MATING TRAIT DIVERGENCE IN *HABRONATTUS AMERICANUS* JUMPING SPIDERS AND SEX RATIO EVOLUTION UNDER SEXUAL CONFLICT

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES
(ZOOLOGY)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)
June 2013

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Abstract

I investigated two topics in evolution. The first concerns the potential role of sexual selection in population divergence of *H. americanus* jumping spiders. Field observations suggest males seldom feed yet travel widely, apparently seeking mates. Males displayed vigorously to females, whereas females appeared highly choosy. The apparent absence of antagonistic behavior during male-male interactions suggests mate competition in this species is mediated by female choice. I assessed if female preferences for local males promote reproductive isolation among three *H. americanus* populations that are each monomorphic for a different male sexual display morph. Supporting this idea, virgin females copulated more often with local compared to foreign males during mate trials. However, the effect of other mating interaction components on mating success remains to be resolved. All crosses produced offspring, ruling out strong intrinsic reproductive barriers among morphs and suggesting divergent female mate preferences may constitute an early source of reproductive isolation. I documented low genetic divergence among several populations, further indicating selection underlies the stark display differences between them. Further, this force appears to counteract gene flow: among-morph population comparisons show “isolation by distance”, despite the fact that phenotypically similar populations, which are scattered widely across the study area, are relatively closely related. This implies a greater exchange of genes between phenotypically similar populations. Collectively, these results implicate divergent selection on, or correlated with, male sexual displays at an early stage of differentiation in this species.

In a separate study, two colleagues and I use genetic models to demonstrate that sex ratio adjustment (SRA) by parents can reduce intralocus sexual conflict (IASC) by directing alleles of a sexually antagonistic trait to the sex of offspring they benefit. If the trait is autosomally inherited, this strategy evolves irrespective of which parent’s genotype SRA is based on. It can also evolve when the trait is sex-linked, provided decisions are based on the genotype of the homogametic sex—SRA based on the heterogametic sex instead promotes fixation of the allele that is detrimental to that sex. These results suggest sexual conflict might account for previously unexplained variation in the occurrence of SRA in nature.
Preface

A version of Chapter 5 has been published as: “Blackburn, G.S.B., Albert, A.Y.K., and Otto, S.P. (2010). The evolution of sex ratio adjustment in the presence of sexually antagonistic selection. The American Naturalist (176) 3, 264-275”. I generated the original idea for the project and developed the final scope of the study with Sally Otto. I built recursion equations for the analytical models. Arianne Albert and Sally isolated model conditions that favor the evolution of sex ratio adjustment (SRA). Sally designed a simulation for the long-term evolution of SRA. I modified the simulation for alternative genetic architectures and Sally and I compiled simulation results. Sally wrote or strongly guided my writing of the Results, and she also created the article figures. I wrote most of the remaining manuscript.

The behavioral experiment in Chapter 3 was approved by the UBC animal care committee (A06-1540). Permits for spider collection were obtained from the Yosemite National Park (YOSE 2007 SCI 0045, YOSE 2010 SCI 0068).
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Acknowledgements

I would like to thank my advisor Wayne Maddison for being a constant source of enthusiasm and encouragement for me. Working with Wayne is a lot of fun and that made a huge difference at every stage of this project, from initial explorations of field sites to ironing out wrinkles in the final writing. I would also like to thank Wayne for choosing such good people to join the lab: Melissa Bodner, Damian Elias, Junxia Zhang, Edi Piascek, Genevieve Leduc-Robert, Ingi Agnarrson, Gustavo Ruiz, Karen Needham, and Peter Midford. They have been a constant source of collegiality and friendship for me.

Wayne and my committee members, Darren Irwin, Sally Otto, Bernie Roitberg, and Dolph Schluter, have patiently stood by me as I tried to grow. I thank them for their guidance. Bernie brought to our meetings the same common sense that I had admired in him during my previous degree at SFU, and this was of particular importance to me during both the planning and writing up of my research. Darren had a helpful way of thinking through problems from scratch and this set a valuable example for me. Dolph has always been generous with his time, and I relied on his advice to help me through some tough research decisions. Dolph also welcomed me into his lab meetings and this put me in contact with a great group of students. I was far more influenced by his approach to science than I have so far been able to bring into my own work. I would especially like to thank Sally. Working with Sally on the sex ratio paper was a truly great experience for me. She had a special way of guiding me through each obstacle while letting me pretend I was getting there on my own. The pace was exhilarating, and I hope I can capture it again.

An amazing part of my experience has been to notice the talent and expertise in my schoolmates. I drew on their advice and examples constantly. A few also went well out of their way for me, and I am truly grateful to them: Rowan Barrett, Melissa Bodner, Gina Conte, Anne Dalziel, Rich Fitzjohn, Aleeza Gerstein, Haley Kenyon, Julie Lee-Yaw, Jon Mee, Diana Rennison, Thor Veen, and Junxia Zhang. Thanks especially to Julie who leapt into my project with both feet and helped me bring it home.

My family and friends outside of UBC offered absolutely unwavering support for me the whole way through this degree. I doubt they realize how much I leaned on them at some stages. At other times, the support or space I demanded as I struggled to grow was pretty clear. No matter what, they were always there. Most of all, Samantha has been right by my
side the whole way, closely sharing all the ups and downs with me. I could not have done this without her, and I wouldn't want to. Mom, dad, Meghan & co., Jason & Amanda, Martin & Christine, Ben, Catriona, Joel, Esperanza, Robb & Jenny, Mike & Erica, Sam – Thank you for never giving up!
For Samantha
Chapter 1: Introduction

Biological evolution is the change of allele frequencies over time in a group of individuals. In combination with environmental effects on trait expression, evolution underlies the diversity of living forms around us. The present thesis describes two separate investigations into the evolutionary process. The first focuses on how evolution promotes population divergence, potentially leading to the formation of new species. Using a combination of field observations, laboratory-based behavioral tests, and population genetic analyses, I ask how selection stemming from mating interactions in a set of phenotypically divergent populations contributes to the buildup of reproductive isolation between them. The second investigation explores evolution within species. In collaboration with two colleagues, I design genetic models to investigate whether sex ratio adjustment by parents can evolve as a means to direct traits with opposing fitness effects in females and males to the offspring they will benefit most.

1.1 Sexual selection and speciation

Among the most striking traits in nature are those directly involved in sexual reproduction. Two characteristics of these traits in particular intrigued Darwin as he was developing his theory of natural selection (Darwin 1859; Darwin 1871). First, the traits often appeared to offer little benefit to their bearers (usually males) in terms of daily growth and survival. Indeed, the traits appeared to impose large performance costs in this respect, given that they were often developed to extreme magnitudes relative to other traits. The inconsistent development of the traits across sexes, and their underdevelopment or absence in juvenile life stages, also seemed at odds with overlapping aspects of their life histories. Second, the traits seemed to feature prominently in sexual contests among adult males or in displays to adult females—that is, they apparently played a central role in competition for mates.

Darwin recognized the unique role these traits appeared to play in determining the fitness of their bearers, and the potentially unique socially-mediated mechanisms by which they might evolve. He defined “sexual selection” as a special form of natural selection deriving specifically from mate competition. Theoretical research on sexual selection has since led to the identification of numerous hypothetical mechanisms for the evolution of traits by means of sexual selection, and empirical evidence for several of these is mounting. At the same time, progress in our understanding of how species form has brought renewed attention to the potential
role of sexual selection in speciation. As a result, sexual selection research has become a central issue in evolutionary biology.

1.1.1 **Trends in the study of sexual selection with respect to speciation**

Research efforts into the evolution of sexual displays and their role in speciation each have extensive and partially separate histories. Sexual displays received their first widespread theoretical attention during the Modern Synthesis of the 1930’s. At that time they were considered to function primarily in “species recognition”—discrimination among genetically differentiated forms as a means of avoiding maladaptive hybridization (Coyne & Orr 2004; e.g. Mayr 1963; Ritchie 2007; reviewed in West-Eberhard 1983). Viewed in this light, sexual selection was considered to play a direct role in speciation through the enhancement of mating barriers among divergent lineages (reviewed in Coyne & Orr 2004; ”reinforcement”, Dobzhansky 1937), with its influence on patterns of diversity depending on the prevalence of natural selection against hybridization in nature.

This view was sidelined in the 1980’s when attention shifted instead to the role of displays in intrapopulation mate competition and the different mechanisms by which this might evolve (reviewed in, Andersson 1994; Kirkpatrick 1982; e.g., Lande 1981). Ushering in this shift was the return to Darwin’s original insight that mate choice can mediate mate competition within species (West-Eberhard 1983), an idea that has now received widespread empirical support (reviewed in Andersson 1994). This era initiated the exploration of a variety of hypothetical models for the evolution of sexual display traits and the mate preferences that shape them (Andersson 1994; reviewed in Kirkpatrick & Ryan 1991). When referring to sexual display traits and mate preferences collectively, I will call them “mating traits” (e.g. Hoskin & Higgie 2010).

Consideration of the most prominent models in the literature highlights the numerous biologically feasible processes by which one or both types of mating trait might evolve within populations and how this might prompt divergence in the dynamics of mating interactions among populations.

The earliest model was put forward verbally by Ronald Fisher (1915; 1930), who suggested mate preferences evolve in concert with display traits simply due to the linkage disequilibrium that automatically forms between the relevant female and male alleles during the process of mate choice (“runaway” selection, Lande 1981; Pomiankowski & Iwasa 1998). Subsequent authors proposed that attractive males might instead provide heritable fitness benefits outside of
the mating advantage itself, either indirectly to offspring (Hamilton & Zuk 1982; Iwasa & Pomiankowski 1999; Iwasa et al. 1991; “good genes”, Zahavi 1975) or directly to their mate (e.g., by alleviating mate search costs or offering enhanced paternal care), resulting in selection favoring the evolution of preferences for male display traits. Focusing instead on the proximate mechanisms of communication, the “sensory drive” model emphasizes the strong potential influence of sensory perception and processing on mate preferences (Endler 1992; Guilford & Dawkins 1991). Consequently, any sensory biases that predispose the choosing sex to respond most strongly to certain types of signal stimuli would favor displays that appeal to these biases (Boughman 2002; Endler 1992; Endler & Basolo 1998; Ryan 1990). Finally, recent years have seen a greater appreciation for the frequently differing reproductive interests between the sexes (e.g. mating rate, Arnvist & Rowe 2005; Bateman 1948; Chippindale et al. 2001; Parker 1979) and attention to the co-evolutionary cycles this can prompt between the sexes (Dawkins & Guilford 1991; Dawkins & Krebs 1978; Holland & Rice 1999; Rice 1996). From this perspective, the “chase-away” model posits that female and male mating traits co-evolve antagonistically via the spread of manipulative signals in one sex and subsequent buildup of resistance to those signals in the opposite sex (Gavrilets 2000; Gavrilets et al. 2001; Holland & Rice 1998).

