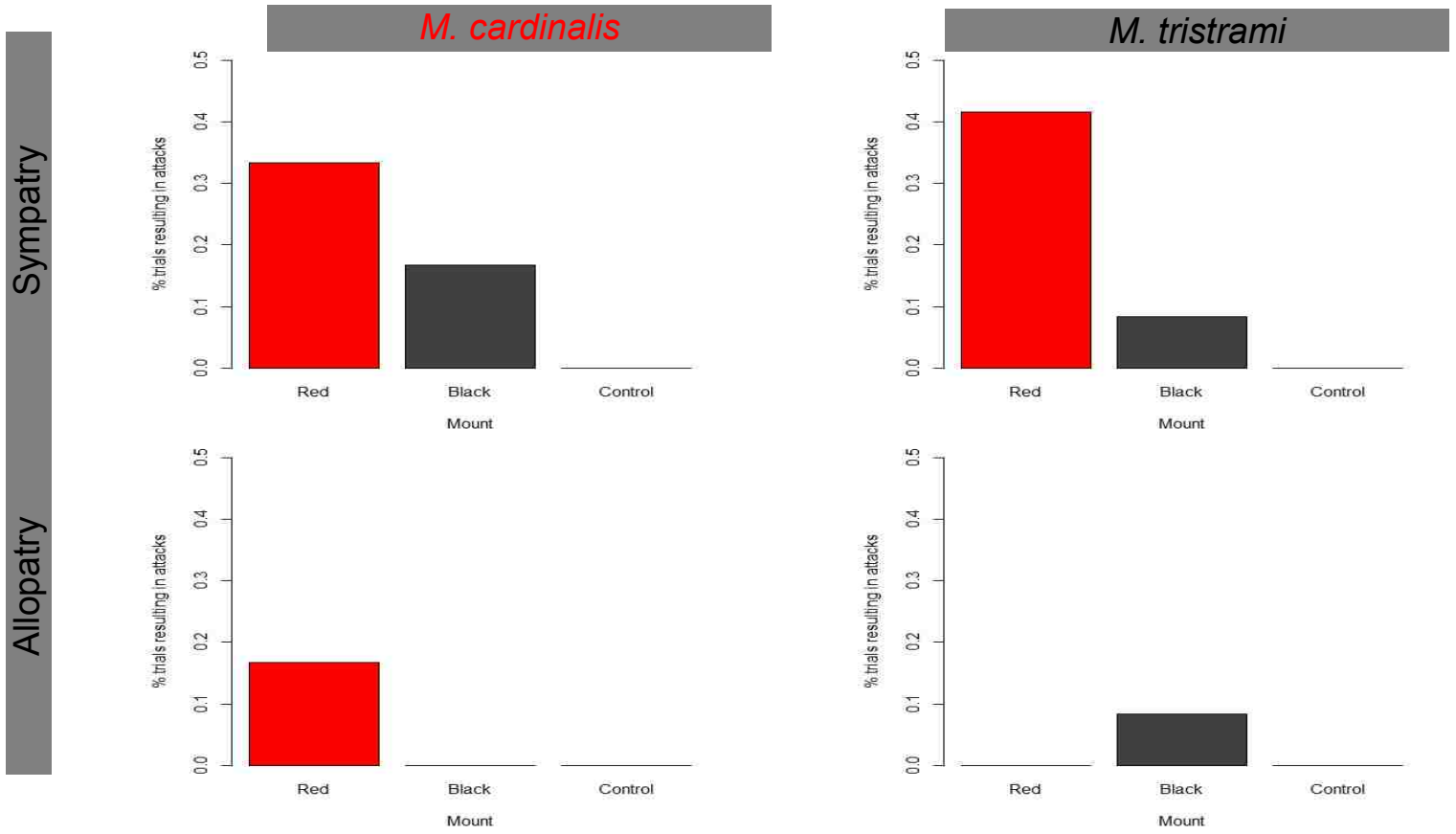
Factor	df	Sum of Squares	F	*P*
Focal species	1	0.00	0.000	0.9881
Mount color	2	5.05	7.344	**0.0078**
Red vs. black				**0.0360**
Red vs. control				**0.0169**
Black vs. control				0.7297
Presence of heterospecifics (sympatry vs. allopatry)	1	3.69	7.344	**0.0081**
Interaction: Mount color x Presence of heterospecifics	2	3.93	3.915	**0.0228**
Residuals	110	55.26		

Supplementary Table S3.1: Loadings for first three principal components of behavioral responses towards simulated territorial incursions.

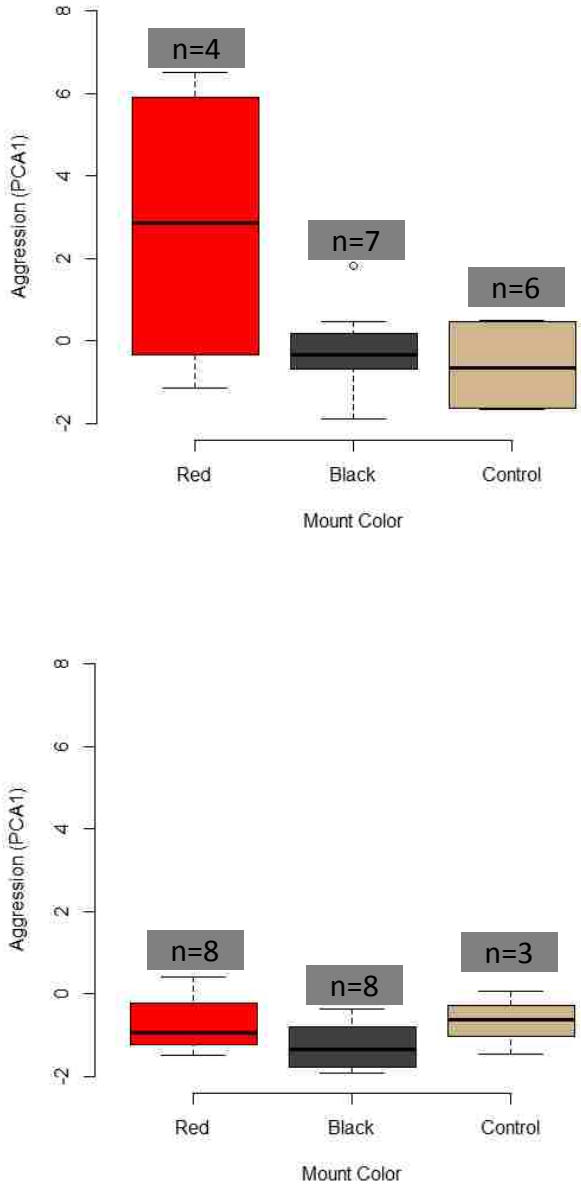
	PC1	PC2	PC3
Standard deviation	1.868	1.303	1.133
Proportion of variance	0.349	0.170	0.128
Loadings:			
% time attacking mount after initial response	0.417	0.302	0.161
% remaining time <15cm from mount	0.470	0.235	0.200
% remaining time 15cm-2m from mount	0.396	0.285	0.080
% remaining time 2-5m from mount	0.319	-0.130	-0.338
% remaining time 5-10m from mount	0.159	-0.521	-0.135
% remaining time 10-20m from mount	0.065	-0.518	0.520
Nearest approach distance	-0.338	0.122	0.377
Number of calls	-0.030	-0.269	0.076
“Aggressive” flights	0.297	-0.188	0.505
“Non-aggressive” flights	0.337	-0.304	-0.350

Supplementary Table 3.2: ANOVA of aggressive response (untransformed PC1) of *Myzomela* males towards simulated territorial intrusion using different color mounts. All nonsignificant interactions, including mount ID nested within mount color were removed from the model. Results of Tukey's HSD test presented for pair-wise comparisons of mount color.

Factor	df	Sum of Squares	F	*P*
Focal species	1	2.2	0.765	0.3836
Mount color	2	34.8	5.920	**0.0036**
Red vs. black				**0.0143**
Red vs. control				**0.0118**
Black vs. control				0.8169
Presence of heterospecifics (sympatry vs. allopatry)	1	37.7	12.827	**0.0005**
Interaction: Mount color x Presence of heterospecifics	2	22.1	3.764	0.0262
Residuals	110	323.1		



Supplementary Figure S3.1: Percent of trials that resulted in attacks towards taxidermy mounts of red *M. cardinalis* males, black *M. tristrami* males, and unrelated ecological competitors. Graphs represent different populations tested: sympatric *M. cardinalis* from Kirakira (upper left), sympatric *M. tristrami* from Kirakira (upper right), allopatric *M. cardinalis* from Ugi (lower left), allopatric *M. tristrami* from Santa Catalina (lower right).



Supplementary Figure S3.2: Aggressive response by *Myzomela* towards taxidermy mounts of red *M. cardinalis* males, black *M. tristrami* males, and unrelated ecological competitors. Top graph represents *Myzomela cardinalis* that responded to experiments targeting *M. tristrami*. Bottom graph represents experiments conducted on allopatric *M. tristrami* from interior Makira. Positive principal component values indicate more intensive response. Box plots represent 25th and 75th percentile of aggression scores towards each mount with mean aggression represented by solid black line.

-

Chapter 4

Rapid ecological character displacement follows secondary contact in island birds

Background

Ecological character displacement ("ECD"), the phenomenon in which selection for reduced competition drives the evolution of increased morphological differentiation between sympatric species, has been hypothesized to be an important driver of phenotypic divergence (Brown and Wilson 1956; Schluter and McPhail 1992; Pfennig and Pfennig 2009). ECD has remained controversial (Stuart and Losos 2013), partly because several models predict that it should only occur under limited circumstances (e.g., Slatkin 1980; Abrams and Matsuda 1994; but see Doebeli 1996; Drossel and McKane 1999), but evidence for the phenomenon exists in a variety of taxa (summarized in Grant 1972; Schluter 2001; Dayan and Simberloff 2005). ECD is most commonly assumed to be driven by resource partitioning, i.e., selection for phenotypes that improve organisms' ability to exploit unoccupied ecological niches (Fjeldså 1983; Schluter et al. 1985; Dayan et al. 1989; Schluter and McPhail 1992; Jones 1997; Reifová et al. 2011). In contrast, much less attention has been given to the potential for agonistic interactions between species to directly drive ECD via interference competition, the phenomenon in which one species actively prevents another from exploiting high-quality resources (Jaeger et al. 2002; Grether et al. 2009; Grether et al. 2013). Putative ECD in size driven by interference competition has been identified in lizards (Melville 2002), carnivorous mammals (Whitehead and Walde 1993; Sidorovich et al. 1999; McDonald 2002), and birds (Diamond et al. 1989). Notably, this latter study identified putative ECD between

species of nectivorous *Myzomela* honeyeaters (*M. pammelaena* and *M. sclateri*) that are believed to have achieved sympatry on a volcanic island during the mid-17th century. However, the date of secondary contact in Diamond et al. (1989) was indirectly inferred from geological evidence, and ECD in that system could not be distinguished from the alternative hypothesis that differences in sizes were a byproduct of founder effects. In this study, we test for evidence of ECD between two different species of *Myzomela* honeyeaters, *M. cardinalis* and *M. tristrami*, which achieved historically recent secondary contact on Makira in the Solomon Islands.

The Makira *Myzomela* represent an excellent system for testing whether ECD can occur over short time scales. The two species exist in sympatry along the coast of Makira, allopatric populations of *M. cardinalis* occur on the satellite islands of Ugi and Three Sisters, and allopatric populations of *M. tristrami* persist in the montane, forested interior of Makira, as well as the satellite islands of Santa Catalina and Santa Ana (Fig 4.1) (see Sardell and Uy 2016 for more description). These species hybridize in sympatry (Sardell and Uy 2016), which could promote phenotypic convergence between them (Grant et al. 2004; Heliconius Genome Consortium 2012; Stern 2013). Alternatively, secondary contact may have resulted in selection for ECD in this system. Notably, interference competition is expected to be high between these species because they feed in the same flowering trees, a limited food source, and are highly aggressive toward one another, with male *M. cardinalis* being dominant (Diamond 2002). Furthermore, size is an important driver of interspecies interactions in honeyeaters. Specifically, larger species dominantly exclude smaller ones from high-quality nectar sources, whereas smaller species can exploit poor-quality nectar sources due to their lower energy requirements (Ford 1979;

-

Beehler 1980; Ford and Paton 1982; Diamond et al. 1989). If interference competition has driven ECD in this system, then we predict that it would predominantly favor reduced body size in *M. tristrami*, the subordinate species, similar to asymmetric ECD patterns found in lizards with high degrees of interference competition (Melville 2002). Taxon cycle theory also predicts similar patterns of asymmetric ECD, with competition selecting for smaller body size in native species following secondary contact with larger invaders (Roughgarden and Pacala 1989).

The Makira *Myzomela* offer an unusual opportunity to test the rate of evolution in natural populations. Historical collection records indicate that secondary contact between *M. cardinalis* and *M. tristrami* occurred naturally around the turn of the 20th century, with *M. cardinalis* colonizing Makira from Ugi and/or Three Sisters (Diamond 2002). Studies from introduced populations provide evidence that body size can evolve over similar time scales due to character release or size convergence (Yom-Tov et al. 1999; Simberloff et al. 2000), and observations of ECD in the Makira *Myzomela* would provide additional data supporting the power of selection to drive rapid evolution of phenotypic differentiation in natural vertebrate populations.

Methods

We captured 283 *Myzomela* from six regions of Makira-Ulawa province, Solomon Islands during annual field expeditions between 2012 and 2015 (Fig. 4.1b). Two of these regions, Ugi and Three Sisters contain allopatric populations of *M. cardinalis*; two regions, Santa Catalina and highland Makira, contain allopatric populations of *M. tristrami*; and two regions, North Makira and Star Harbour, contain

-

populations of both species, with the former site representing an older area of sympatry relative to the latter (Fig. 4.1b). Nearly all birds were captured by erecting mist-nets in areas where *Myzomela* congregated, often at flowering trees.

Seven morphological measurements were obtained from each captured *Myzomela*. Mass was measured with a 60g spring scale (Pesola AG, Switzerland). Bill length from tip of bill to start of nares, bill height at the start of nares, bill width at the start of nares, and tarsus length were obtained based on the median of three measurements for each bird using 150mm dial calipers (Swiss Precision Instruments, Switzerland). Unflattened wing chord length and tail length were measured with a 15cm wing rule (AVINET, Dryden, NY). JMS measured all birds to eliminate observer effects. We took multiple voucher photos of each individual to record its plumage. Finally, blood samples were taken from the brachial vein of each bird and preserved in lysis buffer (Longmire et al. 1997), and genomic DNA was extracted in the lab using DNeasy Blood & Tissue Kits (Qiagen).

We assigned birds to species based on plumage phenotypes as described in Sardell and Uy (2016). Birds possessing abnormal combinations of red and black plumage (e.g., a black body and red back or a black head with patches of red feathers) were classified as phenotypic hybrids. Similarly, birds were assigned to age classes (i.e., adult vs. juvenile) based on plumage. Adult *M. tristrami* are fully melanic with entirely black bills, whereas juveniles possess gray plumage on their underparts and yellow bills. Adult *M. cardinalis* possess distinct bright red plumage and black gapes that distinguishes them from juveniles with duller plumage and yellow gapes. Birds were sexed using molecular PCR-based methods and primers described in Han et al. (2009). Accuracy of molecular sexing was confirmed by checking for consistency with sexing

-

based on plumage phenotype, as well as presence/absence of testes in vouchered specimens.

We expected the seven morphological measurements to be highly correlated, so we used principal component analysis to collapse them into a single "body size" score (per Rising and Somers 1989; Freeman and Jackson 1990) based on all individuals in the data set. The first principal component (PC1) of morphological data explained 58.5 percent of total morphological variation, with all seven measures of size loading equally and in the expected direction for a correlation with body size (Supp. Table S4.1). Use of a single PC1 metric for all individuals allows for direct comparisons of size between sexes. However, morphology may differ between males and females, so we also conducted separate analyses using PC1 values calculated separately for each sex. For both sets of PC1 data, we fitted linear models to the data using the `lm` function in R v.3.2.3 (R Development Team) to test if differences in mean size between populations were statistically significant. We ran two sets of models for males: one which included adults and juveniles with both age and population as fixed factors, and one which included only adults. Because sample size of adult females was very low for some populations, we were only able to test for differences in mean female size between populations using pooled adult and juvenile data, including both age and population as factors in the model.

Results

For *M. cardinalis*, body size as measured by PC1 was significantly larger for allopatric males from Three Sisters than males from other populations (Table 4.1). PC1 for sympatric male *M. cardinalis* from Star Harbour were significantly greater than PC1

-

for males from North Makira (sympatric) and Ugi (allopatric). PC1 for males from the latter two populations were statistically equivalent. Adult males were significantly larger than juveniles. The same patterns held when limiting the analysis to adult males (Supp. Table S4.2). Adult female *M. cardinalis* were also significantly larger than juveniles, and allopatric females from Three Sisters were significantly larger than those from other populations (Table 4.1). Unlike males, sympatric female *M. cardinalis* from Star Harbour were not statistically different in size from females from North Makira or Ugi.