The candidate processes make overlapping predictions regarding the direction of mate preference and display trait evolution, and distinguishing their operation in nature remains a major empirical challenge (reviewed in Andersson & Simmons 2006). Notably, the direction in which mating traits evolve by any of these putative mechanisms is likely to be influenced strongly by the particular source of selection propelling them (e.g., runaway: which display trait first becomes correlated with mating success; direct or indirect benefits: which adaptive benefit is signaled by the display trait; sensory bias or chase-away: which bias is triggered by display traits). Since these sources are themselves likely to vary according to the ecological or genetic context in which they arise, sexual selection might foster an array of different phenotypic outcomes for the mating traits involved (e.g. Lande 1981; Parker & Partridge 1998). Further, this evolution can be rapid, and in many cases might never reach a stable equilibrium (Mead & Arnold 2004). Thus, while the prevalence of different putative mechanisms and their outcomes remains to be clarified, the prediction that sexual selection promotes diversification is well established.
These advances have been accompanied by renewed attention to the relationship between sexual selection and speciation (West-Eberhard 1983; Schluter 2000; Panhuis et al. 2001; Coyne and Orr 2004; Ritchie 2007). The fact that differentiation might stem from intra-population processes broadens substantially the expected scope of sexual selection to contribute to population divergence, potentially resulting in the development of reproductive barriers between separate lineages. This possibility raises several empirical questions (Panhuis et al. 2001). One of these is whether the diverse display traits in nature actually signify sexual isolation among populations. Many alternative outcomes of sexual selection could generate phenotypic differentiation without posing reproductive barriers if populations were to come into contact, and this would seriously restrict the contribution of sexual selection to long-term diversification in general. For example, a given display trait might be mutually appealing across populations or could even appeal most strongly to foreign females, due either to preference evolution within populations that enhanced female response to rare display traits or to pre-existing female sensory biases that are shared across populations. Testing the potential for sexual selection to contribute to speciation therefore entails an account of the mate preferences associated with them. Discrimination against foreign males arising during mating interactions or postcopulatory gametic competition (i.e., forms of “prezygotic” isolation, reviewed in Coyne and Orr 2004) would implicate sexual selection as a reproductive barrier between divergent groups.

A second question, given the presence of prezygotic isolation, is whether this isolation is strong enough to promote ongoing divergence. In particular, whereas even modest sexual selection might drive the divergence of mating traits in isolated populations, considerably stronger selection is needed to promote differentiation of these loci or other parts of the genome if populations come into contact and exchange migrants (Gray & Cade 2000). This calls for estimates of the strength of sexual selection, or at least accounts of how effectively it counters the effects of gene flow.

Finally, a major determinate of the role of sexual selection in speciation concerns the stage at which it contributes relative to other sources of reproductive isolation. Reinforcement involves the buildup of prezygotic isolation as a result of selection against hybrids. While this does not necessarily diminish the importance of sexual selection to the process of speciation, it restricts its scope to a relatively late stage—after alternative reproductive barriers have already formed among related lineages. By contrast, the more recent theory summarized above extends the potential contribution of sexual selection to the outset of population divergence. If it
commonly fills this latter role, sexual selection can in principle contribute to all stages of speciation, or even initiate it. Testing this possibility entails investigating the presence of alternative potential sources of reproductive isolation in systems where divergent sexual selection is at play.

1.1.2 Study system: *Habronattus* jumping spiders (Aranae, Salticidae)

I investigate these questions in a species of jumping spider, *Habronattus americanus*. Jumping spiders (Salticidae) are gaining attention in sexual selection research due to their elaborate male sexual displays (Chan et al. 2008; Clark & Biesiadecki 2002; Lim et al. 2007). The North American genus *Habronattus* consists of about one hundred species that feature a wide array of morphological, sonic, and behavioral traits across species that are prominently displayed to females during courtship (Griswold 1987; Maddison & Stratton 1988; Peckham & Peckham 1889; Peckham & Peckham 1890; Richman 1982). Masta and Maddison (2002) have provided evidence that sexual selection contributes to intraspecific phenotypic divergence. Other research has begun to unravel how female preferences shape male displays (Hebets & Maddison 2005) and to explore the function of the various traits and modalities that comprise them (Elias et al. 2006; Elias 2003).

*H. americanus* is a cursorial, generalist, predator that is known to occur from southern California (USA) to the Yukon (Canada), and at least as far eastward as Ontario (Canada). Adult females are brown and gray in color and similar in appearance to juveniles. Conversely, adult males of this species possess red, blue, and white morphological features on their anterior cephalothorax and legs that are prominently displayed to females during ritualized courtship dances (Griswold 1987; see images in Maddison 1995). A striking aspect of phenotypic variation among *H. americanus* males in this regard is the number of traits comprising the display that are covered with red-coloured bristles. Three discrete display morphs of this red coloration were known to exist in nature at the start of this thesis and they form the focus of the present research: red pedipalps (hereafter referred to as the “P” morph); red pedipalps and red anterior legs (“PL”); red pedipalps, anterior legs, and chelicerae (“PLC”). The P and PLC forms have been reported only within the western USA, whereas the PL morph occurs across most of the species range. Collections have so far revealed only a single morph in each location sampled, suggesting the populations have diverged sharply in these characters.
1.1.3 Research goals

The *H. americanus* system appears to offer insight to each of the three questions raised above. The prominence of the anterior legs, pedipalps and chelicera in courtship implicates sexual selection in the divergence of phenotypically contrasting populations. If the populations are also closely related, processes shaping these mating traits might be among the earliest forces pushing them apart. Finally, in part of the species range, the close geographic proximity of several phenotypically contrasting populations raises the possibility that divergence in this region has arisen or persisted in the presence of gene flow.

I begin the research on this species with an observational field study conducted within a single population (Chapter 2). The goal is to document features of adult female and male life history traits in nature that help characterize the mating system in this species and hence uncover the direction and strength of sexual selection between sexes. At the broadest level, I seek to clarify whether mating interactions are characterized by intersexual interactions—pointing to female choice as a key source of sexual selection—versus direct contests among males. I also qualitatively gauge the strength of sexual selection by documenting the relative effort exhibited by each sex in finding prospective mates. I also take advantage of the presence of a closely related species, *H. ophrys*, in an adjacent population as a means to gain insight to differences in the mode or strength of sexual selection between them.

Having assessed the likely selective context of mating traits in *H. americanus*, I next turn to a set of *H. americanus* populations in the Sierra Nevada Mountains (CA, USA) that each feature a unique male sexual display morph, collectively representing the three morphs described above (Chapter 3). I conduct mate trials in a laboratory setting between individual females and males from different populations in order to determine if and how female mate preferences have diverged among populations. The objective here is to establish if females discriminate against male displays, and hence if female mate preferences pose a source of prezygotic isolation among populations. I assess this across several components of mating interactions (copulation occurrence, latency to copulate, number of female sperm storage organs ["spermatheca"] accessed, and copulation duration) in order to determine potential variation in the contribution of each component to mating outcomes. I also further characterize *H. americanus* mating strategies by testing females both as virgins and following copulation, as well as a sample of field-matured females with unknown sexual histories. Finally, I gather data on the production of offspring.
across mate trials as a means to learn whether strong intrinsic postmating reproductive isolation, in the form of early offspring inviability, exists among populations.

The close proximity of the *H. americanus* populations in the Sierra Nevada Mountains raises the possibility that the phenotypic divergence has arisen, or currently persists, in the presence of gene flow among populations. The system therefore provides a test of the possibility that sexual selection in this system drives divergence despite gene exchange among populations. In the final research effort in this system (Chapter 4), I survey a broad geographic area in the mountain range to determine how regionally widespread is the occurrence of discretely differing morphs. I then conduct a genetic analysis across populations. I assess if the sites are closely related, and hence if the discrete differences in display morph are likely promoted by divergent selection. I also evaluate if there is genetic evidence for gene flow between locations despite the apparent phenotypic divergence between them.

### 1.2 Sex ratio adjustment in the presence of sexually antagonistic selection

The differing gamete sizes that define male versus female sexual roles (i.e., anisogamy) lead to distinct reproductive strategies in each sex: males invest their relatively small and numerous sperm in the fertilization of many offspring; females invest their ova in the production of high-quality offspring (Darwin 1871; Maynard Smith 1978; Parker et al. 1972). This fundamental difference between the sexes translates into numerous life history differences between them, resulting in opposing sex-specific (i.e., sexually antagonistic) selection pressures on many of the traits that they share. Sexually antagonistic selection on traits expressed in both females and males represents intralocus sexual conflict (Bonduriansky & Chenoweth 2009; "IASC", Parker 1979; van Doorn 2009).

#### 1.2.1 Trends in the study of intralocus sexual conflict and sex allocation

Many of the life history differences between the sexes are reflected in the presence of sexually dimorphic traits—familiar examples in humans include male-female differences in adult muscle mass and pelvic structure. Accordingly, theoretical research on the resolution of IASC has focused primarily on identifying genetic mechanisms by which sexual dimorphism can evolve (Connallon & Clark 2011; genomic imprinting, Day & Bonduriansky 2004; gene duplication, Gallach & Betran 2010; e.g., modifiers promoting sex-limited trait expression, Lande 1980; sex linkage, Rice 1984). Several of these mechanisms have gained empirical
support (see also Baker et al. 2012; reviewed in Bonduriansky & Chenoweth 2009; van Doorn 2009; Wyman et al. 2012), suggesting the evolution of sexual dimorphism is both a robust and flexible solution to IASC.

However, despite the widespread occurrence of sexual dimorphism in nature, many dimorphic traits are suboptimally expressed in one or both sexes in terms of the selection pressures acting on them (e.g. Forsman 1995; Harano et al. 2010). Further, numerous other traits with contrasting expression optima among sexes are monomorphic (reviewed in Cox & Calsbeek 2009; e.g. Merila et al. 1997). These cases could reflect genetic constraints on the degree to which sexual dimorphism can evolve, such as strong correlations between female and male trait expression (Lande 1980) or pleiotropic non-sexually antagonistic functions of the trait in question (Badyaev 2002; Ellegren & Parsch 2007; van Doorn 2009). Alternatively, residual levels of IASC might be an inevitable outcome of a limited array of mutational possibilities, even where sexual dimorphism is strong (Bonduriansky & Chenoweth 2009). At least in these cases, selection might strongly favor alternative means to alleviate IASC.

One potential alternative is sex ratio adjustment (SRA). SRA has been documented in a wide array of taxa, often varying across individuals or reproductive efforts within individuals, and in some cases reaching highly skewed proportions of male versus female offspring (reviewed in Cockburn 2002; e.g. Komdeur et al. 1997; West 2009; West et al. 2002). Classical theory explains the evolution of SRA as a means by which parents can optimally allocate resources to each sex of offspring, based on the idea that the ecological conditions of breeding often have differential fitness prospects for male versus female young. A wealth of empirical data supports this notion, with evidence that females may implement SRA in response to their condition or resource availability for offspring during reproduction (Trivers & Willard 1973; West & Sheldon 2002) or the attractiveness of their mate (Burley 1981; Burley 1986). IASC might provide an especially strong source of selection for SRA in this respect, given that parents who can direct sexually antagonistic fitness effects to the sex of offspring that will benefit most from them will simultaneously avoid expression costs in the opposite sex.

The evolution of SRA in response to IASC is unlikely to be constrained by factors limiting the evolution of sexual dimorphism. Moreover, the two mechanisms may act in concert, together reducing IASC to lower levels than either mechanism could achieve on its own. From this perspective, SRA represents a potentially important solution to IASC. At the same time, sexually antagonistic fitness effects might account for some of the variation in SRA occurrence.
in nature noted by previous authors (Cockburn 2002; Krackow 1995; West 2009; West et al. 2002).

1.2.2 Research goals

In the final research chapter of this thesis (Chapter 5), in collaboration with two colleagues I build population genetic models to investigate the evolution of SRA in the presence of IASC. We predict that opposing fitness effects in each sex at a locus will favor SRA because it allows parents to channel sexually antagonistic fitness effects to the sex that will benefit most from them. We consider a one-locus, bi-allelic, sexually antagonistic trait and then determine analytically the conditions that will favor the spread of an SRA allele at a separate locus. We supplement the analytical model with individual-based simulations that allow me to track the evolution of SRA through time. Lastly, we repeat this effort across several different types of genetic architecture for sexual antagonism and SRA—autosomal and sex-linked inheritance—as a means to assess genetic constraints on the evolution of SRA.
Chapter 2: Characterizing sexual selection from natural breeding behavior in two species of jumping spider, Habronattus americanus and H. ophrys

2.1 Summary

Breeding behavior, when observed within its natural ecological context, reveals unique details about mating strategies and courtship interactions that point to potential sources of selection on sexual display traits. I observed the breeding behavior of H. americanus and H. ophrys jumping spiders in their natural settings, addressing three issues that help to characterize the potential mode of sexual selection in these species: 1. Do males invest more than females in mate search?; 2. How frequently do mating interactions occur, and is mate choice limited to females?; 3. Do direct male-male contests occur? In H. americanus, males apparently invested heavily in mate search, travelling further and for proportionately more of their time than females yet eating nothing and exhibiting little hunting behavior. Females encountered on average one male per hour of surface activity and the ensuing mating interactions suggested the presence of distinct gender roles: males courted females in every case by employing a ritualized sequence of display behaviors, whereas females rejected all courting males and terminated each encounter. Conversely, male-male interactions in this species were brief, non-ritualized, and apparently non-aggressive. In contrast to H. americanus, H. ophrys featured similar degrees of travel among sexes. In addition, the few behavioral interactions I observed in this species featured intermittent male display to females, male termination of several intersexual encounters, and male-male aggression. Overall, the findings suggest that H. americanus male sexual display evolution may have been strongly shaped by female choice and costs associated with mate search, while the source or strength of selection acting on male display traits could differ in H. ophrys.

2.2 Introduction

Reproductive success in sexual species is constrained by the distribution of resources needed to reproduce (Emlen & Oring 1977; Trivers 1972; Trivers 1985; Williams 1966) and the environmental conditions in which mating interactions occur (Endler 1992; Endler & Basolo 1998). As a result, ecological context influences both the general strategy used by each sex to secure mates and the specific traits that mediate mating success. Evidence for these effects is widespread. At the level of mating strategy, the intensity of direct male competition and the
degree or direction of mate choice among sexes can vary with factors such as the availability of mates (Lehmann 2007; Mills & Reynolds 2003; Shelly & Bailey 1992; Thornhill 1984), food (Gwynne 1993; Gwynne & Simmons 1990), and nest sites (Borg et al. 2002), and with the presence of predators (Kelly & Godin 2001). Within mating interactions, the form and effectiveness of display traits depend on aspects of the signal medium such as ambient light quality (e.g. Boughman 2001; Seehausen & van Alphen 1998) and sound transmission properties (e.g. Clayton 1990; Hebets 2003; Verzijden & ten Cate 2007). Consequently, examining breeding behavior within its natural ecological context, and thus in the presence of such effects, is an essential step in identifying the forces that shape the evolution of mating traits.

Jumping spiders are gaining attention in sexual selection research due to their elaborate male courtship displays (Chan et al. 2008; Clark & Biesiadecki 2002; Lim et al. 2007). The North American genus Habronattus consists of about one hundred species that feature a wide array of morphological, sonic, and behavioral traits across species that are prominently displayed to females during mating interactions (Griswold 1987; Maddison & Stratton 1988; Peckham & Peckham 1889; Peckham & Peckham 1890; Richman 1982). Masta and Maddison (2002) have provided evidence that sexual selection contributes to this diversity. Other research has begun to unravel how female preferences shape male displays (Hebets & Maddison 2005) and to explore the significance of the multiple traits and modalities that comprise them (Elias et al. 2006; Elias 2003). However, little is known about the breeding ecology of Habronattus jumping spiders in the wild, and no published account exists of natural mating interactions for any species in the genus. In the present study, I observed two closely related species, *H. americanus* and *H. ophrys*, in natural settings. Adult females of both species are drab in color and similar in appearance to juveniles, whereas adult males possess distinctive markings on their face and legs that are prominently displayed during courtship (see images in Maddison 1995). I documented aspects of breeding behavior in these species that help to characterize their mating strategies and identify potential sources of selection on male sexual display traits.

First, I assessed the relative mate search effort in each sex. The costs of mate search may represent an important source of selection on reproductive effort in general because they can constrain the rate of interaction with prospective mates or may compromise energy required for other aspects of reproduction (e.g., courtship, copulation, or parental care) (Kasumovic et al. 2007; Proctor 1992). While *H. americanus* and *H. ophrys* males evidently pay the costs of morphological and behavioral display, the relative effort of each sex in bringing about mating
interactions is unknown in these species. I hypothesized that if males also bear the brunt of mate search costs they would travel more than females and expend less effort in other fitness enhancing activities such as hunting and feeding.

Next, I focused on the relative choosiness of each sex during intersexual encounters. In several *Habronattus* species, including *H. americanus*, a single copulation is sufficient to fertilize several clutches of eggs, and each clutch requires 3-4 weeks of female care (GSB, unpublished data; S. E. Masta, personal communication). Conversely, males appear to contribute only sperm to their offspring and in captivity they can mate with multiple females within a few days. Postcopulatory reproductive investment in these species is clearly higher, and potential reproductive rate is apparently lower, in females compared to males. This dynamic is expected to shift the operational sex ratio toward males (Emlen & Oring 1977; Trivers 1972), resulting in strong male mate competition (Bateman 1948; Jones et al. 2000). Consequently, females should be selective if given a choice of numerous prospective mates, which might impose strong selection on male displays. Nonetheless, choosiness might also be favored in males if they bear significant costs of parental investment under natural conditions (Andersson 1994; Trivers 1972; Trivers 1985; Williams 1966), if they are likely to encounter more females than they can fertilize, or if mating incurs high risks (Emlen & Oring 1977). I assessed the rate of intersexual encounters in the wild in order to assess the potential for either sex to choose among a selection of prospective mates. I predicted that if mate choice is limited to females then males would display to all females they encountered and only females would terminate encounters. I further predicted that if female choosiness is strong then the proportion of encounters leading to copulation would be low. I also noted the environmental conditions of these interactions in order to assess the breadth of signal conditions in which they occurred and thus to identify potential proximate selection pressures on male display traits.

Finally, I assessed whether male-male interactions affect mate competition. In many taxa, physical contests among males determine mating success (Andersson 1994; Beebe 1944; Clutton-Brock & Albon 1979; Jackson 1977; Searcy & Nowicki 2000) and the same traits used to this end can also be used by females when selecting a mate (Berglund et al. 1996). Thus, both intra- and intersexual interactions must be examined to clarify the potential source of sexual selection on display traits. I predicted that if direct contests among males shape display traits then males would engage in combat or ritualized display behavior with each other.
2.3 Methods

I conducted behavioral observations between April 25 and May 28, 2005, at Iona Beach Regional Park, Vancouver, British Columbia, Canada (latitude 49°N 13’12”, longitude 123°W 12’49”, 3-5 m elevation). This time period encompassed the earliest stage of the breeding season of the focal species, which can extend at least into July at this site. *H. ophrys* are found in thick grass at this location while *H. americanus* are found in open, sandy habitat with sparse cover by herbs and other beach plants. I chose a patch within each habitat type and in each I marked a rectangular grid that filled a large fraction of the patch (*H. americanus* grid: 20 × 100 m; *H. ophrys* grid: 8 × 100 m). Searches for spiders were conducted by a single worker (GSB) between 08:00-17:00, the main period of surface activity for both species at this site (see Results). Search effort was distributed roughly evenly throughout this time period and between the two study plots. To begin a search the worker began at a grid coordinate and walked slowly in a straight-line (north, east, south, or west) until either a spider was sighted or the boundary of the habitat patch was reached. In the latter case, the search was resumed beginning with a new coordinate and direction. Coordinates and directions were chosen randomly to approximate an unbiased search effort throughout the grids. The first adult spider sighted during a search was directly observed from a distance of about 1-2 m for up to 30 min or until it was lost from view. Spiders showed no obvious reaction to worker presence under this protocol.

I noted when each subject was mobile and the net distance (cm) and direction (degrees, accurate to the nearest of 16 inter-cardinal directions) it travelled every 5 min. I also recorded the time that each subject spent under partial cover (partially obscured vertical view of the subject) or full cover (totally obscured vertical view) of plants or litter. The behavior of spiders while under full cover was typically unknown. In my view, this is unlikely to bias the observations of *H. americanus*, given that the relatively open habitat structure of this species provided a clear view of the vicinity of each subject. However, I cannot make this claim for *H. ophrys* due to the dense grass cover throughout the habitat patch of this species.