For *M. tristrami*, male body sizes as measured by PC1 in the two allopatric populations, Highland Makira and Santa Catalina, were statistically equivalent (Table 4.2). Male PC1 in both of these populations was significantly greater than PC1 for sympatric male *M. tristrami* from North Makira. Sympatric male *M. tristrami* from Star Harbour were intermediate in size, but not significantly different from any of the three other populations. Adult male *M. tristrami* did not differ significantly in size from juveniles. These patterns continued to hold when the analysis was limited to adult males only (Supp. Table S4.3). In contrast, juvenile *M. tristrami* females were significantly smaller than adults, and mean female size did not different significantly between any of the four populations (Table 4.2).

Analyses which used PC1 values calculated separately for each sex revealed the same patterns of size variation and ECD in males and females as discussed above for PC1 values reflecting all individuals combined (Supp. Tables S4.3-S4.5).

-

Discussion

We found that recent secondary contact has resulted in the evolution of asymmetric ECD in the Makira *Myzomela*, despite ongoing hybridization between the two species, which should favor phenotypic convergence (Grant et al. 2004; Heliconius Genome Consortium 2012). *M. tristrami* males from the population that has been in contact with *M. cardinalis* the longest are significantly smaller than those from allopatric populations, while *M. tristrami* males that have come into contact with *M. cardinalis* during a more recent range expansion are smaller, but not significantly so, as expected if the magnitude of ECD depends on time in sympatry. Several studies have shown that differences in body size can evolve over 100-300 years (e.g.,Diamond et al. 1989; Yom-Tov et al. 1999; Simberloff et al. 2000), but this is the most robust study to date that demonstrated that secondary contact can specifically drive ECD in body size over these time scales, as opposed to size convergence in sympatry or size divergence due to character release in derived allopatric populations.

In contrast to *M. tristrami*, we found no evidence for ECD among sympatric *M. cardinalis* males. Allopatric *M. cardinalis* from Three Sisters were larger than males from other populations, suggesting possible selection for reduced size on Makira if this population was the source for the original colonization. However, we believe that Ugi is a more likely source due to its closer proximity (8km away from Makira versus 20km for Three Sisters). Furthermore, *M. tristrami* vagrants have been observed on Ugi but not Three Sisters (Dutson 2001), indicating greater exchange of migrants between Makira and the former but not the latter. Prior genetic sequencing to examine haplotype sharing between populations did not clarify whether Three Sisters or Ugi was the original source

-

of *M. cardinalis* that colonized Makira (Sardell and Uy 2016). Increased male size in *M. cardinalis* from Star Harbour may be due to general selection for increased sized in this population relative to North Makira, as similar patterns were observed in *M. tristrami*, and may explain why no significant ECD among the latter species was observed in this population. Alternatively, body size is often correlated with increased dispersal ability in birds (Dawideit et al. 2009), so selection may have favored increased size in *M. cardinalis* during its recent range expansion along the coast of Makira.

We hypothesize that asymmetric ECD in the Makira *Myzomela* was driven primarily by interference competition. Larger *M. cardinalis* are predicted to dominate smaller *M. tristrami* during aggressive interactions at flowering trees and prevent the latter species from accessing high-quality nectar sources (Diamond et al. 1989). Smaller size in *M .tristrami* was therefore driven by selection for reduced energy requirements. This hypothesis potentially explains why no ECD was observed in females. Female *M. cardinalis* are statistically equivalent in size to female *M. tristrami*, strongly reducing interference competition between the two, and suggesting that females of both species should be subject to similar levels of interference competition from males. Failure to observe ECD in sympatric females also is consistent with size reductions in sympatric male *M. tristrami* being driven by selection for smaller male size rather than adverse maternal or developmental affects resulting from reliance on low-quality food sources which should affect both sexes.

Similar patterns of asymmetric ECD resulting from interference competition were observed in *Niveoscincus* lizards, where interspecific aggression is hypothesized to select for decreased body size in the smaller species (Melville 2002). This pattern also matches

-

key predictions from taxon cycle theory: that native species on islands will evolve an optimum body size for available resources, that only larger species will be able to successfully invade islands by outcompeting natives for access to these resources, and that these interspecies interactions will result in size displacement in the native species (Roughgarden and Pacala 1989). However, results from the Makira *Myzomela* differ from patterns of putative ECD between *Myzomela* species on Long Island in Papua New Guinea where displacement occurred among both species (Diamond et al. 1989). In contrast to the single colonization on Makira, both species of Long Island *Myzomela* recently colonized the island via long-distance dispersal (Diamond et al. 1989). If long-distance dispersal ability and colonization probability was correlated with increased body size (see Dawideit et al. 2009), then relative increases in body size in the larger species of the Long Island *Myzomela* would be expected for reasons other than ECD.

This study provides some of the strongest evidence to-date that interspecfic competition and agonistic interactions between species can drive rapid asymmetric ECD following secondary contact, even in the face of ongoing hybridization. Additional studies similar to this one are needed to provide insight into this neglected evolutionary process, as are studies that directly quantify the fitness consequences of size differences in sympatric and allopatric populations.

-

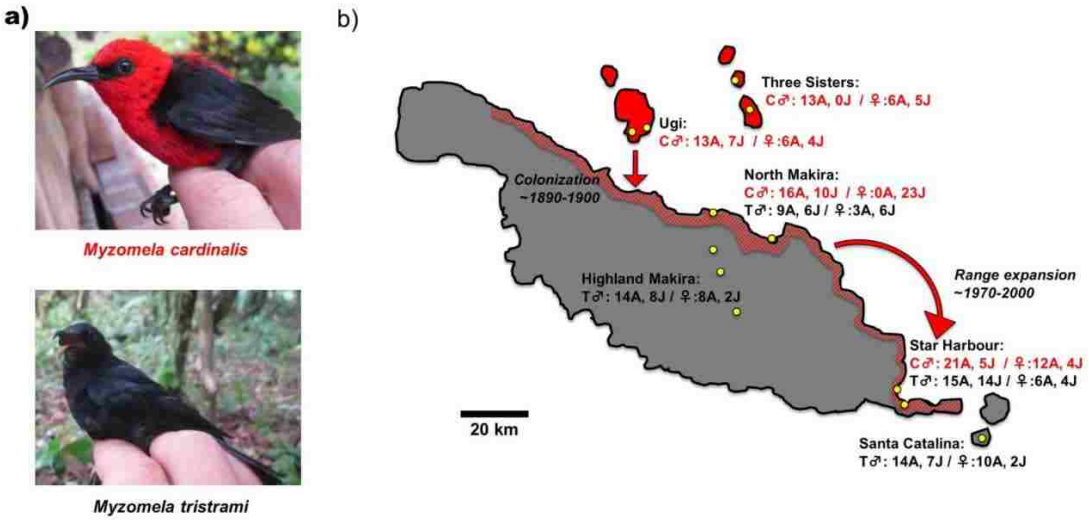


Figure 4.1: (A) Adult male M. cardinalis (top) and adult male M. tristrami (bottom). (B) Map of Makira and satellite islands showing approximate range of M. cardinalis and M. tristrami Sampling localities are shown with sample sizes for each species, sex, and size class indicated ("C" = *M. cardinalis*, "T" = *M. tristrami*, "A" = adult, "J" = juvenile). Arrows show range expansion of *M. cardinalis* with approximate dates indicated.

-

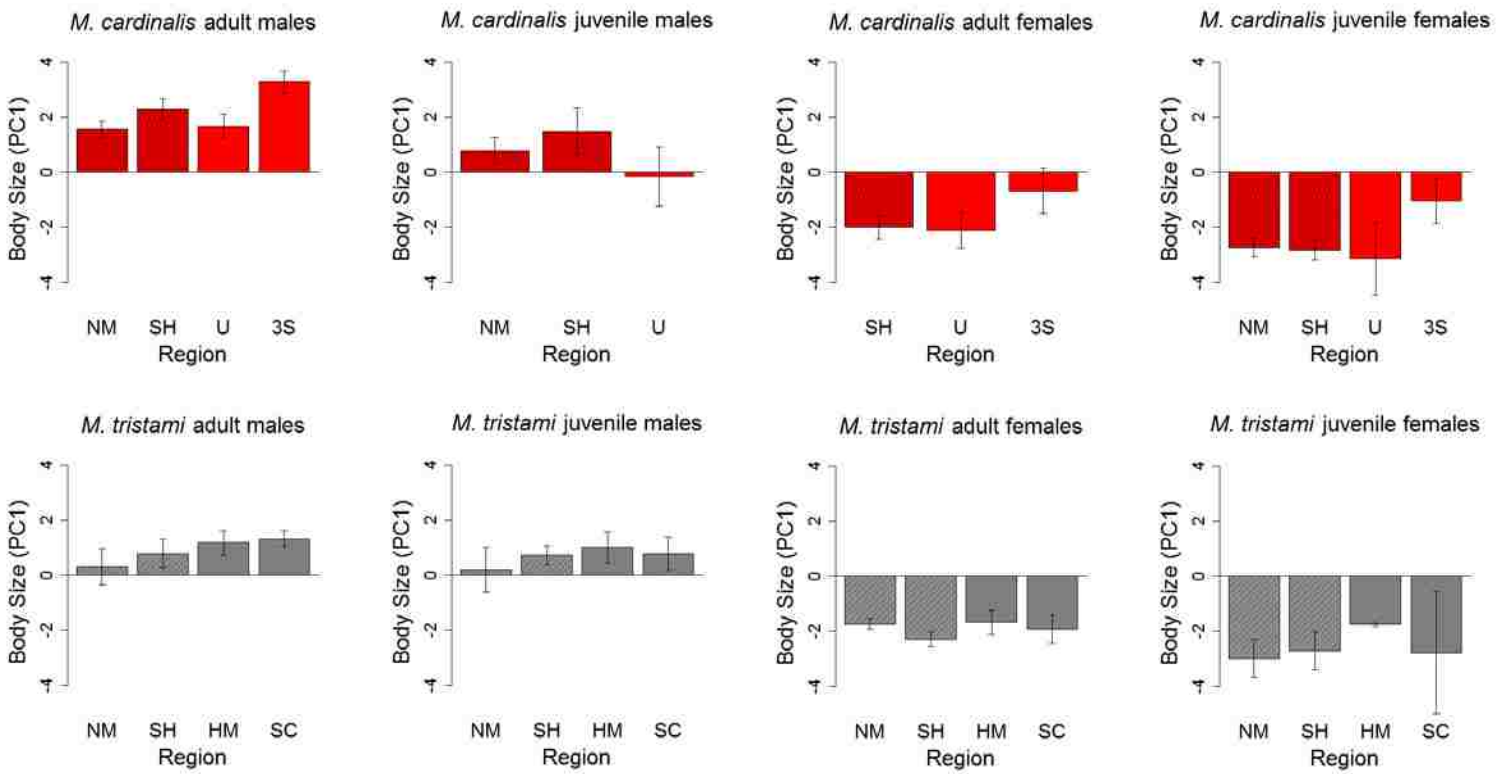


Figure 4.2: Mean body size of *Myzomela* from different populations as measured by first principa1 component of morphology (PC1). From left to right: adult males, juvenile males, adult females, juvenile females. Top row represents *M. cardinalis*. Bottom row represents *M. tristrami*. Guide to populations: NM = North Makira, SH = Star Harbour, U = Ugi, 3S = Three Sisters, HM = Highland Makira, SC = Santa Catalina. Sympatric populations denoted with diagonal hatching. Error bars represent standard error.

-

Table 4.1: Linear models of body size (PC1) based on population and age for male and female *M. cardinalis*. A = allopatric population. S = sympatric population. *p*-values in bold are significant at $p < 0.05$.

Factor	Estimate	Std. Error	t value	*p*-value
Model 1: male *M. cardinalis*				
Age (adult vs. juvenile)	-1.11	0.221	-5.02	**3.1×10^{-6}**
North Makira (S) vs. Star Harbour (S)	0.65	0.240	2.72	**7.9×10^{-3}**
North Makira (S) vs. Ugi (A)	-0.27	0.253	-1.06	0.29
North Makira (S) vs. Three Sisters (A)	1.59	0.301	5.29	**1.1×10^{-6}**
Star Harbour (S) vs. Ugi (A)	-0.92	0.255	-3.60	**5.5×10^{-4}**
Star Harbour (S) vs. Three Sisters (A)	0.94	0.292	3.22	**1.91×10^{-3}**
Ugi (A) vs. Three Sisters (A)	1.86	0.312	5.94	**6.9×10^{-8}**
Model 2: female *M. cardinalis*				
Age (adult vs. juvenile)	0.74	0.292	2.55	**0.014**
North Makira (S) vs. Star Harbour (S)	0.03	0.348	0.08	0.94
North Makira (S) vs. Ugi (A)	0.23	0.360	0.63	0.53
North Makira (S) vs. Three Sisters (A)	-1.48	0.344	-4.32	**6.6×10^{-5}**
Star Harbour (S) vs. Ugi (A)	0.20	0.338	0.59	0.56
Star Harbour (S) vs. Three Sisters (A)	-1.51	0.331	-4.57	**2.9×10^{-5}**
Ugi (A) vs. Three Sisters (A)	-1.71	0.364	-4.71	**1.8×10^{-5}**

-

Table 4.2: Linear models of body size (PC1) based on population and age for male and female *M. tristrami*. A = allopatric population. S = sympatric population. *p*-values in bold are significant at $p < 0.05$.

Factor	Estimate	Std. Error	t value	*p*-value
Model 3: male *M. tristrami*				
Age (adult vs. juvenile)	-0.20	0.179	-1.14	0.26
North Makira (S) vs. Star Harbour (S)	0.51	0.259	1.98	0.05
North Makira (S) vs. Highland Makira (A)	0.85	0.272	3.12	**2.5×10^{-3}**
North Makira (S) vs. Santa Catalina (A)	0.87	0.275	3.16	**2.2×10^{-3}**
Star Harbour (S) vs. Highland Makira (A)	0.34	0.231	1.45	0.15
Star Harbour (S) vs. Santa Catalina (A)	0.36	0.234	1.53	0.13
Highland Makira (A) vs. Santa Catalina (A)	0.02	0.248	0.09	0.93
Model 4: female *M. tristrami*				
Age (adult vs. juvenile)	0.66	0.254	2.59	**0.014**
North Makira (S) vs. Star Harbour (S)	0.07	0.330	0.20	0.84
North Makira (S) vs. Highland Makira (A)	-0.58	0.344	-1.67	0.10
North Makira (S) vs. Santa Catalina (A)	-0.17	0.335	-0.51	0.62
Star Harbour (S) vs. Highland Makira (A)	-0.64	0.318	-2.02	0.05
Star Harbour (S) vs. Santa Catalina (A)	-0.24	0.307	-0.77	0.45
Highland Makira (A) vs. Santa Catalina (A)	0.41	0.301	1.35	0.19

-

Supplementary Table S4.1: First three principal components of morphology in Makira *Myzomela* calculated for all individuals (both males and females)

	PC1	PC2	PC3
Standard deviation	2.023	0.876	0.831
Proportion of variance	0.585	0.110	0.099
Loadings:			
Mass	0.413	0.033	-0.322
Bill length	0.317	0.213	0.834
Bill height	0.373	-0.197	0.291
Bill width	0.288	-0.882	-0.018
Tarsus length	0.411	0.116	-0.127
Wing chord length	0.438	0.207	-0.213
Tail length	0.383	0.283	-0.234

-

Supplementary Table S4.2: Linear models of body size (PC1) based on population, for only adult male *M. cardinalis* and *M. tristrami*. A = allopatric population. S = sympatric population. *p*-values in bold are significant at $p < 0.05$.

Factor	Estimate	Std. Error	t value	*p*-value
Model 5: adult male *M. cardinalis*				
North Makira (S) vs. Star Harbour (S)	0.72	0.249	2.89	**5.3×10^{-3}**
North Makira (S) vs. Ugi (A)	0.12	0.281	0.42	0.68
North Makira (S) vs. Three Sisters (A)	1.72	0.281	6.13	**8.0×10^{-8}**
Star Harbour (S) vs. Ugi (A)	-0.60	0.265	-2.28	**0.027**
Star Harbour (S) vs. Three Sisters (A)	1.00	0.265	3.76	**3.9×10^{-4}**
Ugi (A) vs. Three Sisters (A)	1.60	0.295	5.43	**1.1×10^{-6}**
Model 6: adult male *M. tristrami*				
North Makira (S) vs. Star Harbour (S)	0.49	0.360	1.35	0.18
North Makira (S) vs. Highland Makira (A)	0.88	0.365	2.41	**0.02**
North Makira (S) vs. Santa Catalina (A)	1.02	0.365	2.81	**7.2×10^{-3}**
Star Harbour (S) vs. Highland Makira (A)	0.39	0.317	1.24	0.22
Star Harbour (S) vs. Santa Catalina (A)	0.54	0.317	1.69	0.10
Highland Makira (A) vs. Santa Catalina (A)	0.14	0.323	0.45	0.66

-

Supplementary Table S4.3: First three principal components of morphology in Makira *Myzomela* calculated separately for males and females.

	PC1	PC2	PC3
MALES			
Standard deviation	1.60	1.09	0.98
Proportion of variance	0.365	0.170	0.138
Loadings:			
Mass	0.470	-0.322	0.028
Bill length	-0.003	0.617	-0.683
Bill height	0.240	0.595	0.329
Bill width	0.275	0.369	0.558
Tarsus length	0.408	-0.080	-0.043
Wing chord length	0.518	-0.136	-0.226
Tail length	0.459	0.025	-0.247
FEMALES			
Standard deviation	1.40	1.08	0.98
Proportion of variance	0.279	0.168	0.136
Loadings:			
Mass	0.440	0.115	-0.190
Bill length	0.290	0.077	0.039
Bill height	0.374	0.194	-0.242
Bill width	0.405	0.461	-0.365
Tarsus length	0.456	0.001	0.613
Wing chord length	0.431	-0.526	0.278
Tail length	0.158	-0.674	-0.563

-

Supplementary Table S4.4: Linear models of male body size (sex-specific PC1 calculated for males only) based on age and population. A = allopatric population. S = sympatric population. *p*-values in bold are significant at $p < 0.05$.