Behavioral interactions were considered to occur with any animal that I visually estimated to be within 30 cm of the subject; this represents the maximum approximate distance at which spiders were observed to orient to other individuals in the present study. These encounters were judged to end when at least one individual was ≥30 cm from the other and departing, and the remaining participant was not in pursuit. During conspecific encounters I recorded the duration of the interaction and the behaviors that occurred. When the interactants were of the opposite
sex, I also noted the substrate the interaction occurred on. During heterospecific encounters I noted the reaction of the subject and, in the event of prey capture, prey identity and handling time. For individuals involved in more than one encounter, mean encounter durations were used for analysis purposes. Temperature measurements (in degrees Celsius) were taken from approximately 2 cm height above and below the ground surface at the final position of each subject.

The spiders I observed were not uniquely marked but the risk of re-sampling individuals appears low due to the large habitat patches I studied, the potential recruitment of new adults within each patch during the course of the study, and potential migration between each patch and neighboring habitat. Visual scan surveys conducted in May, 2006 (Appendix A.1), at each habitat patch suggested that population sizes are large (minimum habitat patch population size of adult H. americanus: 163 females, 98 males; H. ophrys: 14 females, 37 males), with the possible exception of H. ophrys females (but these may typically be concealed from view; see Discussion). I therefore assume the data collected from individual spiders are independent. Statistical analyses were conducted with JMP 8.0.1 (SAS Institute Inc.). Non-parametric methods were used in cases where the data failed to meet the assumptions of parametric tests. Sample sizes vary among tests and table entries according to data availability.

2.4 Results

I observed a total of 64 individuals (H. americanus: 13 females, 28 males; H. ophrys: 8 females, 15 males). Eight spiders were lost from view before their 30-minute observation session ended (range 10.6-25.0 min) but the data for these individuals were retained in the analyses under the assumption that the circumstances determining their disappearance were random with respect to the data recorded. Both species were most active in conditions of low wind and clear, sunny skies. This defined a potential activity period beginning at sunrise and continuing until late afternoon, typically with decreased activity for several hours in the middle of the day. Air and ground temperatures broadly overlapped at the two habitat patches during this period, ranging (sometimes within the same day) from 14-32 ºC (air) and 13-45 ºC (ground). A time budget for the behaviors I examined is presented in Figure 2.1; durations, rates, and proportions of time spent performing behaviors are summarized in Table 2.1 and details of intersexual encounters are in Table 2.2.
2.4.1 Factors affecting mate search effort

2.4.1.1 Travel

Paths traversed by males during a complete 30 min period were in general longer than those traversed by females (Figure 2.2). *H. americanus* males spent a greater proportion of time mobile (Welch’s ANOVA: F_{1, 29.771} = 23.508, p < 0.0001) (Figure 2.1, Table 2.1) and travelled at a greater rate (m h^{-1}) than females (Welch’s ANOVA: F_{1, 27.845} = 55.195, p < 0.0001) (Figure 2.3). *H. ophrys* showed no difference among sexes in proportion of time mobile (ANOVA: F_{1, 21} = 0.103, p = 0.751) or travel rate (ANOVA: F_{1, 20} = 2.164, p = 0.157). Both measures were significantly lower for *H. ophrys* than *H. americanus* males (proportion of time mobile: ANOVA: F_{1, 41} = 10.024, p = 0.003; travel rate: Welch’s ANOVA: F_{1, 33.709} = 31.730, p < 0.0001), while there was no difference in these measures among females of the two species (proportion of time mobile: ANOVA: F_{1, 16} = 0.415, p = 0.529; travel rate: ANOVA: F_{1, 16} = 0.031, p = 0.863). This result apparently reflects greater investment by *H. ophrys* males compared to *H. americanus* males in activities while concealed beneath vegetation; they spent significantly more time under full cover than both *H. ophrys* females (ANOVA: F_{1, 21} = 8.61, p = 0.008) and *H. americanus* males (Van der Waerden Test: z = 3.110, p = 0.002) (Figure 2.1, Table 2.1).

2.4.1.2 Feeding and hunting

Three *H. americanus* female subjects were observed feeding. Prey included juvenile crickets (Orthoptera spp.) and small flies (Diptera spp.) (estimated prey lengths 5-10 mm). On seven other occasions, two of these females and two additional females were also observed lunging at potential prey (including the prey species above and also small beetles [Coleoptera spp.] and ants [Hymenoptera spp.]); I assume these instances represented hunting activity. One *H. americanus* male also lunged at a potential prey item but no males were seen feeding. Females spent a significantly greater proportion of time feeding than males (Median test: Z = 2.608, p = 0.009) (Figure 2.1, Table 2.1). Combining the prey capture events and lunges at prey noted above into an estimate of hunting behavior, I find that females also hunted at a greater rate than males (Median Test: Z = 3.347, p = 0.001) (Figure 2.4). Three *H. ophrys* female subjects were observed with prey and a fourth was observed outside of a formal observation period (the latter was omitted from statistical analyses). Prey consisted of leafhoppers (Hemiptera spp.) and aphids (Hemiptera spp.) (estimated prey lengths ≤ 5 mm), and a large fly (Diptera sp.; estimated
prey length 5-10 mm). A male was also informally observed holding a mayfly (Ephemeroptera spp.; estimated prey length 5-10 mm) (omitted from statistical analyses) but no lunges were observed by either sex. The proportion of time feeding and rate of hunting events (as defined above) was greater in *H. ophrys* females than males (Median Test: [proportion of time feeding] $Z = 1.980, p = 0.048$; [rate of hunting events] $Z = 2.243, p = 0.025$).

### 2.4.2 Behavioral interactions

#### 2.4.2.1 Intersexual encounters

I observed 12 encounters among *H. americanus* males and females (including 9 different male subjects) and 9 encounters among *H. ophrys* males and females (including 7 different male subjects), corresponding to an encounter rate of about 1 male per hour in both species (Table 2.1). Intersexual encounters ranged in length from 10-709 seconds in *H. americanus* and 10-1414 seconds in *H. ophrys*.

Behavioral interactions were observed for 11 of the 12 *H. americanus* intersexual encounters (Figure 2.5, Table 2.2). A male was judged to have noticed a female if he faced her directly with his anterior medial (image resolving) eyes at any point during the encounter. There were 6 such cases in *H. americanus* and males courted females using ritualized courtship behavior (described in Appendix A.2) in each case, corresponding to a probability range of display between 0.55-1 (95% Agresti-Coull confidence intervals for proportions; “95% CI”). In four cases, I clearly observed both sexes prior to the start of the interaction. In two of these cases the female appeared to initiate the interaction by approaching the male. Male displays began when the male was between about 1-10 cm from the female, and they occurred on substrates of sand (2 cases), wood (1 case), or prostrate dead grass stems (3 cases). They occurred under open sky (2 cases), partial cover of vegetation or litter (2 cases), or began under partial cover but moved into the open (2 cases). In 2 cases overt aggression by females occurred during male display, in the form of lunges at the male or direct hits with her anterior legs. In a different encounter the male mounted the female for 19 seconds but did not copulate (I could clearly distinguish mounts from copulation behavior). The 95% CI of displays leading to a mount is 0.02-0.58, and of displays leading to copulation is 0-0.45. Females terminated the interaction by departing (defined here as walking away to a distance of at least 30cm) in all encounters in which males displayed, resulting in a probability range of rejection of 0.55-1 (95% CI).
Behavioral interactions were observed for 8 of the 9 H. ophrys intersexual encounters (Figure 2.5, Table 2.2). Males displayed in 3 out of 5 encounters in which the male faced the female; this fraction increases to 3 out of 4 if I exclude the second of a pair of encounters that involved one spider in common (Figure 2.5), corresponding to a probability range of display of 0.29-0.96 (95% CI). Males initiated all 3 of the encounters in which I had a clear view of both sexes prior to the interaction starting. Displays began when the male was approximately 2-20 cm from the female. The setting of courtship behavior was noted in 3 of the 4 instances of display; substrates were sand, dead leaves, and grass. One interaction occurred under partial cover but moved into the open, another occurred under partial cover but moved to full cover, and the last occurred under full cover but moved to partial cover. One H. ophrys encounter involved female aggression directed toward the male. Copulation occurred in this encounter, corresponding to a probability range of copulation of 0.04-0.71 (95% CI) for the 4 encounters in which the male faced the female (excluding the second of a pair of encounters that involved one spider in common). Duration of the copulation event is unknown but the total duration for the mount was 9 seconds. The female terminated this interaction, while the male departed first in the 3 other cases in which the male had faced the female. This corresponds to a probability range of termination by females of 0.04-0.71 (95% CI).

Interactions involving multiple males were noted informally during 2 of the above intersexual encounters. Two males were present at once during one of the H. americanus intersexual encounters but only the focal male was observed to display to the female. At least 3 males were present during the H. ophrys encounter that involved copulation. In this case, the focal male appeared to have the direct attention of the female and he displayed to her while a second male maintained a static “first phase” courtship display stage (see Appendix A.2) at a distance of about 3-5 cm behind the focal male. Inter-male aggression was noted between these two males (described in “Male-male encounters” below) and one of them (identity unconfirmed) then copulated with the female; the third male passed by and departed without facing the female.

2.4.2.2 Male-male encounters

Eight H. americanus male-male encounters (involving six different subjects; range of 1-2 encounters per male) and 7 H. ophrys encounters (involving three different subjects; range of 1-4 encounters per male) were observed in addition to the 2 that occurred during intersexual encounters (see “Intersexual encounters” above; omitted from analyses of male-male encounters)
Male-male encounters ranged between 1-135 seconds in *H. americanus* and 15-703 seconds in *H. ophrys*. *H. ophrys* male-male encounter durations were significantly greater than those of *H. americanus* (Median test: $Z = 2.236$, $p = 0.025$; Table 2.2), despite the fact that intersexual encounter durations did not differ significantly between the two species (Median test: $Z = 0.178$, $p = 0.858$). Male-male encounters in *H. americanus* were significantly shorter in duration than intersexual encounters (Median test: $Z = -2.298$, $p = 0.022$). Conversely, in *H. ophrys*, there was no significant difference in the average duration between male-male and intersexual encounters (Median test: $Z = -0.894$, $p = 0.371$); however, sample sizes for this test were low.