Factor	Estimate	Std. Error	t value	*p*-value
Model 5: male *M. cardinalis*				
Age (adult vs. juvenile)	-1.51	0.295	-5.14	**1.9×10^{-6}**
North Makira (S) vs. Star Harbour (S)	0.65	0.319	2.04	**0.04**
North Makira (S) vs. Ugi (A)	-0.35	0.337	-1.05	0.30
North Makira (S) vs. Three Sisters (A)	2.52	0.401	6.28	**1.7×10^{-8}**
Star Harbour (S) vs. Ugi (A)	-1.00	0.340	-2.95	**4.1×10^{-3}**
Star Harbour (S) vs. Three Sisters (A)	1.87	0.389	4.80	**7.2×10^{-6}**
Ugi (A) vs. Three Sisters (A)	2.87	0.416	6.90	**1.1×10^{-9}**
Model 6: male *M. tristrami*				
Age (adult vs. juvenile)	-0.18	0.200	-0.88	0.38
North Makira (S) vs. Star Harbour (S)	0.45	0.289	1.57	0.12
North Makira (S) vs. Highland Makira (A)	0.95	0.303	3.14	**2.3×10^{-3}**
North Makira (S) vs. Santa Catalina (A)	0.88	0.306	2.87	**5.2×10^{-3}**
Star Harbour (S) vs. Highland Makira (A)	0.50	0.257	1.95	0.05
Star Harbour (S) vs. Santa Catalina (A)	0.43	0.261	1.64	0.11
Highland Makira (A) vs. Santa Catalina (A)	-0.07	0.276	-0.27	0.79

-

Supplementary Table S4.5: Linear models of female body size (sex-specific PC1 calculated for females only) based on age and population. A = allopatric population. S = sympatric population. *p*-values in bold are significant at $p < 0.05$.

Factor	Estimate	Std. Error	t value	*p*-value
Model 5: female *M. cardinalis*				
Age (adult vs. juvenile)	-0.90	0.426	-2.12	**0.038**
North Makira (S) vs. Star Harbour (S)	-0.22	0.508	-0.44	0.66
North Makira (S) vs. Ugi (A)	-0.31	0.526	-0.60	0.55
North Makira (S) vs. Three Sisters (A)	2.10	0.502	4.19	**1.0×10^{-4}**
Star Harbour (S) vs. Ugi (A)	-0.09	0.493	-0.19	0.85
Star Harbour (S) vs. Three Sisters (A)	2.33	0.483	4.81	**1.2×10^{-5}**
Ugi (A) vs. Three Sisters (A)	2.42	0.531	4.55	**3.0×10^{-5}**
Model 6: female *M. tristrami*				
Age (adult vs. juvenile)	-0.78	0.372	-2.10	**0.04**
North Makira (S) vs. Star Harbour (S)	0.04	0.484	0.09	0.93
North Makira (S) vs. Highland Makira (A)	0.98	0.504	1.95	0.06
North Makira (S) vs. Santa Catalina (A)	0.32	0.491	0.65	0.52
Star Harbour (S) vs. Highland Makira (A)	0.94	0.467	2.01	0.05
Star Harbour (S) vs. Santa Catalina (A)	0.28	0.450	0.61	0.54
Highland Makira (A) vs. Santa Catalina (A)	-0.66	0.441	-1.5	0.14

-

Chapter 5

A neo-sex chromosome mediates plumage divergence and speciation in island honeyeaters

Background

Sex chromosomes are predicted to accumulate alleles for traits whose fitness consequences vary by sex (i.e., sexually antagonistic traits) due to selection favoring linkage between such alleles and loci important for sex determination (Fisher 1931; Rice 1984; Albert and Otto 2005). Indeed, many examples of alleles for sexually-selected traits, including those important for mate choice in a variety of species, map disproportionately to sex chromosomes (Reinhold 1998; Lindholm and Breden 2002; Sæther et al. 2007). Because divergence in sexual signals is an important driver of pre-zygotic reproductive isolation in many taxa (Boughman 2001; Coyne and Orr 2004; Price 2008), sex chromosomes are expected to play an important role in the relationship between sexual selection and speciation (Qvarnström and Bailey 2009). Here we provide evidence that a previously-unknown neo-sex chromosome plays a prominent role in the maintenance of plumage divergence, an important sexual signal, between two hybridizing species of honeyeaters (*Myzomela tristrami* and *Myzomela cardinalis pulcherrima*). Using a genome-wide set of 132,351 single-nucleotide polymorphisms (SNPs), we demonstrate that SNPs that map to a neo-sex chromosome exhibit the increased differentiation between species expected of loci important for speciation. The same genomic region is also strongly associated with differences in plumage color between species. We also identified two candidate genes responsible for plumage divergence in *Myzomela*, both of which map to the neo-sex chromosome. These results provide

important evidence supporting the hypothesis that accumulation of novel sex linkage facilitates divergence in sexual traits and speciation.

Darwin first hypothesized that sexual selection can drive phenotypic divergence in traits important for species recognition and mate choice, leading to reproductive isolation and speciation (Darwin 1871). Sexual selection has been hypothesized to play an especially prominent role in avian speciation, because intrinsic postzygotic reproductive incompatibilities accumulate relatively slowly in birds, whereas the sexual signals that mediate prezygotic incompatibilities (e.g., plumage and song) diverge relatively rapidly between bird species (Price 2008; Rabosky and Matute 2013; Seddon et al. 2013). Because many species of birds exhibit notable sexual dimorphism in these signals, theory predicts that the avian sex chromosomes will act as particularly harbor important genomic loci for signal divergence and speciation in birds (Kirkpatrick et al. 2004). Chromosomal synteny is highly conserved across distantly-related birds (Derjusheva et al. 2004; Backström et al. 2008b), particularly in the sex chromosomes (birds have ZW sex determination where females are the heterogametic sex and males are homozygous ZZ), while the loci of sexual selection vary between closely-related species (Wilkinson et al. 2015). Accordingly, it is unclear whether existing sex chromosomes can play a disproportionate role in avian speciation via the on-going accumulation of alleles that are subject to sexual antagonism. One mechanism by which sexually-antagonistic alleles can accumulate novel sex-linkage is via a chromosomal fusion between an ancestral autosome and an existing sex chromosome (i.e., a neo-sex chromosome) (Pennell et al. 2015). Indeed, neo-sex chromosomes have been implicated in behavioral isolation and hybrid sterility that drives speciation in stickleback fish (Kitano et al. 2009).

Only one example of an avian neo-sex chromosome has been previously identified (in Sylvioidea passerines) (Pala et al. 2012), but it remains unknown whether loci associated with speciation or sexually-selected traits accumulate disproportionately on the Sylvioidea neo-sex chromosome. Therefore, whether neo-sex chromosomes can facilitate speciation in birds via the accumulation of divergence in sexual traits has not been tested.

We investigated the genomics of speciation and phenotypic divergence in two congeneric species of honeyeaters that hybridize on Makira in the Solomon Islands, *Myzomela cardinalis pulcherrima* (Cardinal Myzomela) and *Myzomela tristrami* (Sooty Myzomela). These two species fill similar ecological niches in sympatry but are highly divergent in plumage (Fig. 5.1). Both sexes of *M. tristrami* are entirely black (i.e., melanic), while *M. cardinalis* is sexually dimorphic, with males possessing brighter patches of red carotenoid-based plumage, consistent with plumage being a signal that is subject to sexual antagonism in this species (Hill and McGraw 2006; Seddon et al. 2013). We previously established that hybridization has led to introgression in this system, and that the two species exhibit extensive haplotype sharing (Sardell and Uy 2016). Admixture offers the opportunity to identify genomic regions that are associated with speciation and phenotypic divergence (Buerkle and Lexer 2008), as theory predicts that these regions will be resistant to gene flow, resulting in elevated divergence in genomic regions containing speciation genes (Turner et al. 2005; Renaut et al. 2013). We exploited the low levels of background genomic divergence in the Makira *Myzomela* to characterize regions associated with speciation and plumage divergence in this system, and to test the hypothesis that sexually antagonistic loci will accumulate disproportionately in sex chromosomes.

Methods

Sampling and Sequencing

We visited Makira-Ulawa province in the Solomon Islands during 2012 and 2013, and used passive mist-netting to capture and collect blood and feather samples of *Myzomela tristrami* and *Myzomela cardinalis pulcherrima* individuals from allopatric and sympatric populations (Sardell and Uy 2016). Blood samples were stored in lysis buffer (Longmire et al. 1997) and genomic DNA was extracted in the lab using Qiagen DNeasy Blood and Tissue Kit. Molecular sexing of each individual was performed using established protocols (Han et al. 2009). For sequencing, we randomly selected 15 allopatric and 15 sympatric individuals from each species, and supplemented them with 12 additional individuals that had previously been established to be genetically admixed (Sardell and Uy 2016), including seven phenotypically intermediate birds and three *M. tristrami* and two *M. cardinalis* (Supp. Table 5.1). We then prepared three mixed-species paired-end restriction-site associated DNA sequencing (RAD-seq) libraries of 24 individuals each using a modified version of the protocol described in Parchman et al. (2013) (Parchman et al. 2013). In brief, we used EcoRI and MseI enzymes to double digest DNA, and ligated the product to customized barcoded adapters to allow pooling of multiple individuals within a single sequencing lane. Adapter-ligated DNA was purified using an AMPure purificiation kit (Beckman-Coulter cat #A63880), amplified via PCR, size-selected using gel electrophoresis, and then purified again prior to sequencing. Each library underwent paired-end sequencing in a half lane on an Illumina HiSeq 2000 at the core facilities of the University of Miami's John P. Hussman Institute for Human Genomics.

Filtering, Alignment, and SNP calling

We sorted raw Illumina reads by barcode, removed adapter sequences and barcodes, which resulted in 94-bp reads, and filtered for quality (removing tags with phred quality scores < 20) using the process_radtags script implemented in the Stacks package (Catchen et al. 2011). We assembled a *de novo* "reference" using the tags produced from a single library. Using the ustacks program in the Stacks package, we first clustered highly similar (99%) reads within each individual and removed tags with excessive read depth (>50), where the cut-off was determined based on the empirical distribution of read depths in our data. Potentially paralogous loci were identified as those reads that clustered at 90% or higher within any individual using the program CAP3 (Huang and Madan 1999), and were removed to avoid potential alignment errors. Consensus reads for each individual were then pooled and aligned using ustacks and cstacks with relaxed clustering parameters (homologous tags were defined as those with fewer than 10 mismatches) to create a *de novo* alignment reference.

The quality-filtered reads for each individual from all libraries were then mapped back to the consensus "reference" using BWA (Li and Durbin 2010), again with up to 10 mismatches allowed. We used the vcfutils program in the SAMtools package to call SNPs, based on a minimum quality threshold of 20, minimum individual read depth of 5 (to allow for identification of heterozygotes), and a maximum individual read depth threshold of 50 (to remove false SNP calls resulting from aligning paralogs). We then used customized Perl scripts to remove loci with more than two alleles and to remove polymorphisms where the minor allele frequency was below five percent. We also improved the accuracy of heterozygous variant calling within individuals by requiring at

least 20% of reads to contain the minor allele at each heterozygous site. Loci where the minor allele frequency for reads from a single individual was greater than zero and less than 20 percent were reclassified as missing data for that individual due to ambiguity in base calling.

For genotype-phenotype associations, we further excluded any loci for which data were not present in at least eight individuals from each of the following populations: allopatric *M. tristrami*, sympatric *M. tristrami*, sympatric *M. cardinalis*, and allopatric *M. cardinalis*. This resulted in a total data set of 317,767 SNPs ("filtered SNP dataset"). Because sex ratio is male biased in both species, this standard also results in exclusion of all loci located on the W sex chromosome from the filtered SNP data set. To assign SNPs to chromosomes, we aligned our *de novo* reference to the February 2013 assembly (WashU taeGut324/taeGut2) of the zebra finch (*Taeniopygia guttata)* reference genome (Warren et al. 2010) downloaded from the UCSC Genome Browser using BWA with the same parameters described above. The 132,251 SNPs which successfully aligned to the zebra finch genome are hereafter called the "mapped SNP dataset".

Divergence calculations and Genotype-Phenotype Associations

Genetic structure in the filtered SNP dataset was quantified via a principal component analysis (PCA) implemented in PLINK, and the first and second principal components were plotted in R to visualize variation associated with species, sex, and population.

F_{ST} values were calculated by classifying individuals to species based on plumage phenotype regardless of mitochondrial haplotype (see Sardell and Uy 2016), with phenotypically intermediate birds excluded from the calculation. Specially, F_{ST} estimates for each locus in the filtered SNP dataset were calculated using the following equation, with expected heterozygosity ignoring structure (H_T) and average of expected heterozygosity over both subpopulations (H_S) estimated from observed minor allele frequency:

$$F_{ST} = \frac{H_T - H_S}{H_T}$$

To test whether high F_{ST} loci accumulate disproportionately in different genomic regions, we identified the loci with the top 1% and top 0.1% of F_{ST} values in the mapped SNP dataset. The expected random distribution of such high-frequency SNPs throughout the genome was estimated based on the proportion of SNPs that mapped to each contig in the zebra finch genome. Statistical significance of differences in observed versus expected frequency of high-*F*st SNPs was calculated via Bonferroni-corrected Fisher's exact tests for each contig.

Genome wide association studies (GWAS) were carried out using a univariate linear mixed model (LMM) approach implemented in the software package GEMMA v.0.94.1. This program quantifies the association between each locus and individual phenotypes (e.g., plumage color) while controlling for population stratification. To account for population structure and kinship, we ran a PCA in Plink, setting the number of principal components equal to the number of individuals in the analysis, and input the results as a relatedness matrix into GEMMA (see Turner and Harr (2014)). Resulting

Wald test *p*-values were then mapped to the zebra finch genome to identify genomic regions containing loci most strongly associated with plumage color.

Candidate plumage genes were identified as loci where alleles were perfectly associated with phenotype, i.e., all individuals with one plumage phenotype are homozygous for one allele and all individuals with the alternate plumage are either homozygous for the alternate allele or heterozygous. Candidate SNPs were mapped against the annotated zebra finch genome using the UCSC Genome Browser to determine chromosomal position and proximally located genes.

Similar approaches were also used to calculate site-by-site divergence between sexes, treating members of each sex as a single population, regardless of species or plumage phenotype, and identifying genomic regions associated with sex-based genetic variation. GWAS were also performed in GEMMA as described above, using sex as the binary trait of interest. Mean heterozygosity was calculated by sex for each locus in the mapped SNP dataset, based on the percent of individuals that were heterozygous at each locus, and averaged over each contig to identify large-scale genomic regions where male and female heterozygosity differed. The statistical significance of any observed differences in heterozygosity was assessed using a customized randomization test implemented in R, wherein 42 individuals were randomly assigned to the "male" group with the remaining 30 individuals assigned to the "female" group. Differences in mean heterozygosity between sexes were then calculated for each of the 36 chromosomal contigs containing at least 100 SNPs, and this process was repeated 10,000 times to create a distribution of expected values under the null hypotheses (i.e., no difference in mean heterozygosity between males and females).

Results

Using paired-end restriction-site associated DNA sequencing (RAD-seq) of 72 *Myzomela* individuals (Supplemental Table 5.1), we created a *de novo* assembly comprising 661,236 94bp-long tags containing 317,767 total single-nucleotide polymorphisms (SNPs) post-filtering. 132,251 of these SNPs were located in tags that aligned to the most recent annotated assembly of the zebra finch (*Taeniopygia guttata*) reference genome (Warren et al. 2010). A principal component analysis of SNP data revealed that species-level differences explain 80.2% of the genetic variation in this system (PC1), with intermediate PC1 values representing hybrid individuals that possess either phenotypically-intermediate plumage or a mismatch between plumage phenotype and mitochondrial haplotype (Fig. 5.1). Unusually, an additional 6.3% of the genetic variation in this pooled data set (PC2) is strongly correlated with sex regardless of species (Fig. 5.1), suggesting that *Myzomela* sexes possess fundamentally different genotypes, even though all SNPs located on the female-specific W chromosome were removed from the genomic data set during filtering.

If we classify individuals into species based on plumage phenotype, SNPs that show elevated differentiation (i.e., F_{ST}) disproportionately map to the zebra finch Z chromosome (ZFchrZ) as well as zebra finch chromosome 5 (ZFchr5) (Fig. 5.2a). SNPs that map to ZFchrZ and ZFchr5 represent 30.5% and 18.2%, respectively of the 99th-percentile SNPs ranked by F_{ST}, a significant deviation from expected values based on total SNP frequency (ZFchrZ: 3.6% of total SNPs, Fisher's Exact Test: $p < 2.2x10^{-16}$, odds ratio = 9.19; ZFchr5: 10.2% of total SNPs, Fisher's Exact Test: $p = 4.0x10^{-8}$, odds ratio = 1.80). No other chromosome is significantly overrepresented among highly

differentiated SNPs. ZFchrZ (54.5%) and ZFchr5 (26.5%) also are disproportionately represented in the top 99.9th percentile of SNPs ranked by F_{ST} (ZFchrZ: $p < 2.2x10^{-16}$, odds ratio = 14.63; ZFchr5: $p = 0.0021$, odds ratio = 2.70). Genomic regions with elevated differentiation like these are often assumed to be centered on alleles subject to strong selection against introgression, and therefore important for speciation (Turner et al. 2005; Renaut et al. 2013).