Ritualized male display behavior among males was rare and brief in both species. Courtship sequences comparable to those directed toward females during intersexual encounters were not observed. In one *H. americanus* interaction both males briefly raised their first legs and then one male leapt over the other and departed. Similar behavior among adult females and juveniles suggests raising the legs can constitute a threat pose. However, males also frequently raise their first legs when they are alone so it is unknown if the case noted here represented signaling between males. Other behaviors observed during *H. americanus* male-male encounters consisted of males briefly facing each other and then departing (3 cases), potential pursuit from a distance (1 case with a closest encounter distance of approximately 20 cm), temporarily faster movement rate (2 cases with a closest encounter distances of approximately 20 and 25 cm; direct visual contact by both males was not confirmed in either of these cases), or no observed difference in behavior (1 case).

Five of the 7 *H. ophrys* male-male interactions were too obscured by vegetation to be clearly observed. Antagonistic behavior between males was apparent in both of the remaining encounters (each of which featured a different subject male). A male briefly faced the other and raised his first legs in one case when he was pursued by the second male; the two then parted. In the other encounter one male lunged at the other. A lunge was also observed in the multiple-male encounter noted above (see “Intersexual encounters”), and in that case the lunging male interrupted and took over courtship of a female. Although these incidences are few, they confirm that antagonism occurs among *H. ophrys* males both in the presence and absence of adult females.
2.5 Discussion

My observations of natural breeding behavior in *H. americanus* and *H. ophrys* provide insight to the potential selective forces acting on male displays in these species. In the focal population of *H. americanus* studied here, mate choice by females appears to represent the principal source of sexual selection on male displays, given that males invested heavily in mate search and courtship compared to females. Contrary to the patterns seen in *H. americanus*, strong patterns of male-biased mate search and female-biased mate choice were lacking in *H. ophrys*, and preliminary observations suggest direct male-male contests may be involved in mate competition. My observations provide initial evidence that ambient light conditions during courtship may also differ between the two species. I discuss each of these issues below with respect to their implications for the evolution of male display traits.

2.5.1 Mate search effort

*H. americanus* males travelled further than females (Figure 2.2a, 2.3) but were never observed feeding during this period, while females frequently hunted (Figure 2.4) and spent over 10% of their time feeding (Figure 2.1, Table 2.1). It is possible that males sought a rare prey type. However, captive males from this population have been observed to readily consume a variety of commercial and wild-caught prey (GSB, unpublished data), arguing against this possibility. The simplest interpretation of these results is that breeding males prioritize finding female mates rather than foraging. In contrast to *H. americanus*, travel rate was similar among sexes in *H. ophrys* (Figure 2.3). *H. ophrys* males may invest relatively less in mate search. Alternatively, they may monitor stationary, concealed females, as has been observed in another species of salticid (Jackson 1977). The latter possibility could explain why *H. ophrys* males spent a high proportion of time under cover (Figure 2.1), and why relatively few *H. ophrys* females were sited.

2.5.2 Intersexual mate choice

Females of both species encountered on average one male per hour of surface activity (Table 2.1), suggesting they interact with numerous prospective mates throughout the breeding season. This likely affords them a high degree of choosiness which may impose strong selection on male display traits if males vary detectably in heritable aspects of condition (e.g., parasite load: Hamilton & Zuk 1982) or other cues (Andersson 1994; Fisher 1930; Kirkpatrick & Ryan 1991).
The refusal of *H. americanus* females to mate in the six encounters that involved male display (Figure 2.5, Table 2.2) supports this view whereas persistent mate search and consistent readiness to display by males suggests male reproductive success in this population is limited chiefly by mating rate. It is possible the *H. americanus* females I observed were already fertilized and this lowered their willingness to mate. Nonetheless, it was apparently worthwhile for males to court, suggesting receptive females were potentially available. In line with this possibility, I observed juvenile spiders throughout the course of the present study, indicating new adult females may recruit to the population throughout the breeding season.

The relatively high travel rates and the lack of prey pursuit exhibited by *H. americanus* males may constrain other aspects of their reproductive investment, potentially providing sources of phenotypic variation by which females choose among prospective mates. Production and maintenance costs of display traits are known to constrain display magnitude (Hill 1991; Hill 1996; Hill 2000; Kotiaho et al. 1998; Kotiaho et al. 1996; Kotiaho et al. 1999) or to compromise overall paternal quality. Consequently, the morphological and behavioral display traits *H. americanus* males present to females during courtship may signal the differential ability of males to sustain endurance demands of mate search or solicitation (Clutton-Brock & Albon 1979; Kotiaho 2000; Parri et al. 2002; Vehrencamp et al. 1989) and hence provide a quality cue on which females base mate choice (reviewed in Andersson 1994; Fisher 1915; Zahavi 1975). Mate trials conducted on males subject to contrasting experimental feeding regimes or travel rates would provide a means to explore costs associated with mate search and their effects on male mating success.

In *H. ophrys*, the occurrence of male display was inconsistent across the five intersexual encounters that involved male visual contact with the female, and males also terminated several of these interactions (Figure 2.5, Table 2.2). The data are too few to estimate the frequency of these outcomes. Nonetheless, I can confirm that the consistent and contrasting sex roles that were apparent in *H. americanus* mating interactions are lacking in *H. ophrys*. Males of this species may experience physiological constraints (e.g., sperm limitation) or contribute post-fertilization paternal investment (e.g., protection of eggs) that serves to balance the operational sex ratio and lower the overall intensity of mate competition among males. Another possibility is that viability costs (e.g., predation risk) trade off with the benefits of display. Alternatively, other aspects of mate competition such as direct intrasexual contests (discussed below) may mediate *H. ophrys* male mating success.
2.5.3 Direct contests among males

Intrasexual contests can pose a source of sexual selection if they determine access to mates or other resources that are critical for reproduction (Emlen & Oring 1977). Alternatively, the display traits used in contests may themselves be subject to selection via mate choice if they reveal quality differences among the contestants (Berglund et al. 1996). In H. americanus, two lines of evidence argue against a role for direct male-male interactions in mate competition. First, eight of the nine observed intersexual interactions involved only a single male and female, indicating the opportunity for male contests in the presence of females may be rare. Further, in the eight male-male interactions I observed no obvious aggression or ritualized display behavior occurred, indicating that the complex behavioral sequences directed towards females and the ornaments they highlight have probably evolved primarily for intersexual courtship rather than intrasexual contest purposes. Second, H. americanus female behavior suggests the ecological context of breeding is unfavorable for male-male contests in this population. Females hunted a variety of prey and on several occasions were observed to retire alone and in apparently unspecialized sites, requiring only a curled leaf or loose substrate in which to spin their silk rest sacs. Accordingly, captive H. americanus females consume diverse invertebrate prey types and show little selectivity among a variety of substrates (soil, sand, leaves, cardboard) for constructing rest sacs or egg sacs (GSB, unpublished data). The open habitat of H. americanus in the present study provided a clear view of the surroundings of individual spiders, letting me further confirm that females were typically solitary while active on the surface. These findings suggest that males have little opportunity to monopolize multiple females or to control female access to reproductive resources such as food or shelter. Supporting this view, movement paths of males during 30-minute periods did not appear to demarcate territories (Figure 2.2a).

Unlike H. americanus males, H. ophrys males showed apparently aggressive behavior toward each other in the form of close pursuits or lunges, including just prior to the single copulation event that I observed. Intrasexual male encounters were also significantly longer in this species compared to H. americanus. These behavioral patterns suggest male-male interactions may play a relatively greater role in mate competition in H. ophrys compared to H. americanus. More observations of behavioral interactions among males in both species are required to verify this pattern. Moreover, confirming the relative fitness consequences of female choice versus male-
male contests will entail establishing how each aspect of mate competition affects mating success.

2.5.4 Environmental signal conditions during courtship

The abiotic conditions in which mating interactions occur can vary within or among populations, altering the optimal design of sexual displays (Boughman 2001; Boughman 2002; Endler 1992; Endler & Basolo 1998; Seehausen et al. 2008). Identifying sources of selection on display traits thus requires an understanding of the breadth of signal conditions in which they are used, whether the goal is to assess the significance of individual traits within multi-component displays or phenotypic differences among species. My preliminary observations of *H. americanus* and *H. ophrys* mating interactions suggest the environmental conditions of male display might contrast between species (described in Results). The six observed instances of *H. americanus* display toward females all occurred in partial to full sunlight. Further, males spent a high proportion of time travelling in the open, suggesting mating interactions might typically occur in that setting. Conversely, in *H. ophrys*, both inter- and intrasexual interactions took place under partial or full cover of vegetation and in general males were significantly more often concealed than *H. americanus* males (Figure 2.1). These results raise the possibility that display differences between the two species are shaped in part by environmental signal conditions during courtship.

In other salticid species, the trait modalities emphasized during courtship depend on the environmental context of display. For example, *Phidippus johnsoni* males display to females by using a dance when on the ground surface and vibrations when in the low light conditions of female nests (Jackson 1977), while *Trite planiceps* use a dance when courting on leaf surfaces and vibrations when in the darkness of rolled-up leaves (Taylor & Jackson 1999). My observations raise the interesting possibility that selection for signal efficacy under differing light conditions has contributed to differences in *H. americanus* and *H. ophrys* visual display design (e.g., Clark & Uetz 1993). It is interesting to observe that the red and blue ornaments of *H. americanus* males are expected to appear brightest and contrast most strongly in sunlight (Endler 1992). Conversely, the green fringes on the legs of male *H. ophrys* should reflect most strongly if light beneath vegetation is shifted toward green wavelengths, whereas the black and white patches on their pedipalps should contrast strongly with each other under even low light conditions (Endler 1992). More observations of natural behavioral interactions are needed to
verify the context of display in these species, and also to assess the potential role of sonic signals during courtship (Elias et al. 2005; Maddison & Stratton 1988).

2.5.5 Conclusions

The present study helps to characterize broad aspects of male and female mating strategies that are expected to affect the direction and strength of sexual selection in *H. americanus* and *H. ophrys*. A comparison of male and female behavior in *H. americanus* suggests female mate choice could account for a large proportion of variation in male mating success, potentially posing strong sexual selection on males. Males appear to prioritize mate search over foraging for food. This contrasts with females who frequently pursued prey and spent a substantial amount of time feeding. In the few mating interactions observed *H. americanus* males always courted females actively, whereas females appeared choosy, rejecting male advances and always terminating interactions that involved courtship. Sex roles appear less contrasting in *H. ophrys*, given the lower travel rates in males, inconsistent display to females, and evidence for direct conflict between males. In both species, further observations are needed to assess the relative importance of female versus male mate choice, and also male-male contests, in mate competition. Further, confirming the evolutionary significance of these aspects of mate competition will require estimates of their individual fitness effects. The present results suggest that efforts in these directions may reveal contrasting modes of sexual selection underlie divergence in the elaborate male displays of these closely related species, which highlights the importance of establishing the ecological context of breeding behavior when studying sexual display evolution.

2.6 Acknowledgements

I would like to thank the Greater Vancouver Regional District for permission to conduct this research at Iona Beach Park, and D. E. Irwin, W. P. Maddison, S. P. Otto, M. Salomon, and D. Schluter for helpful comments on the manuscript. Funding for this research was provided by the National Science and Engineering Research Council through a Postgraduate Grant (GSB) and an NSERC Canada Discovery Grant to W. P. Maddison.
Figure 2.1. Summary of female and male time budgets. The behaviors listed are hypothesized to collectively reflect differences in mating strategies. Bars reflect the mean (SE) proportion of time spent by females (white bars) and males (gray bars) performing each behavior. The behaviors are not mutually exclusive. See Results for an assessment of differences between sexes and species in these behaviors.
Figure 2.2. Female and male travel paths (m). Individual paths (lines) were plotted from individual locations, marked every five minutes of each behavior watch. Final locations (points) of each individual are coloured to depict sex (female: black; male: gray) (a) *Habronattus americanus* and (b) *H. ophrys*. All paths within each panel are depicted starting from a common point of origin to facilitate comparison. Only individuals who were observed for a complete 30 min period are shown (*H. americanus*: 12 females, 20 males; *H. ophrys*: 5 females, 12 males).
Figure 2.3. Female and male travel rate (m/h). Mean (SE) travel rate of females (white bars) and males (gray bars) (*H. americanus*: 10 females, 22 males; *H. ophrys*: 8 females, 14 males). *H. americanus* males travelled at a significantly greater rate than *H. americanus* females. They also travelled at a significantly greater rate than *H. ophrys* males, while females of the two species did not differ in travel rate.

Figure 2.4. Female and male hunting behavior (prey pursuits/h). In both species, a significantly greater hunting rate is apparent in males compared to females. Bars express mean (SE) hunting rate for females (white) and males (gray) (*H. americanus*: 10 females, 27 males; *H. ophrys*: 8 females, 12 males).
Figure 2.5. Summary of behavioral outcomes of intersexual encounters. Each open circle represents an individual encounter (i.e., <30 cm distance) between a male and female, and the progress of each encounter in which the male faced the female is represented by a solid line that extends to the furthest behavioral stage (vertical dashed line) achieved by the male; line shade indicates which sex terminated each encounter (black: females; gray: males). Encounters known to involve one individual in common are connected.
Table 2.1. Activity budget summary data.

<table>
<thead>
<tr>
<th></th>
<th><em>H. americanus</em></th>
<th></th>
<th><em>H. ophrys</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>Mean (SE) n</td>
<td>Mean (SE) n</td>
<td>Mean (SE) n</td>
</tr>
<tr>
<td><strong>Travel</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of time mobile</td>
<td>0.20 (0.04) 10</td>
<td>0.51 (0.05) 28</td>
<td>0.24 (0.05) 8</td>
</tr>
<tr>
<td>Distance travelled (m) per hour</td>
<td>2.39 (0.59) 10</td>
<td>13.04 (1.30) 22</td>
<td>1.79 (0.49) 8</td>
</tr>
<tr>
<td><strong>Feeding and hunting</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of time feeding</td>
<td>0.13 (0.07) 13</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>Duration of feeds (sec)</td>
<td>1008 (278) 3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hunting events per hour</td>
<td>2.07 (0.83) 10</td>
<td>0.12 (0.12) 27</td>
<td>1.25 (0.62) 8</td>
</tr>
<tr>
<td><strong>Conspecific encounters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of time in intersexual enc. a</td>
<td>0.03 (0.02) 10</td>
<td>0.04 (0.02) 25</td>
<td>0.08 (0.06) 7</td>
</tr>
<tr>
<td>Intersexual encounters per hour a</td>
<td>0.97 (0.67) 10</td>
<td>0.55 (0.26) 25</td>
<td>0.86 (0.59) 7</td>
</tr>
<tr>
<td>Male displays to females per hour</td>
<td>0.18 (0.18) 10</td>
<td>0.47 (0.25) 25</td>
<td>0.29 (0.29) 7</td>
</tr>
<tr>
<td>Proportion of time in male-male enc. a</td>
<td>—</td>
<td>—</td>
<td>0.01 (0.00) 24</td>
</tr>
<tr>
<td>Male-male encounters per hour a</td>
<td>—</td>
<td>—</td>
<td>0.64 (0.25) 25</td>
</tr>
<tr>
<td><strong>Time under full cover</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of time under full cover</td>
<td>0.39 (0.14) 10</td>
<td>0.29 (0.06) 28</td>
<td>0.29 (0.07) 8</td>
</tr>
<tr>
<td>Duration of periods under full cover (sec)</td>
<td>180 (87) 8</td>
<td>220 (98) 24</td>
<td>234 (95) 8</td>
</tr>
</tbody>
</table>

a. Includes all cases in which interactants were within 30 cm of each other.
Table 2.2. Conspecific encounter outcomes.

<table>
<thead>
<tr>
<th></th>
<th>H. americanus</th>
<th></th>
<th>H. ophrys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male within</td>
<td>Male displayed</td>
<td>Male within</td>
</tr>
<tr>
<td></td>
<td>30 cm</td>
<td>n</td>
<td>duration (sec)</td>
</tr>
<tr>
<td>Intersexual encounters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SE) duration (sec)</td>
<td>237 (82)</td>
<td>9</td>
<td>331 (103)</td>
</tr>
<tr>
<td>Proportion male mounted</td>
<td>0.11</td>
<td>9</td>
<td>0.17</td>
</tr>
<tr>
<td>Proportion male copulated</td>
<td>0</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Proportion female terminated</td>
<td>0.89</td>
<td>9</td>
<td>1.00</td>
</tr>
<tr>
<td>Male-male encounters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SE) duration (sec)</td>
<td>24 (13)</td>
<td>6</td>
<td>—</td>
</tr>
</tbody>
</table>
Chapter 3: Divergence in female mate choice and male sexual displays among conspecific jumping spider populations, despite the absence of strong postmating barriers

3.1 Summary

Empirical evidence associating sexual selection with diversification has grown rapidly in recent years, but accounts of its proximate role in this process are still rare. Here, I use *Habronattus americanus* jumping spider populations that each possess one of three unique male sexual display morphs to assess the presence of prezygotic sexual isolation among populations, its order of evolution compared to other reproductive barriers, and the behavioral pathways underlying this form of divergence. I conducted one-on-one mate trials in all nine pairwise population combinations, documenting mating success of local or foreign morphs across several components of mating interactions. I observe partial evidence for female discrimination against foreign phenotypic displays. Virgin females permitted copulation more frequently with local males on average, suggesting female mate preferences result in prezygotic isolation among phenotypically contrasting populations. However, the results also expose several additional potential sources of variation in male mating success: differential spermatheca access by local versus foreign males; female copulation with multiple sires; differences in female sexual receptivity among populations. The effect of these aspects of mating interactions on male mating success will require quantification before I can resolve the net degree of prezygotic sexual isolation among morphs. First-clutch hatch success and early offspring survival did not differ strongly among mate trial treatments, suggesting the observed divergence in components of mate choice have arisen before the evolution of strong intrinsic reproductive barriers. Overall, the study indicates that the buildup of sexual isolation occurs at an early stage of divergence by complex interactions between female mate preferences and male sexual displays.

3.2 Introduction

Sexual display traits represent some of the most ornate and diverse traits in nature, often varying dramatically among even closely related taxa. The high pace of evolution that this pattern implies, combined with the role of sexual display traits in negotiating mating interactions, suggests that sexual selection—selection arising from differences in reproductive success caused
by competition over mates (Andersson 1994; Darwin 1871)—might often play an integral role in the buildup of reproductive isolation between lineages, ultimately leading to speciation (Coyne & Orr 2004; Lande 1981; Panhuis et al. 2001; Ritchie 2007; West-Eberhard 1983). Central questions about the role of sexual selection in this respect concern its direction, timing, and strength of action. Does it promote increased reproductive isolation among divergent forms? Does it initiate divergence prior to the establishment of other reproductive barriers? Is it strong enough to promote durable divergence, sustaining population differences even in the presence of gene flow? (Butlin et al. 2012; Panhuis et al. 2001). Answering these questions entails a description of the traits that mediate mating interactions and their influence on fitness across closely related lineages.

In the present study, I pursue these issues in a set of populations of *Habronattus americanus* jumping spiders (Araneae: Salticidae) that exhibit divergence in male sexual display traits in the northern Sierra Nevada Mountains (California, USA). Females of this species are grayish brown in color and do not perform courtship displays. Conversely, adult males possess red, blue, and white morphological features on their anterior cephalothorax and legs that are prominently displayed to females during ritualized courtship dances (Figure 3.1). Populations in the Sierra Nevada Mountains differ discretely in the number of red-coloured traits comprising the male display: pedipalps (“P”); pedipalps and anterior legs (“PL”); pedipalps, anterior legs, and chelicerae (“PLC”). I will use “morph” to identify both males and females in terms of the male sexual display phenotype (P, PL, or PLC) in their population of origin.

The stark divergence in displays and potential geographic contact among populations suggests strong sexual selection might underlie their early state of divergence. I staged one-on-one mate trials between females and males collected from this region, representing all nine pairwise combinations of the three morphs, in order to assess the direction and strength of female mate preferences between populations. My aim was to evaluate whether female mate preferences are a source of sexual isolation among morphs and whether this is likely to sustain phenotypic divergence in the face of potential gene flow among populations. No information currently exists regarding which components of mating interactions affect male mating success, or how female sexual history affects mate choice. I therefore noted the outcome of various components of individual mating interactions, and across several degrees of female mating experience. Following mate trials, I monitored female egg production and early offspring survival to determine whether strong, intrinsic postzygotic reproductive isolation exists—ruling
out this possibility would support the idea that any sexual isolation arising from mate preferences represents an early reproductive barrier among populations.