A GWAS used to identify genes associated with plumage phenotypes also indicates a strong correlation between plumage color and SNPs located on ZFchr5 and ZFchrZ (Fig. 5.2b). Furthermore, only 18 of the 317,767 total SNPs are fixed (including three pairs of linked SNPs that are located within 50bp of each other), such that all individuals of one species are homozygous for one allele, while all individuals of the other species are either homozygous for the alternate allele or heterozygous. Four of the 18 fixed SNPs map to the zebra finch genome (Supp. Table 5.2): two to non-genic regions on ZFchrZ, and two to ZFchr5. One of the latter SNPs falls within an intron of the Zebra Finch homolog of eukaryotic translation initiation factor gamma 2 (*EIF4G2*), expression of which is associated with carotenoid-based plumage in house finches (*Carpodacus mexicanus*) (Balenger et al. 2015). A second fixed SNP on ZFchr5 falls within an intron of the zebra finch homolog to diacylglycerol kinase zeta-like (*DGKZ*), a gene that affects melanin expression in human melanocytes (Kawaguchi et al. 2012) and pigmentation in mice (Agin et al. 1991). An additional fixed SNP that did not successfully map to the zebra finch genome with our default alignment parameters, nonetheless mapped with a high degree of confidence on the UCSC Genome Browser (BLAT score = 50) to a non-coding region of ZFchr5 located approximately 30kbp

downstream from the zebra finch homolog of DNA damage-binding protein 2 (*DDB2*), a gene associated with xeroderma pigmentosum in humans, a disorder that results in extreme sensitivity of skin cells to ultraviolet light (Itoh et al. 2001).

To investigate sex-linked genetic structure in this system, we again used F_{ST} as a measure of population divergence to identify genomic regions exhibiting significant population structuring, pooling all individuals and defining "sex" rather than "species" as the populations of interest. High sex-linked F_{ST} loci mapped disproportionately to a 42Mbp region of ZFchr5, including 82.7% of the top 1% of SNPs (versus 16.7% expected, Fisher's exact test $p < 2.2x10^{-16}$, odds ratio = 5.32) and 75% of the top 0.1% SNPs ($p = 6.6x10^{-13}$, odds ratio = 12.94) (Fig. 5.3a). In addition, SNPs that align to four additional contigs in the zebra finch genome are also disproportionately represented in the top 1% of sex-linked SNPs: one also associated with ZFchr5, one also associated with ZFchrZ, and two unanchored contigs. A GWAS with sex as the trait of interest reveals that sex is highly associated with the same contigs identified in the F_{ST} outlier analysis, including the same region of ZFchr5 that shows elevated F_{ST} by sex (Fig. 5.3b). Heterozygosity on ZFchr5 is also significantly elevated in females, the heterogametic sex in birds, relative to males, the homogametic sex, ($P<0.0072$ based on a Bonferroni-corrected randomization test) as expected for young sex chromosomes that have diverged but not yet undergone significant deterioration (Yoshida et al. 2014) (Fig. 5.4). Indeed, female heterozygosity was ≥0.9 in 1.4% (168/12023) of the SNPs mapping to ZFchr5, while male heterozygosity was <0.075 in the same SNPs. Together these results are consistent with ZFchr5 acting as a neo-sex chromosome in *Myzomela*, likely resulting from a chromosomal fusion between the autosome and an ancestral sex chromosome.

Discussion

Using genome-wide data from hybridizing taxa, we have demonstrated that alleles associated with both speciation and divergence in a sexual signal, i.e., plumage color, disproportionately map to a previously unknown neo-sex chromosome along with the ancestral Z sex-chromosome in *Myzomela* honeyeaters. This observation provides important support for the hypothesis that sex-linkage is a key mechanism for promoting divergence within sexually selected traits, which can in turn drive pre-zygotic reproductive isolation and speciation. Sex-linkage previously has been hypothesized to be important for the evolution of the extraordinary diversity of sexual signals in birds (Kirkpatrick et al. 2004), and this study provides novel evidence that the accumulation of sex-linkage via the evolution of a neo-sex chromosome is associated with plumage divergence in an avian system.

The particular mechanism responsible for generating novel sex-linkage in the *Myzomela* homolog of ZFchr5 remains unknown, because visualization of karyotypes using fluorescence in situ hybridization for this system was not possible due to logistical constraints imposed by working in the Solomon Islands. The most parsimonious explanation is that sex-linkage resulted from the fusion of ZFchr5 with an existing sex chromosome, as similar fusion events are believed to be responsible for the origin of neo-sex chromosomes in Sylvioidea birds (Pala et al. 2012), as well as a many fishes, reptiles, and mammals (Pennell et al. 2015) Alternatively, sex chromosome turnover in this system may have followed either *de novo* evolution of a sex-determining locus (SDL) on ZFchr5 or a translocation event involving an existing SDL. However, evolutionary models predict that these events are highly unlikely to occur in systems with significant

sex chromosome degeneration, such as birds, because natural selection will strongly oppose decoupling of the primary SDL from loci located on the ancestral sex chromosome that are subject to strong sexually-antagonistic selection (Van Doorn and Kirkpatrick 2007).

Furthermore, it is tempting to speculate that the evolution of the *Myzomela* neo-sex chromosome may have played a broader role in diversification in this genus, the most species-rich genus in the unusually-complex Meliphagidae honeyeater radiation, and the 26th-most species-rich of the 1,258 genera of Passerine songbirds (Gill and Donsker 2014). Unlike nearly all other species of honeyeaters, many species of *Myzomela* possess bright red, sexually-dichromatic, carotenoid-based plumage, consistent with plumage color being a sexually antagonistic trait in this genus. By increasing the number of sex-linked loci in the genome, evolution of the neo-sex chromosome in this genus may have enhanced the potential for sexual antagonism at previously non-sex-linked loci to be resolved through sex-specific trait expression. Such relaxation of evolutionary constraints imposed by sexual antagonism could in turn increase the potential for sexual traits that are favored exclusively in males to elaborate and diverge rapidly, resulting in the noteworthy diversity of plumages observed across the *Myzomela* radiation. Preliminary phylogenetic data indicates that *M. tristrami* and *M. cardinalis* are not sister species (Sardell and Uy 2016), suggesting that the neo-sex chromosome is likely shared by many other taxa in this genus. Future studies examining the phylogenetic distribution of the *Myzomela* sex chromosome will provide further insight into the potential for sex chromosome rearrangements to drive rapid diversification in this system.

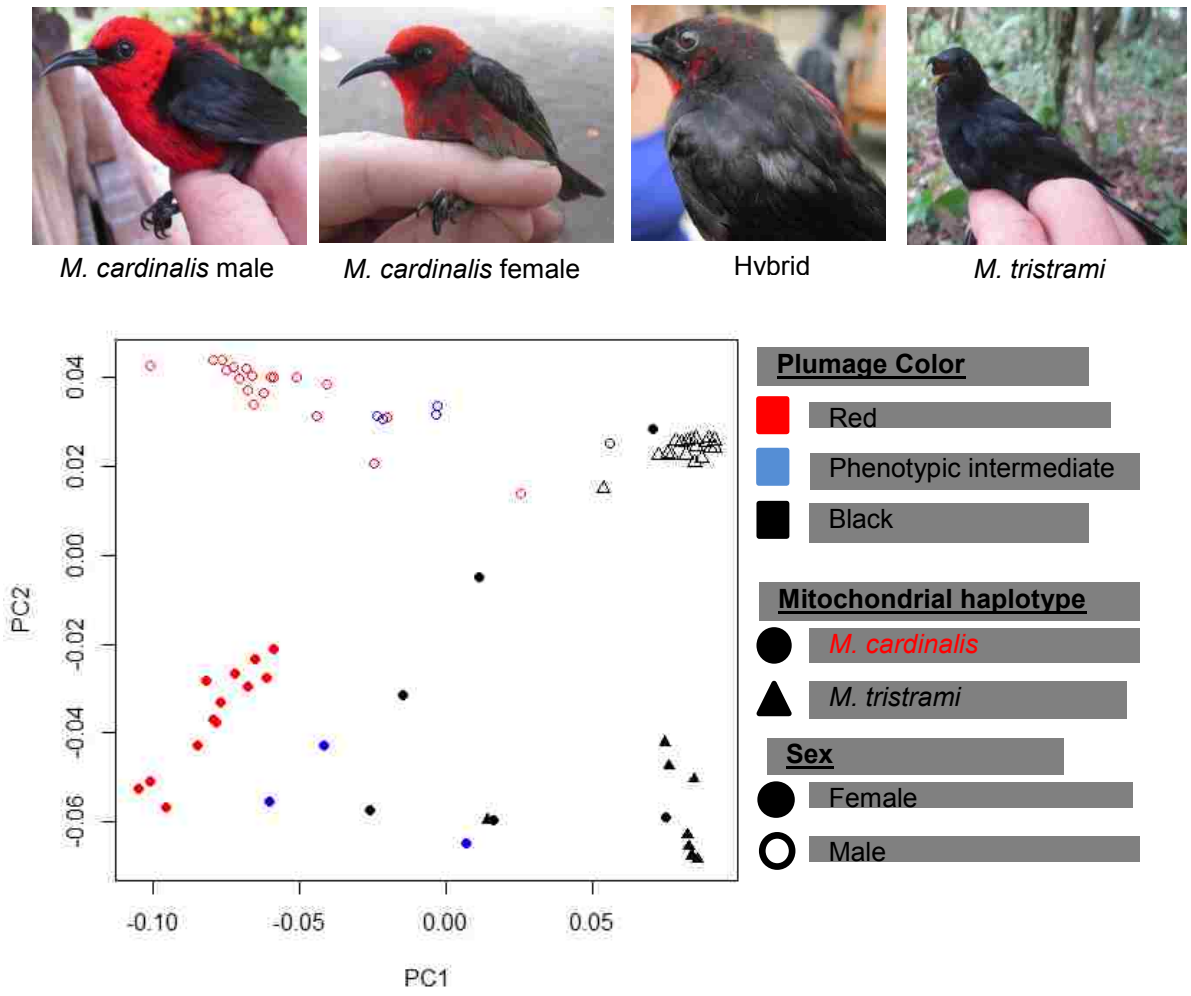


Figure 5.1: (top) Representative plumage phenotypes for, from right to left, adult male *Myzomela cardinalis*, adult female *M. cardinalis*, phenotypically intermediate hybrid, adult *M. tristrami*. (bottom) First two principal components of PCA of genetic variation for 38 *M. tristrami*, 37 *M. cardinalis*, and 7 phenotypic intermediates sequenced at 317,767 SNPs. PC1 and PC2 explain 80.2 percent and 6.3 percent of total genetic variation, respectively.

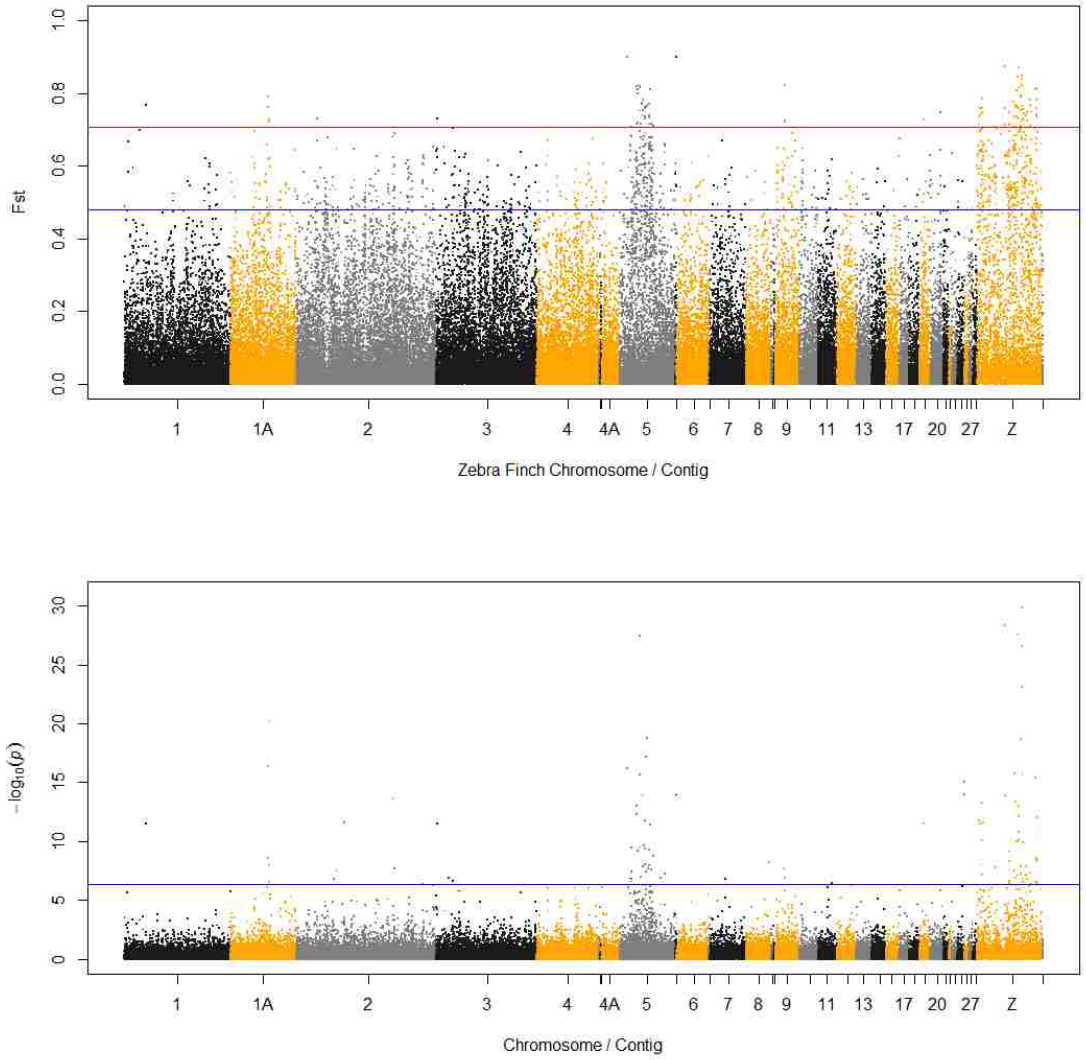


Figure 5.2: Identification of genomic regions associated with plumage divergence in *Myzomela* via SNP mapping to chromosomal contigs from the zebra finch genome. Contigs containing fewer than 100 SNPs are excluded from plots. Top: SNP-by-SNP F_{ST} comparisons for *M. cardinalis* versus *M. tristrami* individuals with standard plumage phenotypes. Blue line represent 99th percentile of SNPs by F_{ST}. Red line represents 99.9th percentile of SNPs. Bottom: SNP-by-SNP Wald P values resulting from genome-wide associate study (GWAS) for plumage phenotype, i.e., red vs. melanic. Blue line represents Bonferroni-corrected line of significance (P=0.05).

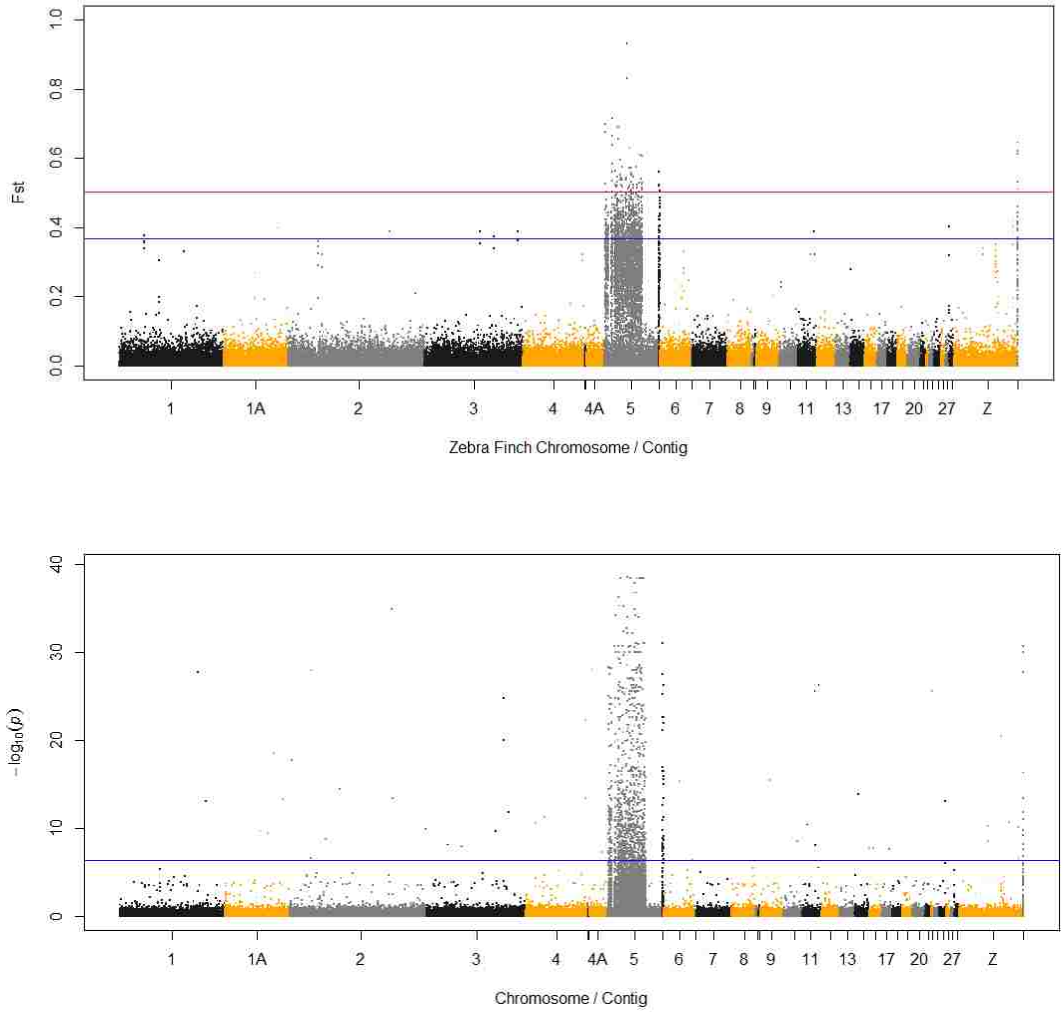


Figure 5.3: Identification of sex-linked genomic regions in *Myzomela* via SNP mapping to chromosomal contigs from the zebra finch genome. Contigs containing fewer than 100 SNPs are excluded from plots. Top: SNP-by-SNP F_{ST} comparisons for males versus females. Blue line represent 99th percentile of SNPs by F_{ST}. Red line represents 99.9th percentile of SNPs. Bottom: SNP-by-SNP Wald P values resulting from genome-wide associate study (GWAS) for sex. Blue line represents Bonferroni-corrected line of significance (P=0.05).

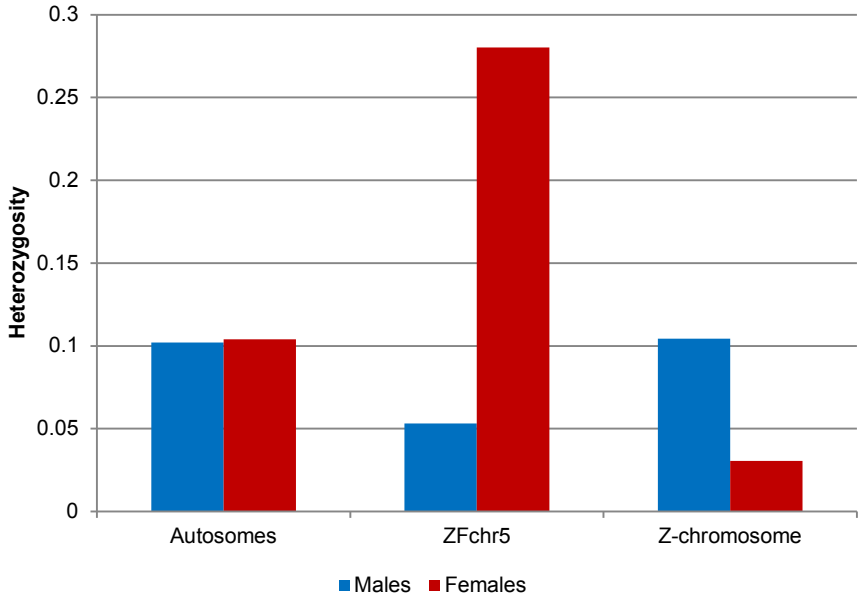


Figure 5.4: Mean male and female heterozygosity for *Myzomela* SNPs that map to different chromosomal regions in the zebra finch genome. Observed heterozygosity values greater than zero for SNPs mapping to the Z chromosome in females are likely artifacts of sequencing and alignment error.

Supplemental Table 5.1: Breakdown of *Myzomela* sequenced for SNP dataset.

Species	Population	Males	Females	Total
M. tristrami	Allopatric (Highland Makira)	9	6	15
M. tristrami	Sympatric (Coastal Makira)	10	8	18
M. cardinalis	Allopatric (Ugi)	9	6	15
M. cardinalis	Sympatric (Coastal Makira)	10	7	17
Phenotypic intermediates	Sympatric (Coastal Makira)	4	3	7

Supplemental Table 5.2: Candidate plumage loci perfectly fixed by phenotype.

Chromosomal Location of SNP on Zebra Finch Genome	Sequenced *M. cardinalis* haplotypes	Sequenced *M. tristrami* haplotypes
chr5:8,250,298	T/T - 23, C/T - 3	C/C - 31
chr5:21,784,005	G/G – 14, G/C - 16	C/C - 31
chr5:22,313,536	G/G - 22, G/T - 6	T/T - 32
chrZ:31,068,488	G/G - 25, G/A - 4	A/A - 31
chrZ:50,039,192	A/A - 24, A/G -6	G/G - 32

Chapter 6

Summary of dissertation findings

For my Ph.D. dissertation, I examined the evolutionary consequences of recent secondary contact between two honeyeater species, *Myzomela cardinalis* and *Myzomela tristrami*, which came into contact on Makira in the Solomon Islands approximately 120 years ago. Under the traditional allopatric model of speciation, secondary contact is the "moment of truth" for whether or not phenotypic and genotypic divergence between two previously isolated populations is sufficient to result in reproductive isolation (Mayr 1942; Brown and Wilson 1956; Harrison 1993; Noor 1999; Mayr and Diamond 2001). If not, hybridization and gene flow is traditionally expected to lead to convergence between two taxa (Dobzhansky 1937; Mayr 1942; Rhymer and Simberloff 1996; Grant et al. 2004; Taylor et al. 2006). Alternatively, secondary contact can act to promote phenotypic divergence between taxa, e.g., via mechanisms such as character displacement and reinforcement (Dobzhansky 1940; Brown and Wilson 1956; Grant 1972; Grether et al. 2009; Ortiz-Barrientos et al. 2009; Pfennig and Pfennig 2009). Insights into the potential evolutionary outcomes of secondary contact are important for our understanding of the processes which have historically governed the evolution of biodiversity. However, most such knowledge comes from studies that compared allopatric populations either to populations with long-standing sympatry (Caraway et al. 2001; Shaw 2002; Cianchi et al. 2003; Larsen et al. 2010; Nunes et al. 2010; Lavretsky et al. 2015) or to recently sympatric populations that resulted from anthropogenic species introductions (Rhymer and Simberloff 1996; Mooney and Cleland 2001; Fitzpatrick et al. 2010), both of which

may be unrepresentative of the dynamics of initial secondary contact events (Strauss et al. 2006). Few examples of natural, historically-recent secondary contact between closely-related taxa have been well studied, and Mayr and Diamond (2001) first noted the unusual potential for the Makira *Myzomela* to offer unique insights into this rarely observed, but important stage of the speciation process. The data and results that I presented in the previous chapters represent the first study examining the genetics, behavioral ecology and evolutionary dynamics of this noteworthy system.

In Chapter 2, I tested whether reproductive isolation is complete between the Makira *Myzomela*, or whether hybridization has had potentially important consequences for the maintenance of phenotypic and genotypic divergences in this system. By documenting phenotypically intermediate birds in the field, I confirmed on-going hybridization between the two species even at site where both are common. This observation conflicts with Mayr and Diamond's (2001) hypothesis that prior hybridization in this system only occurred when mating opportunities between *M. cardinalis* were very rare during the generations immediately following secondary contact. By sequencing birds at one mitochondrial and six nuclear markers, I confirmed that sympatric populations contained admixed individuals. However, hybridization was asymmetric with all individuals with admixed ancestry descending from the *M. cardinalis* matriline.

I further discovered that asymmetric reproductive isolation in this system has resulted in low-levels of nuclear introgression, but no mitochondrial introgression into sympatric *M. cardinalis*. In contrast, both *M. cardinalis* mitochondrial and nuclear haplotypes have introgressed into sympatric *M. tristrami*. This pattern was opposite to

that found in nearly all shifting avian hybrid zones, where introgression is predominantly from the native species to the invading one. However, I proposed an alternative interpretation of the data that is consistent with the standard pattern of introgression: that gene flow should be viewed as an introgression of *M. tristrami* plumage alleles into the genotypic background of *M. cardinalis*. Indeed, evidence of nuclear introgression of *M. cardinalis* alleles to *M. tristrami* was nearly exclusively limited to "cryptic" hybrids, i.e., birds that possessed melanic plumage typical of *M. tristrami* but *M. cardinalis* mitochondrial haplotypes. These results were consistent with a lack of backcrossing in this direction, and strong reproductive isolation between *M. tristrami* and the phenotypically-similar, black cryptic hybrids. Therefore, secondary contact which results in hybridization had important evolutionary consequences for associations between phenotype and genetic compatibility in this system.

In Chapter 3, I experimentally tested whether phenotypic divergence between *Myzomela* species on Makira influenced agonistic interactions during male-male competition in sympatry. To do so, I used taxidermy mounts to simulate territorial intrusions and recorded the behavioral responses of males that responded. I discovered that males of both *Myzomela* species exhibited significantly increased aggressiveness towards mounts of *M. cardinalis* mounts relative to mounts of *M. tristrami* as well as an unrelated ecological competitor (*Dicaeum tristrami*). This result was contrary to the hypothesis that males should be most aggressive towards individuals with whom they compete most strongly for mating opportunities and resources conspecifics (Mikami et al. 2004; Seehausen and Schluter 2004; van Doorn et al. 2004; Uy et al. 2009a; Uy et al. 2009b). Notably, *M. tristrami* were aggressive towards *M.* cardinalis males, even though

genetic data from Chapter 2 indicated that *M. tristrami* males do not compete with *M. cardinalis* males for mating opportunities with *M. tristrami* females. Instead, I hypothesized that agonistic interactions between the nectivorous *Myzomela* species were predominantly driven by competition for limited food resources (i.e., nectar sources such as flowering trees) rather than competition for mates. I showed that sympatric *M. cardinalis* males were significantly larger than sympatric *M. tristrami* males, and therefore should dominate inter-species conflicts. Furthermore, hybrids were intermediate in size, and therefore would also be expected to lose most agonistic interactions with *M. cardinalis*. These results provided a potential explanation for the introgression of melanic plumage alleles from *M. tristrami* to *M. cardinalis* that I identified in Chapter 2. Namely, black plumage may be favored in hybrids because it reduces the probability of eliciting aggressive responses from larger, dominant *M. cardinalis* males.

I did not detect any evidence of significantly biased aggression toward any mount types among allopatric males of either species, suggesting that aggression biases of *M. tristrami* toward *M. cardinalis* likely represented an artifact of secondary contact rather than ancestral secondary bias. These results indicated that population-level shifts in behavioral response in this system occurred rapidly over the 120 years since secondary contact, either due to selection for increased aggression towards *M. cardinalis* or as a learned response to previously-experienced agonistic interactions. Increased aggression in sympatric *M. cardinalis* may also have been driven by increased competition following secondary contact, although we cannot dismiss the possibility that these differences originated during colonization (e.g., if increased dispersal ability is pleiotropically associated with aggression).

Although I did not detect any significant differences in overall aggression between species, as expected if *M. cardinalis* is dominant over *M. tristrami*, my experiments were limited in their ability to detect any such differences. For example, biases in data collection would have tended to overestimate aggression in *M. tristrami* relative to *M. cardinalis*. Moreover, my experimental design did not test for any differences in aggression that result in one species being less likely to respond aggressively to audio stimuli. Accordingly, these data did not contradict the hypotheses that difference in overall aggression between *Myzomela* species has driven shifts in behavioral responses and phenotypic introgression of plumage-related alleles.

In Chapter 5, I investigated whether secondary contact has had evolutionary consequences for size divergence in *Myzomela*. I captured 283 total *Myzomela* from two sympatric populations and two allopatric populations of each species, and measured morphology and determined the sex and age class (adult vs. juvenile) of each individual. I found that hybridization between *Myzomela* has not driven phenotypic convergence via the evolution of reduced size differences in sympatry in this system. Instead, secondary contact has resulted in asymmetric ecological character displacement, with mean size of male *M. tristrami* from the oldest sympatric population being significantly smaller than males form allopatric populations. Furthermore, males from the more recent area of secondary contact were intermediate in size, as expected if evolution of character displacement is gradual. *M. cardinalis* males from the oldest sympatric population were statistically identical in size to those from the presumed colonization source, indicative of a lack of character displacement in size in the larger, colonizing species. Lack of character displacement in sympatric *M. cardinalis* also suggests that decrease in sizes of

sympatric male *M. tristrami* is likely due to secondary contact rather than site-specific environmental effects.

As with phenotypic introgression and aggression biases described in Chapter 2 and 3, I hypothesize that character displacement in this system was also driven primarily by interspecies competition (i.e., "agonistic character displacement") (Grether et al. 2009; Grether et al. 2013). Specifically, theory predicts that interference competition for limited food sources in which the larger dominant species (i.e., *M. cardinalis*) prevents the smaller species (i.e., *M. tristrami*) from accessing high-quality food sources will result in selection for decreased size and lower resource requirements in the smaller species (Dayan and Simberloff 2005). No character displacement was observed in females of either species, which are equivalent in size and expected to receive reduced aggression relative to males. Lack of character displacement in females also indicated that the reduction in body size in male *M. tristrami* was driven by sex-specific selection, rather than maternal or developmental affects caused by reduced access to high-quality food sources. Although other studies have found that population-level mean sizes can evolve over 100-300 years, my results provide the best evidence to-date that rapid agonistic character displacement can occur over these time scales.

Finally, in Chapter 6, I leveraged admixture resulting from hybridization between the Makira *Myzomela* to identify genomic regions that were resistant to introgression and associated with speciation and plumage divergence in this system. Using double-digest RAD-seq, I generated a set of 317,767 genome-wide single nucleotide polymorphisms, 132,251 of which occurred in sequences that mapped to the zebra finch genome. During the course of this research, I discovered that *Myzomela* possess a previously-unknown

neo-sex chromosome that originated from a fusion between chromosome 5 and an existing sex chromosome, only the second known example of this phenomenon in birds (Pala et al. 2012). A 23 Mbp region on this chromosome exhibited significantly elevated heterozygosity in females and reduced heterozygosity in males, as predicted of young sex chromosomes that have experienced a reduction in recombination but which have not undergone significant degeneration. SNPs in this region also showed significantly increased genetic divergence between sex (as measured by F_{ST}), and were highly associated with sex in a genome wide association study, consistent with it acting as a neo-sex chromosome.

SNPs exhibiting high differentiation in hybridizing populations are assumed to represent genomic regions that are resistant to introgression and which are important for speciation (Turner et al. 2005; Nadeau et al. 2012; Nosil and Feder 2012; Renaut et al. 2012; Renaut et al. 2013; but see Cruickshank and Hahn 2014; Burri et al. 2015). High F_{ST} SNPs in the Makira *Myzomela* disproportionately mapped to the neo-sex chromosome, as did SNPs that were highly associated with plumage color, a sexually-antagonistic train, in a genome-wide associate study. Additionally, of the four SNPs that were "fixed" for plumage phenotype and which map to the zebra finch genome, two were located on the Z sex chromosome, and two were located on the neo-sex chromosome within introns of candidate genes known to be associated with coloration in other organisms. Theory predicts that genes under sexually-antagonistic selection should accumulate on sex chromosomes, and that genomic regions with reduced recombination will feature prominently in speciation (Fisher 1931; Rice 1984, 1987; Kirkpatrick et al. 2004; Kirkpatrick and Barton 2006; Van Doorn and Kirkpatrick 2007). This study

represents only the third known example of a system where neo-sex chromosomes are linked with speciation (Kitano et al. 2009; Nguyen et al. 2013), and only the second example linking neo-sex chromosomes with secondary sexual traits (Kitano et al. 2009).

With this project, I have established the Makira *Myzomela* as a potentially important model system for future studies examining the evolutionary consequences of secondary contact, as well as the genomics of speciation, sexual selection, and sex chromosome evolution. Moreover, I used this system to test several key evolutionary hypotheses, and uncovered unusual patterns of asymmetric introgression, behavioral ecology, and character displacement. Together, these results provide novel insights into the rarely observed immediate outcomes of recent secondary contact, as well as the processes that govern the evolution and maintenance of biodiversity.

References

Abbott, R., D. Albach, S. Ansell, J. W. Arntzen, S. J. Baird, N. Bierne, J. Boughman, A. Brelsford, C. A. Buerkle, R. Buggs, R. K. Butlin, U. Dieckmann, F. Eroukhmanoff, A. Grill, S. H. Cahan, J. S. Hermansen, G. Hewitt, A. G. Hudson, C. Jiggins, J. Jones, B. Keller, T. Marczewski, J. Mallet, P. Martinez-Rodriguez, M. Möst, S. Mullen, R. Nichols, A. W. Nolte, C. Parisod, K. Pfennig, A. M. Rice, M. G. Ritchie, B. Seifert, C. M. Smadja, R. Stelkens, J. M. Szymura, R. Väinölä, J. B. W. Wolf, and D. Zinner. 2013. Hybridization and speciation. J. Evol. Biol. 26:229-246.

Abrams, P. A. and H. Matsuda. 1994. The evolution of traits that determine ability in competitive contests. Evol. Ecol. 8:667-686.

Agin, P., J. Dowdy, and M. Costlow. 1991. Diacylglycerol-induced melanogenesis in Skh-2 pigmented hairless mice. Photodermatol. Photoimmunol. Photomed 8:51-56.