3.3 Methods

3.3.1 Spider collection and care

Adult male and both adult (‘field’) and subadult (‘lab-matured’) female *H. americanus* spiders were collected during two field efforts, May 11-23 and June 7-18, 2007. Male and female spiders representing the P morph were collected from three separate populations (Table 3.1) due to the relatively small numbers captured at each location. The three locations contributed approximately equal numbers of females to the total sample (P-1a: 29; P-1b: 26; P2: 24) and these were assigned randomly to mate trial treatment groups (below). PL and PLC morphs were each collected from a single sample site (Table 3.1). Distances between populations range from approximately 3–46 kilometers (Table B.1). Captive spiders were housed individually in 50ml clear plastic vials. Each vial was perforated at the top to allow air exchange and contained a folded piece of cardboard to provide a retreat shelter. The vials were stored on trays of up to 25. Males and females were kept on separate trays and cardboard barriers were placed between all vials to maintain each spider in visual isolation from others. Within a few days of capture, spiders were transferred to a temporary field lab where they were maintained under alternating periods of 14 hours of full-spectrum lighting and 10 hours of darkness. Ambient temperature in this environment varied between 17-22 degrees Celsius. The spiders were fed a variety of wild-caught insect prey and given a small drop of water every few days. Within three weeks of capture, all spiders were transported to a permanent laboratory setting in Vancouver, BC, for long-term care and mate trials. The spiders were maintained under the same conditions as described above, with the following changes: ambient temperature in the lab ranged from about 23-27 degrees Celsius during the light cycle and about 18-22 degrees Celsius in the dark; the spiders were provisioned in the lab with an approximately serially alternating diet of 4-6 adult fruit flies (*Drosophila* spp., reared on standard fruit fly rearing medium supplemented with brewer’s yeast), 1-2 larval cabbage moths (*Trichoplusia ni*, reared on a standard protein- and vitamin- supplemented rearing medium), and one adult blow-fly (Calliphoridae spp., reared on liver and skim milk powder and maintained on sugar and water during the week prior to sacrifice). Subadult females were monitored every 2 days for molts. All vial trays were repositioned on the shelves haphazardly following each check in an attempt to
randomize among vials any shelf effects that might exist (e.g., variation in light influx or ambient temperature).

### 3.3.2 Mate trials

Lab-matured females of each morph were randomly assigned to a male display morph, subject to the constraint that an approximately even number of females from each of the three morphs were tested with each male morph (nine combinations). Females were tested at least 7 days following their final molt. I staged mate trials between individual male-female pairs because this social scenario appears to typify courtship interactions in nature (although female encounters with multiple males also occur; Chapter 1; GSB, pers. obs.). This test format also avoids confounding mate choice with male-male competition (e.g., Shackleton et al. 2005). I conducted trials between 0900-1700 hours, which encompasses the daily active period that has been observed in natural populations of *H. americanus* during the breeding season (GSB, pers. obs.). All mate trials were conducted on the floor of a 10cm-wide, clear plastic, cylindrical arena. The arena floor was lined with white laboratory-grade filter paper and the walls were lined on the outside with a white paper sheath. The top of the arena was open and illuminated by two incandescent, 40W, full-spectrum light bulbs positioned 15cm directly above the cylinder floor—a setup assumed to permit high signal transmission between test subjects. The inner wall of the arena was coated with Insect-a-Slip (BioQuip Products), which is applied wet and dries within minutes to form a smooth white patina that is usually too slippery for the spiders I tested to climb. The coating was always applied at least one day before trials took place in order to minimize any lingering odor from this product.

At the start of a mating trial, a female was dropped from a clean plastic vial onto the arena floor and left alone to habituate to her surroundings for two minutes. A male was then dropped onto the arena floor. Behavioral interactions were monitored remotely via a video camera that was positioned above the arena lights. Males typically displayed vigorously to females, engaging in a ritualized courtship dance (see description in Chapter 1) within a few seconds of facing them. The interaction was then allowed to proceed for 4.5 minutes from the instant a female was directly facing the male with her anterior median eyes—the principle image resolving eyes in salticids—and the male was displaying to the female. This time period captures 3 standard deviations above the mean latency period to copulate in pairs where mating occurred in the present study. Following the trial period, the vial used to introduce the female to
the arena was gently placed over her and, once she had climbed into it, the vial was removed from the arena. Following each trial, the arena, arena floor, and vials used to introduce the spiders to the arena were replaced with clean equipment to ensure that subsequent mate trials were not affected by lingering chemical residues.

Females occasionally attacked and killed males before courtship commenced (approximately 3% of local and 1% of foreign mate trials), and on rare occasions males failed to display to females within the first three minutes of entering the arena. In either of these situations, the trial was terminated and repeated using a different male on the following day.

Each female was re-tested the day after her first mating trial in order to quantify the effect of female sexual status (virgin versus mated) on the outcome of mating interactions and to assess the potential for postcopulatory sexual selection to act on multiple sires. The protocol for mate trials of previously tested females was identical to that described above for virgin females. Male display morph was maintained in the second trial but employing a different male. Males were used in mate trials at most twice, with the exception of two males that were used in three different trials. All re-tested males were given at least four days to rest between trials. In all analyses presented below, I assume the number of trials a male participated in had no effect on the outcome of mating interactions.

Finally, I also tested field-matured females in order to assess the effect of natural sexual experience on female sexual receptivity and mating dynamics. These females were assigned to the same treatments as lab-matured females and subject to a single mate trial. An initial set of trials (n=28) was allowed to proceed in the same manner as the trials involving lab females. Mounts always led to copulation in these trials. I therefore assumed mounts to signify female acceptance and interrupted the remaining mounts prior to copulation. This step allowed me to identify the sexual status of each field-matured female following her test (below).

3.3.3 Predicted patterns of mate choice

My interest was to gauge how females ranked prospective mates ("preference functions", Jennions & Petrie 1997; Lande 1981)—here, assessed in terms of female mate choice at the population level among local versus foreign males. However, the laboratory setting of the mate trials might influence female effort in mate assessment ("choosiness", Jennions & Petrie 1997). The design cannot distinguish this potential effect on mate choice. I proceed under the assumption that the standardized test conditions across trials exert equivalent effects across test
subjects, and hence that the resulting patterns of mate choice reflect comparable degrees of female mate preferences.

I assay mate choice across four components of mating interactions: copulation (yes or no); latency to copulate (seconds following point at which a female faced a displaying male); number of spermatheca (see below) accessed during copulation (one or two), and; copulation duration (seconds). I predicted that if female choice poses a premating barrier among divergent populations then local males would achieve copulations more frequently and quickly than foreign males.

The fitness implications of components of mating interactions that occur during copulation are less certain, as few studies have addressed fertilization rates in spiders, and no data exist for salticids. The female genital structure (epigynum) features two openings, one on each side of her ventral midline, that each lead to a separate sperm storage organ (spermatheca). Copulation involves a male inserting the sperm transfer organ (embolus) at the tip of one of his pedipalp into a spermatheca. In several species of orb weavers, individual pedipalp insertions have been shown to generally reflect high or complete sperm transfer to virgin spermatheca (Bukowski et al. 2001; Jones & Elgar 2008; Snow & Andrade 2005), and a study that assessed fertilization patterns in both spermatheca of virgin females reported equal sperm transfer to each side (Danielson-Francois & Bukowski 2005). Assuming these patterns are relevant to *H. americanus*, and that males attempt to achieve maximum fertilization during copulation, I predicted local males would achieve greater spermatheca access if this component poses a form of sexual isolation among populations. In addition, if sperm transfer during copulation is time-constrained, I predicted local males would achieve longer copulation durations.

### 3.3.4 Fertilization status

I euthanized the field females that permitted a mount during mate trials. I dissected their two sperm storage organs (“spermatheca”) in a saline water medium (contact lens solution, Bausche and Lomb Inc.), and surveyed the organs for the presence of sperm using a dissecting microscope. Any sperm present in the spermatheca were clearly visible when the organs were gently squashed beneath a microscope slide. In cases where the organs were fresh, many sperm became motile. In specimens that were first frozen and processed following termination of the experiment, individual sperm could not typically be resolved but a sperm mass was clearly visible in the spermatheca when the organ was squashed—this assertion was confirmed to be true.
using blind comparisons of fresh and frozen spermatheca from a sample of twenty *H. americanus* females of known sexual status (sperm presence or absence was correctly identified in every case, Binomial test: $p < 0.0001$).

### 3.3.5 Postzygotic reproductive success

To assess postzygotic success, I recorded the proportion of females hatching eggs in 2007 and the occurrence of offspring surviving to second instar in 2008. I maintained lab-matured females following mate trials under an intensified feeding regime in 2007 (fed every 2 days with a drop of water and a rotating diet of 6-10 fruit flies, larger cabbage loopers, or a single blow-fly). Eggs of a single clutch are typically laid within a one-day period within a few weeks following copulation and are tightly wrapped in a silken egg sac (GSB, pers. obs.). Females then enclose themselves and their egg sac in a silken ‘natal chamber’ and remain there for most or all of the 20- to 25-day incubation period. During incubation I ceased food supplements but added a drop of water to the vials every few days in order to maintain ambient humidity. Eggs hatched within a few days of each other, at which point the mother exited the natal chamber and paid no further obvious attention to spiderlings. Hatched eggs were counted and the spiderlings were euthanized.

In December of 2007, I induced diapause among all surviving females by moving them to an outdoor facility that provided shelter from precipitation but exposed the spiders to natural local temperature and light conditions. Daily temperatures typically ranged between approximately 0-10 degrees Celsius during this period. Vials were monitored every week and a drop of water was provided to the few females that were active (i.e., outside of sleep sacs). Most females remained within sealed sleep sacs for the entire outdoor phase. In May 2008 all vials were returned indoors, and indoor temperature was increased by increments of roughly 5-degree Celsius over several days until the regular ambient lab temperature range (above) was reached. Laboratory light, temperature, and intensive feeding regime described above were resumed. Nearly half of the females surviving in May 2008 with substrate (sand, dirt, or leaves) or a larger housing container in order to assess the potential for increased fecundity under different housing conditions. However, these treatments were assigned haphazardly and produced unbalanced housing conditions across phenotypic comparisons. I therefore limit the account of 2008 postmating success to an account of second-instar occurrence across all test groups, ignoring differences in offspring housing conditions prior to their emergence from natal chambers.
Eggs laid in 2008 were maintained until hatch as in 2007, at which point the natal chamber containing the spiderlings was removed from its vial and placed into a clean glass vial with a cork lid. Spiderlings underwent their first molt within approximately the first week inside their egg sac, and then vacated the egg sac and natal chamber. At this point they were separated into individual glass vials and each spiderling was given a tiny drop of water and fed a single tiny cabbage looper every 2 days. Upon second molt, spiderlings were counted and euthanized.

3.3.6 Statistical analyses

I constructed generalized linear models (“GLM”) in JMP (v. 9.0.2, SAS Institute Inc.). The primary interest in model output was the differential mating success of local versus foreign male morphs across different female types. I therefore modeled ‘general’ male morph (local/foreign), as well as ‘specific’ female type (P, PL, or PLC) and the interaction between these two terms. In the event of a significant effect of male morph, I further assessed the potential for differential contributions of specific male morphs to the model. I included Julian test date as a covariate in all models in order to control for any extant confounding effects of female breeding phenology or time in captivity on the results.