Albert, A. Y. and S. P. Otto. 2005. Sexual selection can resolve sex-linked sexual antagonism. Science 310:119-121.

Andersen, M. J., A. Naikatini, and R. G. Moyle. 2014. A molecular phylogeny of Pacific honeyeaters (Aves: Meliphagidae) reveals extensive paraphyly and an isolated Polynesian radiation. Mol. Phylogen. Evol. 71:308-315.

Anderson, C. N. and G. F. Grether. 2010. Interspecific aggression and character displacement of competitor recognition in *Hetaerina* damselflies. Proc. R. Soc. Lond. B 277:549-555.

Anderson, E. 1949. Introgressive hybridization. John Wiley & Sons, New York.

Anderson, E. and G. Stebbins Jr. 1954. Hybridization as an evolutionary stimulus. Evolution 8:378-388.

Arnold, M. L. 1997. Natural hybridization and evolution. Oxford University Press.

Arnold, M. L. 2004. Transfer and origin of adaptations through natural hybridization: were Anderson and Stebbins right? The Plant Cell 16:562-570.

Baack, E. J. and L. H. Rieseberg. 2007. A genomic view of introgression and hybrid speciation. Curr. Opin. Genet. Dev. 17:513-518.

Backström, N., S. Fagerberg, and H. Ellegren. 2008a. Genomics of natural bird populations: a gene-based set of reference markers evenly spread across the avian genome. Mol. Ecol. 17:964-980.

Backström, N., N. Karaiskou, E. H. Leder, L. Gustafsson, C. R. Primmer, A. Qvarnström, and H. Ellegren. 2008b. A gene-based genetic linkage map of the collared flycatcher (*Ficedula albicollis*) reveals extensive synteny and gene-order conservation during 100 million years of avian evolution. Genetics 179:1479-1495.

Baker, M. C. 1991. Response of male indigo and lazuli buntings and their hybrids to song playback in allopatric and sympatric populations. Behaviour 119:225-242.

Baker, M. C. and A. E. M. Baker. 1990. Reproductive behavior of female buntings: isolating mechanisms in a hybridizing pair of species. Evolution 44:332-338.

Balakrishnan, C. N. and M. D. Sorenson. 2006. Song discrimination suggests premating isolation among sympatric indigobird species and host races. Behav. Ecol. 17:473-478.

Baldassarre, D. T. and M. S. Webster. 2013. Experimental evidence that extra-pair mating drives asymmetrical introgression of a sexual trait. Proc. R. Soc. Lond. B 280:20132175.

Baldassarre, D. T., T. A. White, J. Karubian, and M. S. Webster. 2014. Genomic and morphological analysis of a semi-permeable avian hybrid zone suggests asymmetrical introgression of a sexual signal. Evolution 68:2644-2657.

Balenger, S. L., C. Bonneaud, S. A. Sefick, S. V. Edwards, and G. E. Hill. 2015. Plumage color and pathogen-induced gene expression in a wild bird. Behav. Ecol. 26:1100-1110.

Barraclough, T. G., P. H. Harvey, and S. Nee. 1995. Sexual selection and taxonomic diversity in passerine birds. Proc. R. Soc. Lond. B 259:211-215.

Beehler, B. 1980. A comparison of avian foraging at flowering trees in Panama and New Guinea. Wilson Bull. 92:513-519.

Bolnick, D. I., M. Turelli, H. Lopez-Fernandez, P. C. Wainwright, and T. J. Near. 2008. Accelerated mitochondrial evolution and "Darwin's corollary": asymmetric viability of reciprocal F1 hybrids in Centrarchid fishes. Genetics 178:1037-1048.

Boughman, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. Nature 411:944-948.

Brelsford, A. and D. E. Irwin. 2009. Incipient speciation despite little assortative mating: The yellow-rumped warbler hybrid zone. Evolution 63:3050-3060.

Brelsford, A., B. Mila, and D. E. Irwin. 2011. Hybrid origin of Audubon's warbler. Mol. Ecol. 20:2380-2389.

Brown, K. M., L. M. Burk, L. M. Henagan, and M. A. Noor. 2004. A test of the chromosomal rearrangement model of speciation in *Drosophila pseudoobscura*. Evolution 58:1856-1860.

Brown, W. L. and E. O. Wilson. 1956. Character displacement. Systematic Zoology 5:49-64.

Brumfield, R. T., R. W. Jernigan, D. B. McDonald, and M. J. Braun. 2001. Evolutionary implications of divergent clines in an avian (Manacus: Aves) hybrid zone. Evolution 55:2070-2087.

Buerkle, C. A. and C. Lexer. 2008. Admixture as the basis for genetic mapping. Trends Ecol. Evol. 23:686-694.

Burri, R., A. Nater, T. Kawakami, C. F. Mugal, P. I. Olason, L. Smeds, A. Suh, L. Dutoit, S. Bureš, and L. Z. Garamszegi. 2015. Linked selection and recombination rate variation drive the evolution of the genomic landscape of differentiation across the speciation continuum of Ficedula flycatchers. Genome Res. 25:1656-1665.

Butlin, R. K. 2010. Population genomics and speciation. Genetica 138:409-418.

Cain, A. J. and I. C. J. Galbraith. 1956. Field notes on birds of the eastern Solomon Islands. Ibis 98:100-134.

Caraway, V., G. D. Carr, and C. W. Morden. 2001. Assessment of hybridization and introgression in lava-colonizing Hawaiian *Dubautia* (Asteraceae: Madiinae) using RAPD markers. Am. J. Bot. 88:1688-1694.

Catchen, J. M., A. Amores, P. Hohenlohe, W. Cresko, and J. H. Postlethwait. 2011. Stacks: building and genotyping Loci de novo from short-read sequences. G3 (Bethesda, Md.) 1:171-182.

Cianchi, R., A. Ungaro, M. Marini, and L. Bullini. 2003. Differential patterns of hybridization and introgression between the swallowtails *Papilio machaon* and *P. hospiton* from Sardinia and Corsica islands (Lepidoptera, Papilionidae). Mol. Ecol. 12:1461-1471.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9:1657-1659.

Coluzzi, M., V. Petrarca, and M. A. di Deco. 1985. Chromosomal inversion intergradation and incipient speciation in *Anopheles gambiae*. Ital. J. Zool. 52:45-63.

Coulson, F. I. and J. G. Vedder. 1986. Geology of the central and western Solomon Islands. Pp. 59-87 *in* J. G. Vedder, K. S. Pound, and S. Q. Boundy, eds. Geology and offshore resources of Pacific Island arcs - central and western Solomon Islands. Circum-Pacific Council for Energy and Mineral Resources, Houston, TX.

Coyne, J. and H. A. Orr. 2004. Speciation. Sinauer Associates Inc., Sunderland, MA.

Coyne, J. A. and T. D. Price. 2000. Little evidence for sympatric speciation in island birds. Evolution 54:2166-2171.

Cruickshank, T. E. and M. W. Hahn. 2014. Reanalysis suggests that genomic islands of speciation are due to reduced diversity, not reduced gene flow. Mol. Ecol. 23:3133-3157.

Currat, M., M. Ruedi, R. J. Petit, and L. Excoffier. 2008. The hidden side of invasions: massive introgression by local genes. Evolution 62:1908-1920.

Darwin, C. 1859. On the origins of species by means of natural selection. London: Murray.

Darwin, C. R. 1871. The descent of man, and selection in relation to sex. John Murray, London.

Dawideit, B. A., A. B. Phillimore, I. Laube, B. Leisler, and K. Böhning-Gaese. 2009. Ecomorphological predictors of natal dispersal distances in birds. J. Anim. Ecol. 78:388-395.

Dayan, T. and D. Simberloff. 2005. Ecological and community-wide character displacement: the next generation. Ecol. Lett. 8:875-894.

Dayan, T., D. Simberloff, E. Tchernov, and Y. Yom-Tov. 1989. Inter-and intraspecific character displacement in mustelids. Ecology 70:1526-1539.

DeMarais, B. D., T. E. Dowling, M. E. Douglas, W. L. Minckley, and P. C. Marsh. 1992. Origin of *Gila seminuda* (Teleostei: Cyprinidae) through introgressive hybridization: implications for evolution and conservation. Proc. Natl. Acad. Sci. USA 89:2747-2751.

Derjusheva, S., A. Kurganova, F. Habermann, and E. Gaginskaya. 2004. High chromosome conservation detected by comparative chromosome painting in chicken, pigeon and passerine birds. Chromosome Res. 12:715-723.

Diamond, J., S. L. Pimm, M. E. Gilpin, and M. LeCroy. 1989. Rapid evolution of character displacement in myzomelid honeyeaters. Am. Nat. 134:675-708.

Diamond, J. M. 2002. Dispersal, mimicry, and geographic variation in northern Melanesian birds. Pac. Sci. 56:1-22.

Dijkstra, P. D., O. Seehausen, M. E. Pierotti, and T. Groothuis. 2007. Male–male competition and speciation: aggression bias towards differently coloured rivals varies between stages of speciation in a Lake Victoria cichlid species complex. J. Evol. Biol. 20:496-502.

Dingle, C., J. W. Poelstra, W. Halfwerk, D. M. Brinkhuizen, and H. Slabbekoorn. 2010. Asymmetric response patterns to subspecies-specific song differences in allopatry and parapatry in the gray-breasted wood-wren. Evolution 64:3537-3548.

Dobzhansky, T. 1937. Genetics and the origin of species. Columbia University Press, New York.

Dobzhansky, T. 1940. Speciation as a stage in evolutionary divergence. Am. Nat. 74:312-321.

Doebeli, M. 1996. An explicit genetic model for ecological character displacement. Ecology 77:510-520.

Dowling, T. E. and C. L. Secor. 1997. The role of hybridization and introgression in the diversification of animals. Annu. Rev. Ecol. Syst. 28:593-619.

Driskell, A. C. and L. Christidis. 2004. Phylogeny and evolution of the Australo-Papuan honeyeaters (Passeriformes, Meliphagidae). Mol. Phylogen. Evol. 31:943-960.

Drossel, B. and A. McKane. 1999. Ecological character displacement in quantitative genetic models. J. Theor. Biol. 196:363-376.

Drummond, A. J. and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7:214.

Duckworth, R. A. and A. V. Badyaev. 2007. Coupling of dispersal and aggression facilitates the rapid range expansion of a passerine bird. Proc. Natl. Acad. Sci. USA 104:15017-15022.

Ducrest, A.-L., L. Keller, and A. Roulin. 2008. Pleiotropy in the melanocortin system, coloration and behavioural syndromes. Trends Ecol. Evol. 23:502-510.

Dutson, G. 2001. New distributional ranges for Melanesian birds. Emu 101:237-248.

Dutson, G. 2011. Birds of Melanesia: Bismarcks, Solomons, Vanuatu and New Caledonia. Princeton University Press, Princeton, NJ.

Ellison, C. K. and R. S. Burton. 2008. Interpopulation hybrid breakdown maps to the mitochondrial genome. Evolution 62:631-638.

Ellison, C. K., O. Niehuis, and J. Gadau. 2008. Hybrid breakdown and mitochondrial dysfunction in hybrids of *Nasonia* parasitoid wasps. J. Evol. Biol. 21:1844-1851.

Excoffier, L., M. Foll, and R. J. Petit. 2009. Genetic consequences of range expansions. Annu. Rev. Ecol., Evol. Syst. 40:481-501.

Feder, J. L., S. P. Egan, and P. Nosil. 2012. The genomics of speciation-with-gene-flow. Trends Genet. 28:342-350.

Fisher, R. A. 1931. The evolution of dominance. Biol. Rev. 6:345-368.

Fitzpatrick, B. M., J. R. Johnson, D. K. Kump, J. J. Smith, S. R. Voss, and H. B. Shaffer. 2010. Rapid spread of invasive genes into a threatened native species. Proc. Natl. Acad. Sci. USA 107:3606-3610.

Fjeldså, J. 1983. Ecological character displacement and character release in grebes Podicipedidae. Ibis 125:463-481.

Flockhart, D. T. and K. L. Wiebe. 2009. Absence of reproductive consequences of hybridization in the Northern Flicker (*Colaptes auratus*) hybrid zone. Auk 126:351-358.

Ford, H. A. 1979. Interspecific competition in Australian honeyeaters—depletion of common resources. Aust. J. Ecol. 4:145-164.

Ford, H. A. and D. C. Paton. 1982. Partitioning of nectar sources in an Australian honeyeater community. Aust. J. Ecol. 7:149-159.

Freeman, S. and W. M. Jackson. 1990. Univariate metrics are not adequate to measure avian body size. Auk 107:69-74.

Gardner, J. L., J. W. H. Trueman, D. Ebert, L. Joseph, and R. D. Magrath. 2010. Phylogeny and evolution of the Meliphagoidea, the largest radiation of Australasian songbirds. Mol. Phylogen. Evol. 55:1087-1102.

Gause, G. F. 1934. The struggle for existence. Williams and Wilkins, Baltimore, MD.

Gil, D. 1997. Increased response of the Short-Toed Treecreeper *Certhia brachydactyla* in sympatry to the playback of the song of the Common Treecreeper *C. familiaris*. Ethology 103:632-641.

Gill, F. and D. Donsker. 2014. IOC World Bird List (version 4.4).

Gill, F. B. 1970. Hybridization in Norfolk Island white-eyes (Zosterops). Condor 72:481-482.

Gompert, Z., J. A. Fordyce, M. L. Forister, A. M. Shapiro, and C. C. Nice. 2006. Homoploid hybrid speciation in an extreme habitat. Science 314:1923-1925.

Grant, B. R. and P. R. Grant. 2002. Simulating secondary contact in allopatric speciation: an empirical test of premating isolation. Biol. J. Linn. Soc. 76:545-556.

Grant, P. R. 1972. Convergent and divergent character displacement. Biol. J. Linn. Soc. 4:39-68.

Grant, P. R. and B. R. Grant. 2009. The secondary contact phase of allopatric speciation in Darwin's finches. Proc. Natl. Acad. Sci. USA 106:20141-20148.

Grant, P. R. and B. R. Grant. 2014. Synergism of natural selection and introgression in the origin of a new species. Am. Nat. 183:671-681.

Grant, P. R., B. R. Grant, and J. Deutsch. 1996. Speciation and hybridization in Island birds [and discussion]. Philos. Trans. R. Soc. Lond. B 351:765-772.

Grant, P. R., B. R. Grant, J. A. Markert, L. F. Keller, and K. Petren. 2004. Convergent evolution of Darwin's finches caused by introgressive hybridization and selection. Evolution 58:1588-1599.

Grant, P. R., B. R. Grant, and K. Petren. 2005. Hybridization in the recent past. Am. Nat. 166:56-67.

Greene, E., B. E. Lyon, V. R. Muehter, L. Ratcliffe, S. J. Oliver, and P. T. Boag. 2000. Disruptive sexual selection for plumage coloration in a passerine bird. Nature 407:1000-1003.

Greig, E. I., D. T. Baldassarre, and M. S. Webster. 2015. Differential rates of phenotypic introgression are associated with male behavioral responses to multiple signals. Evolution 69:2602-2612.

Grether, G. F., C. N. Anderson, J. P. Drury, A. N. Kirschel, N. Losin, K. Okamoto, and K. S. Peiman. 2013. The evolutionary consequences of interspecific aggression. Ann. N. Y. Acad. Sci. 1289:48-68.

Grether, G. F., N. Losin, C. N. Anderson, and K. Okamoto. 2009. The role of interspecific interference competition in character displacement and the evolution of competitor recognition. Biol. Rev. 84:617-635.

Hamilton, W. D. and M. Zuk. 1982. Heritable true fitness and bright birds: a role for parasites? Science 218:384-387.

Han, J.-I., J.-H. Kim, S. Kim, S.-R. Park, and K.-J. Na. 2009. A simple and improved DNA test for avian sex determination. Auk 126:779-783.

Hansen, B. T. and T. Slagsvold. 2003. Rival imprinting: interspecifically cross-fostered tits defend their territories against heterospecific intruders. Anim. Behav. 65:1117-1123.

Hardin, G. 1960. The competitive exclusion principle. Science 131:1292-1297.

Harrison, R. G. 1993. Hybrid zones and the evolutionary process. Oxford University Press, Oxford, UK.

Hedrick, P. W. 2011. Genetics of populations. Jones & Bartlett Publishers, Sudbury, MA.

Hedrick, P. W. 2013. Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. Mol. Ecol. 22:4606-4618.

Heliconius Genome Consortium. 2012. Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. Nature 487:94-98.

Hermansen, J. S., S. A. Sæther, T. O. Elgvin, T. Borge, E. Hjelle, and G. P. Sætre. 2011. Hybrid speciation in sparrows I: phenotypic intermediacy, genetic admixture and barriers to gene flow. Mol. Ecol. 20:3812-3822.

Heslewood, M. M., M. S. Elphinstone, S. C. Tidemann, and P. R. Baverstock. 1998. Myoglobin intron variation in the Gouldian Finch *Erythrura gouldiae* assessed by temperature gradient gel electrophoresis. Electrophoresis 19:142-151.

Higgins, P., L. Christidis, and H. Ford. 2008. Family Meliphagidae (Honeyeaters) *in* J. del Hoyo, A. Elliott, and D. A. Christie, eds. Handbook of the birds of the world, volume 13: Penduline-tits to Shrikes. Lynx Edicions, Barcelona, Spain.

Hill, G. E. 1991. Plumage coloration is a sexually selected indicator of male quality. Nature 350:337-339.

Hill, G. E. 2006. Female mate choice for ornamental coloration. Pp. 137-200 *in* G. E. Hill, and K. J. McGraw, eds. Bird coloration, Volume II: Function and coloration. Harvard University Press, Cambridge, MA.

Hill, G. E. and J. D. Johnson. 2013. The mitonuclear compatibility hypothesis of sexual selection. Proc. R. Soc. Lond. B 280:20131314.

Hill, G. E. and K. J. McGraw. 2006. Bird Coloration. Volume II. Function and Evolution. Harvard University Press, Cambridge, MA.

Hill, G. E. and R. Montgomerie. 1994. Plumage colour signals nutritional condition in the house finch. Proc. R. Soc. Lond. B 258:47-52.

Höbel, G. and H. C. Gerhardt. 2003. Reproductive character displacement in the acoustic communication system of green tree frogs (Hyla cinerea). Evolution 57:894-904.

Hoffmann, A. A. and L. H. Rieseberg. 2008. Revisiting the impact of inversions in evolution: from population genetic markers to drivers of adaptive shifts and speciation? Annu. Rev. Ecol., Evol. Syst. 39:21.

Huang, X. and A. Madan. 1999. CAP3: A DNA sequence assembly program. Genome Res. 9:868-877.

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

Itoh, T., A. Nichols, and S. Linn. 2001. Abnormal regulation of DDB2 gene expression in xeroderma pigmentosum group E strains. Oncogene 20:7041-7050.

Jaeger, R. G., E. D. Prosen, D. C. Adams, and W. Montgomery. 2002. Character displacement and aggression in two species of terrestrial salamanders. Copeia 2002:391-401.

Jankowski, J. E., S. K. Robinson, and D. J. Levey. 2010. Squeezed at the top: interspecific aggression may constrain elevational ranges in tropical birds. Ecology 91:1877-1884.

Johnstone, R. A. 1997. The evolution of animal signals. Pp. 155-178 *in* J. R. Krebs, and N. B. Davies, eds. Behavioural ecology: An evolutionary approach. Blackwell Science, Ltd., Malden, MA.

Jones, M. 1997. Character displacement in Australian dasyurid carnivores: size relationships and prey size patterns. Ecology 78:2569-2587.

Joseph, L., A. Toon, Á. S. Nyári, N. W. Longmore, K. Rowe, T. Haryoko, J. Trueman, and J. L. Gardner. 2014. A new synthesis of the molecular systematics and biogeography of honeyeaters (Passeriformes: Meliphagidae) highlights biogeographical and ecological complexity of a spectacular avian radiation. Zool. Scr. 43:235-248.

Kaneshiro, K. Y. 1976. Ethological isolation and phylogeny in the *planitibia* subgroup of Hawaiian *Drosophila*. Evolution 30:740-745.

Kaneshiro, K. Y. 1980. Sexual isolation, speciation and the direction of evolution. Evolution 34:437-444.

Kawaguchi, M., J. C. Valencia, T. Namiki, T. Suzuki, and V. J. Hearing. 2012. Diacylglycerol kinase regulates tyrosinase expression and function in human melanocytes. J. Invest. Dermatol. 132:2791-2799.

Kimball, R. T., E. L. Braun, F. K. Barker, R. C. K. Bowie, M. J. Braun, J. L. Chojnowski, S. J. Hackett, K.-L. Han, J. Harshman, and V. Heimer-Torres. 2009. A well-tested set of primers to amplify regions spread across the avian genome. Mol. Phylogen. Evol. 50:654-660.

Kirkpatrick, M. and N. Barton. 2006. Chromosome inversions, local adaptation and speciation. Genetics 173:419-434.

Kirkpatrick, M., D. W. Hall, and P. Dunn. 2004. Sexual selection and sex linkage. Evolution 58:683-691.

Kirschel, A. N., D. T. Blumstein, and T. B. Smith. 2009. Character displacement of song and morphology in African tinkerbirds. Proc. Natl. Acad. Sci. USA 106:8256-8261.

Kitano, J., J. A. Ross, S. Mori, M. Kume, F. C. Jones, Y. F. Chan, D. M. Absher, J. Grimwood, J. Schmutz, and R. M. Myers. 2009. A role for a neo-sex chromosome in stickleback speciation. Nature 461:1079-1083.

Koopman, K. F. 1957. Evolution in the genus Myzomela (Aves: Meliphagidae). Auk 74:49-72.

Kraaijeveld, K., F. J. L. Kraaijeveld-Smit, and M. E. Maan. 2011. Sexual selection and speciation: the comparative evidence revisited. Biol. Rev. 86:367-377.

Kroodsma, D. E., B. E. Byers, E. Goodale, S. Johnson, and W.-C. Liu. 2001. Pseudoreplication in playback experiments, revisited a decade later. Anim. Behav. 61:1029-1033.

Küpper, C., M. Stocks, J. E. Risse, N. dos Remedios, L. L. Farrell, S. B. McRae, T. C. Morgan, N. Karlionova, P. Pinchuk, and Y. I. Verkuil. 2016. A supergene determines highly divergent male reproductive morphs in the ruff. Nat. Genet. 48:79-83.

Lack, D. 1947. Darwin's finches. Cambridge University Press, Cambridge, UK.

Lamichhaney, S., J. Berglund, M. S. Almén, K. Maqbool, M. Grabherr, A. Martinez-Barrio, M. Promerová, C.-J. Rubin, C. Wang, N. Zamani, B. R. Grant, P. R. Grant, M. T. Webster, and L. Andersson. 2015. Evolution of Darwin's finches and their beaks revealed by genome sequencing. Nature 518:371-375.

Lamichhaney, S., G. Fan, F. Widemo, U. Gunnarsson, D. S. Thalmann, M. P. Hoeppner, S. Kerje, U. Gustafson, C. Shi, and H. Zhang. 2016. Structural genomic changes underlie alternative reproductive strategies in the ruff (*Philomachus pugnax*). Nat. Genet. 48:84-88.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. Proc. Natl. Acad. Sci. USA 78:3721-3725.

Larsen, P. A., M. R. Marchán-Rivadeneira, and R. J. Baker. 2010. Natural hybridization generates mammalian lineage with species characteristics. Proc. Natl. Acad. Sci. USA 107:11447-11452.

Lavretsky, P., A. Engilis, J. M. Eadie, and J. L. Peters. 2015. Genetic admixture supports an ancient hybrid origin of the endangered Hawaiian duck. J. Evol. Biol. 28:1005-1015.

Lexer, C., C. A. Buerkle, J. A. Joseph, B. Heinze, and M. F. Fay. 2007. Admixture in European Populus hybrid zones makes feasible the mapping of loci that contribute to reproductive isolation and trait differences. Heredity 98:74-84.

Li, H. and R. Durbin. 2010. Fast and accurate long-read alignment with Burrows–Wheeler transform. Bioinformatics 26:589-595.

Librado, P. and J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451-1452.

Ligon, R. A. and G. E. Hill. 2009. Do adult eastern bluebird, *Sialia sialis*, males recognize juvenile-specific traits? Anim. Behav. 77:1267-1272.

Lindholm, A. and F. Breden. 2002. Sex chromosomes and sexual selection in poeciliid fishes. Am. Nat. 160:S214-S224.

Longmire, J. L., M. Maltbie, and R. J. Baker. 1997. Use of "lysis buffer" in DNA isolation and its implication for museum collections. Occasional Papers - Museum of Texas Tech University 163.

Losos, J. B. and R. E. Ricklefs. 2009a. Adaptation and diversification on islands. Nature 457:830-836.

Losos, J. B. and R. E. Ricklefs. 2009b. The theory of island biogeography revisited. Princeton University Press, Princeton, NJ.

Lowry, D. B. and J. H. Willis. 2010. A widespread chromosomal inversion polymorphism contributes to a major life-history transition, local adaptation, and reproductive isolation. PLoS Biol. 8:e1000500.

Luyendyk, B. P., W. Bryan, and P. Jezek. 1974. Shallow structure of the New Hebrides island arc. Geol. Soc. Am. Bull. 85:1287-1300.

Lynch, A. and A. J. Baker. 1991. Increased vocal discrimination by learning in sympatry in two species of chaffinches. Behaviour 116:109-125.

Maan, M. E. and O. Seehausen. 2011. Ecology, sexual selection and speciation. Ecol. Lett. 14:591-602.

MacArthur, R. H. and E. O. Wilson. 1967. The theory of island biogeography. Princeton University Press, Princeton.

Mallet, J. 2005. Hybridization as an invasion of the genome. Trends Ecol. Evol. 20:229-237.

Mallet, J. 2007. Hybrid speciation. Nature 446:279-283.

Marshall, D. C. and J. R. Cooley. 2000. Reproductive character displacement and speciation in periodical cicadas, with description of a new species, 13-year *Magicicada neotredecim*. Evolution 54:1313-1325.

Mayr, E. 1932. Birds collected during the Whitney South Sea Expedition. XVIII. Notes on Meliphagidae from Polynesia and the Solomon islands. Am. Mus. Novit. 516.

Mayr, E. 1942. Systematics and the origin of species. Columbia University Press, New York.

Mayr, E. 1945. Birds of the Southwest Pacific. Macmillan, New York.

Mayr, E. and J. Diamond. 2001. The birds of northern Melanesia. Speciation, ecology, and biogeography. Oxford University Press, Oxford.

McDonald, D. B., R. P. Clay, R. T. Brumfield, and M. J. Braun. 2001. Sexual selection on plumage and behavior in an avian hybrid zone: experimental tests of male-male interactions. Evolution 55:1443-1451.

McDonald, R. A. 2002. Resource partitioning among British and Irish mustelids. J. Anim. Ecol. 71:185-200.

McEntee, J. P. 2014. Reciprocal territorial responses of parapatric African sunbirds: species-level asymmetry and intraspecific geographic variation. Behav. Ecol. 25:1380-1394.

Melville, J. 2002. Competition and character displacement in two species of scincid lizards. Ecol. Lett. 5:386-393.

Michel, A. P., S. Sim, T. H. Powell, M. S. Taylor, P. Nosil, and J. L. Feder. 2010. Widespread genomic divergence during sympatric speciation. Proc. Natl. Acad. Sci. USA 107:9724-9729.

Mikami, O. K., M. Kohda, and M. Kawata. 2004. A new hypothesis for species coexistence: male–male repulsion promotes coexistence of competing species. Popul. Ecol. 46:213-217.

Mooney, H. A. and E. E. Cleland. 2001. The evolutionary impact of invasive species. Proc. Natl. Acad. Sci. USA 98:5446-5451.

Moore, W. S. 1987. Random mating in the northern flicker hybrid zone: implications for the evolution of bright and contrasting plumage patterns in birds. Evolution 41:539-546.

Nadeau, N. J., A. Whibley, R. T. Jones, J. W. Davey, K. K. Dasmahapatra, S. W. Baxter, M. A. Quail, M. Joron, R. H. ffrench-Constant, M. L. Blaxter, J. Mallet, and C. D. Jiggins. 2012. Genomic islands of divergence in hybridizing *Heliconius* butterflies identified by large-scale targeted sequencing. Philos. Trans. R. Soc. Lond. B 367:343-353.

Newman, M. M., P. J. Yeh, and T. D. Price. 2006. Reduced territorial responses in dark-eyed juncos following population establishment in a climatically mild environment. Anim. Behav. 71:893-899.

Nguyen, P., M. Sýkorová, J. Šíchová, V. Kůta, M. Dalíková, R. Č. Frydrychová, L. G. Neven, K. Sahara, and F. Marec. 2013. Neo-sex chromosomes and adaptive potential in tortricid pests. Proc. Natl. Acad. Sci. USA 110:6931-6936.

Ninnes, C. and S. Andersson. 2014. Male receiver bias for red agonistic signalling in a yellow-signalling widowbird: a field experiment. Proc. R. Soc. Lond. B 281:20140971.

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

Noor, M. A., K. L. Grams, L. A. Bertucci, and J. Reiland. 2001. Chromosomal inversions and the reproductive isolation of species. Proc. Natl. Acad. Sci. USA 98:12084-12088.

Nosil, P. 2008. Speciation with gene flow could be common. Mol. Ecol. 17:2103-2106.

Nosil, P. 2012. Ecological speciation. Oxford University Press, Oxford.

Nosil, P. and J. L. Feder. 2012. Genomic divergence during speciation: causes and consequences. Philosophical Transactions of the Royal Society B: Biological Sciences 367:332-342.

Nosil, P., D. J. Funk, and D. Ortiz-Barrientos. 2009. Divergent selection and heterogeneous genomic divergence. Mol. Ecol. 18:375-402.

Nunes, M. D. S., P. Orozco-Ter Wengel, M. Kreissl, and C. Schlötterer. 2010. Multiple hybridization events between *Drosophila simulans* and *Drosophila mauritiana* are supported by mtDNA introgression. Mol. Ecol. 19:4695-4707.

Nyári, Á. S. and L. Joseph. 2013. Comparative phylogeography of Australo-Papuan mangrove-restricted and mangrove-associated avifaunas. Biol. J. Linn. Soc. 109:574-598.

Nylander, J. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University 2.

Ortiz-Barrientos, D., A. Grealy, and P. Nosil. 2009. The genetics and ecology of reinforcement. Ann. N. Y. Acad. Sci. 1168:156-182.

Pala, I., S. Naurin, M. Stervander, D. Hasselquist, S. Bensch, and B. Hansson. 2012. Evidence of a neo-sex chromosome in birds. Heredity 108:264-272.

Panhuis, T. M., R. Butlin, M. Zuk, and T. Tregenza. 2001. Sexual selection and speciation. Trends Ecol. Evol. 16:364-371.

Papadopulos, A. S., W. J. Baker, D. Crayn, R. K. Butlin, R. G. Kynast, I. Hutton, and V. Savolainen. 2011. Speciation with gene flow on Lord Howe Island. Proc. Natl. Acad. Sci. USA 108:13188-13193.

Parchman, T., Z. Gompert, M. Braun, R. Brumfield, D. McDonald, J. Uy, G. Zhang, E. Jarvis, B. Schlinger, and C. Buerkle. 2013. The genomic consequences of adaptive divergence and reproductive isolation between species of manakins. Mol. Ecol. 22:3304-3317.

Patten, M. A., J. T. Rotenberry, and M. Zuk. 2004. Habitat selection, acoustic adaptation, and the evolution of reproductive isolation. Evolution 58:2144-2155.

Pearce, D., S. R. Pryke, and S. C. Griffith. 2011. Interspecific aggression for nest sites: model experiments with Long-tailed Finches (*Poephila acuticauda*) and endangered Gouldian Finches (*Erythrura gouldiae*). Auk 128:497-505.

Pearson, S. F. and S. Rohwer. 2000. Asymmetries in male aggression across an avian hybrid zone. Behav. Ecol. 11:93-101.

Pennell, M. W., M. Kirkpatrick, S. P. Otto, J. C. Vamosi, C. L. Peichel, N. Valenzuela, and J. Kitano. 2015. Y Fuse? Sex Chromosome Fusions in Fishes and Reptiles. PLoS Genetics 11::e1005237.

Pettersson, M., M. Bylund, and A. Alderborn. 2003. Molecular haplotype determination using allele-specific PCR and pyrosequencing technology. Genomics 82:390-396.

Pfennig, D. W. and K. S. Pfennig. 2010. Character displacement and the origins of diversity. Am. Nat. 176:S26-S44.

Pfennig, K. S. and D. W. Pfennig. 2009. Character displacement: ecological and reproductive responses to a common evolutionary problem. Q. Rev. Biol. 84:253.

Prescott, D. R. 1987. Territorial responses to song playback in allopatric and sympatric populations of alder (*Empidonax alnorum*) and willow (*E. traillii*) flycatchers. Wilson Bull.:611-619.

Price, T. D. 2008. Speciation in birds. Roberts and Company, Greenwood Village, CO.

Primmer, C. R., T. Borge, J. Lindell, and G. P. Sætre. 2002. Single-nucleotide polymorphism characterization in species with limited available sequence information: high nucleotide diversity revealed in the avian genome. Mol. Ecol. 11:603-612.

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

Prum, R. O. 2014. Interspecific social dominance mimicry in birds. Zool. J. Linn. Soc. 172:910-941.

Quinn, V. S. and D. K. Hews. 2000. Signals and behavioural responses are not coupled in males: aggression affected by replacement of an evolutionarily lost colour signal. Proc. R. Soc. Lond. B 267:755-758.

Qvarnström, A. and R. I. Bailey. 2009. Speciation through evolution of sex-linked genes. Heredity 102:4-15.

Qvarnström, A., A. M. Rice, and H. Ellegren. 2010. Speciation in *Ficedula* flycatchers. Philos. Trans. R. Soc. Lond. B 365:1841-1852.

Qvarnström, A., N. Vallin, and A. Rudh. 2012. The role of male contest competition over mates in speciation. Current Zoology 58.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rabosky, D. L. and D. R. Matute. 2013. Macroevolutionary speciation rates are decoupled from the evolution of intrinsic reproductive isolation in Drosophila and birds. Proc. Natl. Acad. Sci. USA 110:15354-15359.

Ramsay, E. 1883. Notes on the zoology of the Solomon Islands - Part IV. Proc. Linn. Soc. N.S.W. 7:16-43.

Real, L. 1990. Search theory and mate choice. I. Models of single-sex discrimination. Am. Nat. 136:376-405.

Reifová, R., J. Reif, M. Antczak, and M. W. Nachman. 2011. Ecological character displacement in the face of gene flow: Evidence from two species of nightingales. BMC Evol. Biol. 11:1.

Reinhold, K. 1998. Sex linkage among genes controlling sexually selected traits. Behav. Ecol. Sociobiol. 44:1-7.

Renaut, S., C. J. Grassa, S. Yeaman, B. T. Moyers, Z. Lai, N. C. Kane, J. E. Bowers, J. M. Burke, and L. H. Rieseberg. 2013. Genomic islands of divergence are not affected by geography of speciation in sunflowers. Nat. Commun. 4:1827.

Renaut, S., N. Maillet, E. Normandeau, C. Sauvage, N. Derome, S. M. Rogers, and L. Bernatchez. 2012. Genome-wide patterns of divergence during speciation: the lake whitefish case study. Philos. Trans. R. Soc. Lond. B 367:354-363.

Rheindt, F. E. and S. V. Edwards. 2011. Genetic introgression: an integral but neglected component of speciation in birds. Auk 128:620-632.

Rhymer, J. M. and D. Simberloff. 1996. Extinction by hybridization and introgression. Annu. Rev. Ecol. Syst. 27:83-109.

Rice, W. R. 1984. Sex chromosomes and the evolution of sexual dimorphism. Evolution 38:735-742.

Rice, W. R. 1987. The accumulation of sexually antagonistic genes as a selective agent promoting the evolution of reduced recombination between primitive sex chromosomes. Evolution 41:911-914.

Richards, D. G. 1979. Recognition of neighbors by associative learning in rufous-sided towhees. Auk 96:688-693.

Rieseberg, L. H. 2001. Chromosomal rearrangements and speciation. Trends Ecol. Evol. 16:351-358.

Rieseberg, L. H. and C. A. Buerkle. 2002. Genetic mapping in hybrid zones. Am. Nat. 159:S36-S50.

Rieseberg, L. H., S.-C. Kim, R. A. Randell, K. D. Whitney, B. L. Gross, C. Lexer, and K. Clay. 2007. Hybridization and the colonization of novel habitats by annual sunflowers. Genetica 129:149-165.

Rising, J. D. and K. M. Somers. 1989. The measurment of overall body size in birds. Auk 106:666-674.

Ritchie, M. G. 2007. Sexual selection and speciation. Annu.Rev.Ecol.Evol.Syst. 38:79-102.

Rothschild, W. and E. Hartert. 1908. On a collection of birds from San Christoval, Solomon Islands. Novit. Zool. 15:359-365.

Roughgarden, J. and S. Pacala. 1989. Taxon cycle among *Anolis* lizard populations: review of evidence. Pp. 403-432. Speciation and its consequences. Sinauer Associates, Sunderland, MA.

Rundle, H. D., S. F. Chenoweth, P. Doughty, and M. W. Blows. 2005. Divergent selection and the evolution of signal traits and mating preferences. PLoS Biol. 3:e368.

Sæther, S. A., G.-P. Sætre, T. Borge, C. Wiley, N. Svedin, G. Andersson, T. Veen, J. Haavie, M. R. Servedio, and S. Bureš. 2007. Sex chromosome-linked species recognition and evolution of reproductive isolation in flycatchers. Science 318:95-97.

Sætre, G. P., T. Moum, S. Bures, M. Král, M. Adamjan, and J. Moreno. 1997. A sexually selected character displacement in flycatchers reinforces premating isolation. Nature 387:589-592.

Safran, R. J., E. S. C. Scordato, L. B. Symes, R. L. Rodríguez, and T. C. Mendelson. 2013. Contributions of natural and sexual selection to the evolution of premating reproductive isolation: a research agenda. Trends Ecol. Evol. 28:643-650.

Sardell, J. M. 2016. Recent dispersal events among Solomon Islands bird species reveal differing potential routes of island colonization. Pac. Sci. 70:201-208.

Sardell, J. M. and J. A. C. Uy. 2016. Hybridization following recent secondary contact results in asymmetric genotypic and phenotypic introgression between island species of *Myzomela* honeyeaters. Evolution 70:257-267.

Schluter, D. 2001. Ecological character displacement. Pp. 265-276. Evolutionary ecology: concepts and cases studies. Oxford University Press, Oxford, UK.

Schluter, D. and J. D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. Am. Nat. 140:85-108.

Schluter, D., T. D. Price, and P. R. Grant. 1985. Ecological character displacement in Darwin's finches. Science 227:1056-1059.

Seddon, N., C. A. Botero, J. A. Tobias, P. O. Dunn, H. E. Macgregor, D. R. Rubenstein, J. A. Uy, J. T. Weir, L. A. Whittingham, and R. J. Safran. 2013. Sexual selection accelerates signal evolution during speciation in birds. Proc. R. Soc. Lond. B 280:20131065.

Seddon, N. and J. A. Tobias. 2010. Character displacement from the receiver's perspective: Species and mate recognition despite convergent signals in suboscine birds. Proc. R. Soc. Lond. B 277:2475-2483.

Seehausen, O. 2004. Hybridization and adaptive radiation. Trends Ecol. Evol. 19:198-207.

Seehausen, O. and D. Schluter. 2004. Male–male competition and nuptial–colour displacement as a diversifying force in Lake Victoria cichlid fishes. Proc. R. Soc. Lond. B 271:1345-1353.

Ser, J. R., R. B. Roberts, and T. D. Kocher. 2010. Multiple interacting loci control sex determination in Lake Malawi cichlid fish. Evolution 64:486-501.

Servedio, M. R. and M. A. F. Noor. 2003. The role of reinforcement in speciation: theory and data. Annu. Rev. Ecol., Evol. Syst. 34:339-364.

Shaw, K. L. 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: what mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. Proc. Natl. Acad. Sci. USA 99:16122-16127.

Short, L. L. 1969. Taxonomic aspects of avian hybridization. Auk 86:84-105.

Sidorovich, V., H. Kruuk, and D. Macdonald. 1999. Body size, and interactions between European and American mink (*Mustela lutreola* and *M. vison*) in Eastern Europe. J. Zool. 248:521-527.

Simberloff, D., T. Dayan, C. Jones, and G. Ogura. 2000. Character displacement and release in the small Indian mongoose, *Herpestes javanicus*. Ecology 81:2086-2099.

Slade, R. W., C. Moritz, A. Heideman, and P. T. Hale. 1993. Rapid assessment of single-copy nuclear DNA variation in diverse species. Mol. Ecol. 2:359-373.

Slatkin, M. 1980. Ecological character displacement. Ecology 61:163-177.

Smith, C. E. and C. E. Filardi. 2007. Patterns of molecular and morphological variation in some Solomon Island land birds. Auk 124:479-493.

Smith, M. W. and S. J. O'Brien. 2005. Mapping by admixture linkage disequilibrium: advances, limitations and guidelines. Nature Reviews Genetics 6:623-632.

Sorenson, M. D., J. C. Ast, D. E. Dimcheff, T. Yuri, and D. P. Mindell. 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. Mol. Phylogen. Evol. 12:105-114.

Steeves, T. E., R. F. Maloney, M. L. Hale, J. M. Tylianakis, and N. J. Gemmell. 2010. Genetic analyses reveal hybridization but no hybrid swarm in one of the world's rarest birds. Mol. Ecol. 19:5090-5100.

Stein, A. C. and J. A. C. Uy. 2006. Unidirectional introgression of a sexually selected trait across an avian hybrid zone: A role for female choice? Evolution 60:1476-1485.

Stephens, M., N. J. Smith, and P. Donnelly. 2001. A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. 68:978-989.

Stephenson, B. P. and A. Ramírez-Bautista. 2012. Did sexually dimorphic dorsal coloration evolve by a pre-existing bias in males in the lizard *Sceloporus minor*? Evol. Ecol. 26:1277-1291.

Stern, D. L. 2013. The genetic causes of convergent evolution. Nat. Rev. Genet. 14:751-764.

Strauss, S. Y., J. A. Lau, and S. P. Carroll. 2006. Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? Ecol. Lett. 9:357-374.

Stuart, Y. E. and J. B. Losos. 2013. Ecological character displacement: glass half full or half empty? Trends Ecol. Evol. 28:402-408.

Svedin, N., C. Wiley, T. Veen, L. Gustafsson, and A. Qvarnström. 2008. Natural and sexual selection against hybrid flycatchers. Proc. R. Soc. Lond. B 275:735-744.

Taylor, E., J. Boughman, M. Groenenboom, M. Sniatynski, D. Schluter, and J. Gow. 2006. Speciation in reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus aculeatus*) species pair. Mol. Ecol. 15:343-355.

Tobias, J. A. and N. Seddon. 2009. Signal design and perception in *Hypocnemis* antbirds: evidence for convergent evolution via social selection. Evolution 63:3168-3189.

Toon, A., J. Hughes, and L. Joseph. 2010. Multilocus analysis of honeyeaters (Aves: Meliphagidae) highlights spatio-temporal heterogeneity in the influence of biogeographic barriers in the Australian monsoonal zone. Mol. Ecol. 19:2980-2994.

Trier, C. N., J. S. Hermansen, G.-P. Sætre, and R. I. Bailey. 2014. Evidence for mito-nuclear and sex-linked reproductive barriers between the hybrid Italian sparrow and its parent species. PLoS Genetics 10:e1004075.

Turelli, M. and L. C. Moyle. 2007. Asymmetric postmating isolation: Darwin's corollary to Haldane's rule. Genetics 176:1059-1088.

Turner, T. L., M. W. Hahn, and S. V. Nuzhdin. 2005. Genomic islands of speciation in *Anopheles gambiae*. PLoS biology 3:e285.

Tuttle, E. M., A. O. Bergland, M. L. Korody, M. S. Brewer, D. J. Newhouse, P. Minx, M. Stager, A. Betuel, Z. A. Cheviron, and W. C. Warren. 2016. Divergence and functional degradation of a sex chromosome-like supergene. Curr. Biol. 26:344-350.

Tynkkynen, K., J. S. Kotiaho, M. Luojumäki, and J. Suhonen. 2005. Interspecific aggression causes negative selection on sexual characters. Evolution 59:1838-1843.

Uy, J. A. C., R. G. Moyle, and C. E. Filardi. 2009a. Plumage and song differences mediate species recognition between incipient flycatcher species of the Solomon Islands. Evolution 63:153-164.

Uy, J. A. C., R. G. Moyle, C. E. Filardi, and Z. A. Cheviron. 2009b. Difference in plumage color used in species recognition between incipient species is linked to a single amino acid substitution in the Melanocortin-1 Receptor. Am. Nat. 174:244-254.

Uy, J. A. C. and R. J. Safran. 2013. Variation in the temporal and spatial use of signals and its implications for multimodal communication. Behav. Ecol. Sociobiol. 67:1499-1511.

Vallin, N., A. M. Rice, R. I. Bailey, A. Husby, and A. Qvarnström. 2012. Positive feedback between ecological and reproductive character displacement in a young avian hybrid zone. Evolution 66:1167-1179.

Van Doorn, G. and M. Kirkpatrick. 2007. Turnover of sex chromosomes induced by sexual conflict. Nature 449:909-912.

van Doorn, G. S., U. Dieckmann, and F. J. Weissing. 2004. Sympatric speciation by sexual selection: a critical reevaluation. Am. Nat. 163:709-725.

Vaurie, C. 1957. Systematic notes on Palaearctic birds, no. 26, Paridae: the *Parus caeruleus* complex. Am. Mus. Novit. 1833:1-15.

Warren, B. H., E. Bermingham, Y. Bourgeois, L. K. Estep, R. P. Prys-Jones, D. Strasberg, and C. Thébaud. 2011. Hybridization and barriers to gene flow in an island bird radiation. Evolution 66:1490-1505.

Warren, W. C., D. F. Clayton, H. Ellegren, A. P. Arnold, L. W. Hillier, A. Künstner, S. Searle, S. White, A. J. Vilella, and S. Fairley. 2010. The genome of a songbird. Nature 464:757-762.

Weir, J. and D. Schluter. 2008. Calibrating the avian molecular clock. Mol. Ecol. 17:2321-2328.

Werren, J. H., S. Richards, C. A. Desjardins, O. Niehuis, J. Gadau, J. K. Colbourne, G. Nasonia Genome Working, L. W. Beukeboom, C. Desplan, C. G. Elsik, C. J. Grimmelikhuijzen, P. Kitts, J. A. Lynch, T. Murphy, D. C. Oliveira, C. D. Smith, L. van de Zande, K. C. Worley, E. M. Zdobnov, M. Aerts, S. Albert, V. H. Anaya, J. M. Anzola, A. R. Barchuk, S. K. Behura, A. N. Bera, M. R. Berenbaum, R. C. Bertossa, M. M. Bitondi, S. R. Bordenstein, P. Bork, E. Bornberg-Bauer, M. Brunain, G. Cazzamali, L. Chaboub, J. Chacko, D. Chavez, C. P. Childers, J. H. Choi, M. E. Clark, C. Claudianos, R. A. Clinton, A. G. Cree, A. S. Cristino, P. M. Dang, A. C. Darby, D. C. de Graaf, B. Devreese, H. H. Dinh, R. Edwards, N. Elango, E. Elhaik, O. Ermolaeva, J. D. Evans, S. Foret, G. R. Fowler, D. Gerlach, J. D. Gibson, D. G. Gilbert, D. Graur, S. Grunder, D. E. Hagen, Y. Han, F. Hauser, D. Hultmark, H. C. t. Hunter, G. D. Hurst, S. N. Jhangian, H. Jiang, R. M. Johnson, A. K. Jones, T. Junier, T. Kadowaki, A. Kamping, Y. Kapustin, B. Kechavarzi, J. Kim, B. Kiryutin, T. Koevoets, C. L. Kovar, E. V. Kriventseva, R. Kucharski, H. Lee, S. L. Lee, K. Lees, L. R. Lewis, D. W. Loehlin, J. M. Logsdon, Jr., J. A. Lopez, R. J. Lozado, D. Maglott, R. Maleszka, A. Mayampurath, D. J. Mazur, M. A. McClure, A. D. Moore, M. B. Morgan, J. Muller, M. C. Munoz-Torres, D. M. Muzny, L. V. Nazareth, S. Neupert, N. B. Nguyen, F. M. Nunes, J. G. Oakeshott, G. O. Okwuonu, B. A. Pannebakker, V. R. Pejaver, Z. Peng, S. C. Pratt, R. Predel, L. L. Pu, H. Ranson, R. Raychoudhury, A. Rechtsteiner, J. T. Reese, J. G. Reid, M. Riddle, H. M. Robertson, J. Romero-Severson, M. Rosenberg, T. B. Sackton, D. B. Sattelle, H. Schluns, T. Schmitt, M. Schneider, A. Schuler, A. M. Schurko, D. M. Shuker, Z. L. Simoes, S. Sinha, Z.

Smith, V. Solovyev, A. Souvorov, A. Springauf, E. Stafflinger, D. E. Stage, M. Stanke, Y. Tanaka, A. Telschow, C. Trent, S. Vattathil, E. C. Verhulst, L. Viljakainen, K. W. Wanner, R. M. Waterhouse, J. B. Whitfield, T. E. Wilkes, M. Williamson, J. H. Willis, F. Wolschin, S. Wyder, T. Yamada, S. V. Yi, C. N. Zecher, L. Zhang and R. A. Gibbs. 2010. Functional and evolutionary insights from the genomes of three parasitoid *Nasonia* species. Science 327:343-348.

West-Eberhard, M. J. 1983. Sexual selection, social competition, and speciation. Q. Rev. Biol. 58:155-183.

While, G. M., S. Michaelides, R. J. Heathcote, H. E. MacGregor, N. Zajac, J. Beninde, P. Carazo, G. Pérez i de Lanuza, R. Sacchi, and M. A. Zuffi. 2015. Sexual selection drives asymmetric introgression in wall lizards. Ecol. Lett. 18:1366-1375.

Whitehead, H. and S. Walde. 1993. Territoriality and the evolution of character displacement and sexual dimorphism. Ethol. Ecol. Evol. 5:303-318.

Whitlock, M. and D. Schluter. 2015. The analysis of biological data. Roberts & Company Publishers, Greenwood Village, CO.

Wilkinson, G. S., F. Breden, J. E. Mank, M. G. Ritchie, A. D. Higginson, J. Radwan, J. Jaquiery, W. Salzburger, E. Arriero, and S. Barribeau. 2015. The locus of sexual selection: moving sexual selection studies into the post-genomics era. J. Evol. Biol. 28:739-755.

Wilson, D. S. and A. Hedrick. 1982. Speciation and the economics of mate choice. Evol.Theory 6:15-24.

Wirtz, P. 1999. Mother species–father species: unidirectional hybridization in animals with female choice. Anim. Behav. 58:1-12.

Yom-Tov, Y., S. Yom-Tov, and H. Moller. 1999. Competition, coexistence, and adaptation amongst rodent invaders to Pacific and New Zealand islands. J. Biogeogr. 26:947-958.

Yoshida, K., T. Makino, K. Yamaguchi, S. Shigenobu, M. Hasebe, M. Kawata, M. Kume, S. Mori, C. L. Peichel, and A. Toyoda. 2014. Sex chromosome turnover contributes to genomic divergence between incipient stickleback species. PLoS Genet. 10:e1004223.

Zahavi, A. 1975. Mate selection—a selection for a handicap. J. Theor. Biol. 53:205-214.