I guarded against inflated false positives across multiple models by adjusting the probabilities obtained from each analysis to “q-values” (Black 2004; Storey 2002; Storey et al. 2004; reviewed in Verhoeven et al. 2005). This provision let me maintain the false discovery rate across all significant results below the intended Type I error rate (α = 0.05)(Benjamini & Hochberg 1995). I derived q-values in R (v. 2.12.2, R Foundation for Statistical Computing) using the package QVALUE (v. 1.1, Storey & Tibshirani 2003), and employing the “bootstrap” option (Storey et al. 2004) due to the relatively small number of p-values involved (Dabney & Storey 2004). I estimated these values by considering simultaneously the p-values for the three factors and the date covariate (above) across all models. This approach attempts to impose the maximum possible penalization within the q-value framework for multiple testing across the primary analyses, although I acknowledge that terms within individual models violate the assumption of independent tests (Storey & Tibshirani 2003). For the small number of post hoc analyses conducted, I employed Bonferroni corrections within each analysis.
3.4 Results

Sample sizes considered in all analyses are presented in Table 3.2, varying mainly according to individuals available for the type of analysis considered, but also occasionally due to spider deaths or trials compromised by technical errors (e.g. spider escapes from the test arena, video malfunction).

Courtship behavior during mate trials closely resembled that seen under natural conditions in the present study area (GSB, pres. obs.) as well as other populations (Chapter 1). Males typically displayed vigorously to females, irrespective of female population of origin, indicating they were highly motivated to mate. To the contrary, females merely faced courting males, often turning away in the middle of male courtship behavior. During courtship females often attacked—and occasionally killed—males by hitting, grappling, and biting. Conversely, male attacks on females were rare, restricted to cases in which the female persistently faced away from the male, and never escalated beyond hits. I observed no sign of injuries inflicted on females during the mate trials.

During copulation, a male would mount the female anteriorly. To access the epigynum, he would sweep the female’s third and fourth legs on one side of her body toward her anterior and then use his pedipalp to swivel her abdomen approximately 30-90° along a dorsal-abdominal axis to expose her ventral side. Following copulation at the spermatheca on that side, the male would commonly withdraw his pedipalp, access the opposite side of the female’s body, and attempt to inseminate her second spermatheca with the opposite pedipalp. The entire sequence of copulation behaviors appears to require female consent since females occasionally dislodged mounted males by wriggling or using their front legs to pull the male off.

3.4.1 Mating success

3.4.1.1 Copulation occurrence

The mean (SD) proportion of mate trials involving virgin females that resulted in copulation was generally high (0.876 [0.142] across all male-female type combinations; n= 204) (Figure B.1). Invariant mate acceptance across some treatment groups (i.e., all females permitted copulation) constitutes “quasi-complete separation” in the GLM, in which the dependent variable (e.g., copulate [yes/no]) partially separates the predictor variable (e.g., male origin [local/foreign]). To obtain a model solution under this circumstance, I employed a penalized maximum likelihood method in the models.
Local male morphs exhibited a higher rank proportion of males achieving copulation than foreign morphs across all three female types (Figure 3.2a), reflected in a significant contribution of the general male morph term to the copulation (Table 3.3). Trials involving PLC females apparently contributed relatively strongly to this result (Figure 3.2a), although the apparent difference in local and foreign male success in those trials was not significant when judged against a Bonferroni corrected alpha level for three tests ($\alpha = 0.05/3 = 0.017$). When foreign males were instead considered in terms of their specific morphs (P, PL, PLC), the pattern remained that local males were preferred (5/6 cases) or equivalent (1/6 cases) in success to each foreign type (Figure B.1a). In trials involving PLC females, the lower apparent probability of P males achieving copulation compared to PL or PLC males was marginally significant at the Bonferroni corrected alpha level for nine tests ($\alpha = 0.05/9 = 0.006$; Table B.2). Despite this result, the general male morph term in the original GLM remained significant even with PLC × P trials omitted from the analysis (not shown); implying the local male effect in that model is due not only to this comparison but also the generally higher success of local versus foreign males across other comparisons.

Females that had mated with a male in their first trial represented nearly the entire sample (91%) of lab-matured females in second trials. I therefore focused on this subset of individuals to assess mating patterns of lab-reared females with previous sexual experience. The mean proportion of trials resulting in copulation (0.508 ± 0.147 [SD] across male-female pairwise morph combinations; n= 117) appeared lower compared to trials involving virgin females across most female type combinations (Figure 3.2b) although this apparent change was not tested statistically due to non-independence of part of the sample between the two female sexual states. There was no significant difference between local and foreign male morphs in the proportion of males achieving copulation in these trials (Table 3.3). However, females exhibited differences in sexual receptivity that were independent of male morph, reflected in a significant ‘female type’ model term effect. This effect primarily reflects a significantly lower proportion of P females permitting males to copulate compared to PL females (Pearson Chi square: $\chi^2 = 8.352$, p = 0.004) (Figure B.1b)—other female types were not significantly different from each other (P versus PLC: $\chi^2 = 2.875$, p = 0.090; PL versus PLC: $\chi^2 = 0.346$, p = 0.556). Sample sizes are relatively limited for mate trials involving mated PLC females. However, the same qualitative results as reported above were obtained when these trials were omitted from analyses (not shown).
Excluding five females of unmeasured fertilization status, spermatheca assessments indicate that only 21.0% (n=157) of females captured in the field as adults were unfertilized upon capture. This small proportion of females permitted male mounts at a high frequency (86.7%) during lab trials, similar to the copulation rate observed in virgin lab-reared females (above). Of the fertilized field-matured females, only 4.9% permitted a mount (Figure 3.2c)—no formal analyses were performed on this small test group.

3.4.1.2 Latency to copulate

A significantly positive effect of test date on latency to copulate emerged in trials involving virgin females (Table 3.3, Figure B.2; ANOVA, $F_{1,167} = 13.850, p < 0.001$). Controlling for this effect, I observe no significant difference in latency to copulate between local and foreign males (Table 3.3). Too few matings occurred among mated and field females to formally analyze latency to copulate, but the pattern in mated females is broadly comparable to that observed in virgin females (Figure B.3).

3.4.1.3 Spermatheca access

I analyzed only spermatheca access that occurred during first mounts as single mounts are likely to be common in natural mating interactions involving copulation (GSB, pers. obs.) and characterized the majority of trials in which copulation occurred in the present study (virgin females: 52%; mated females: 65%). The occurrence of virgin two-sided matings produced an opposite pattern to that predicted under the assumption of a correlation between spermatheca access and mate preference—local male morphs consistently showed lower rank proportions of two-sided matings than foreign males across female types (Figure 3.3a). This is associated with a significant general male morph term (Table 3.3). Post hoc tests did not reveal individual female types contributing significantly to this result (Figure B.4, Table B.4). A significant female type effect is also present in the model (Table 3.3) due to the relatively low proportion of two-sided matings in comparisons involving PL females (Figure B.4a; Likelihood ratio $\chi^2 = 7.267, p = 0.0264$). Sample sizes for previously mated females (Figure B.4b) were too low to formally analyze spermatheca access.
3.4.1.4 Copulation duration

I focus on copulation duration within first mounts, excluding four trials in which first mounts did not involve copulation. There was no effect of mating combinations on copulation duration (Table 3.3). I included as a covariate a term for the number of spermatheca accessed by males (1 or 2), due to the unequal occurrence of one- versus two-sided matings observed among mating combinations. This term produced a significant effect in the model, due to the significantly lower mean duration of one- compared to two-sided matings (8.1 ± 1.0 [SD] and 19.5 ± 0.6 seconds, respectively; n = 167)(Welch’s ANOVA: F_{0.05(1),162} = 246.37, p < 0.0001). However, an alternative analysis (not shown) conducted only on copulations in which two-sided matings occurred (76% of cases) produced the same qualitative results. I did not analyze the data for lab-reared females in their second trial due to low sample sizes (Table 3.2).

3.4.2 Postzygotic reproductive success

Females who laid eggs in 2007 typically produced only one clutch, so I limited analyses to first clutches—including the few subsequent clutches in analyses did not alter the qualitative results (not shown). The different test dates across females resulted in different lengths of time available for them to lay eggs in 2007. See Appendix B.2 for criteria followed to select the 162 females included in the present analyses under this circumstance. A model term expressing the number of males each female had copulated with (i.e., 1 or 2) revealed no effect of that covariate on the proportion of mated females to hatch eggs (not shown) so it was not considered further. Test date contributed significantly to the model (Table 3.3), reflecting a decline in the proportion of eggs hatched over time (Figure B.5). Controlling for the effect of test date, no terms were significantly correlated with female tendency to hatch eggs. Most of the females tested latest in 2007 did not hatch eggs (Figure B.5). However, the model results were unchanged if these females were excluded from the analyses (not shown). I did not analyze clutch characteristics due to the limited number of clutches produced.

Thirty clutches hatched eggs in 2008, representing all but one of the nine mate trial treatments (PLC female × P male; Table 2.2). No difference was apparent in the proportion hatched from mate trials involving local (31%; n = 16) versus foreign males (29%; n = 24; Pearson Chi square = 0.020, p = 0.889). The number of eggs hatched ranged from 1-13. All spiderlings appeared healthy and readily captured prey. Survival from hatch to second instar was one hundred percent for every clutch.
3.5 Discussion

I find that *H. americanus* female mate preferences, estimated in three populations that feature divergent male sexual displays, have diverged in several components of putative mate choice. Local males experienced a higher average probability of copulation compared to foreign males, supporting the idea that there exists at least partial prezygotic isolation among phenotypically contrasting populations. Further, the production of viable young from each cross supports the idea that this difference has accrued at a relatively early stage of differentiation, prior to the buildup of strong intrinsic reproductive barriers. However, I also observed differential spermatheca access among local and foreign males, female acceptance of multiple mates, and differences in female sexual strategies among populations. The effects of these factors on local versus foreign male mating success, as well as several features of the mate trial protocol, are presently unknown. These will require quantification before it is possible to establish the net degree of prezygotic sexual isolation between populations, and hence the role of sexual selection in promoting population divergence.

3.5.1 Factors affecting prezygotic sexual isolation

I observed a significantly higher copulation occurrence by local compared to foreign males across female types in trials involving virgin females (Table 3.3, Figure 3.2a; for individual population pairwise comparisons see Figure B.1a). Although individual pairwise population comparisons were significant in only one case (PLC females P males; Figure B.1a, Table B.3), all comparisons showed equal or higher rank probability of copulation in local males, collectively suggesting local male advantage might be a common feature of mating interactions across populations. The average advantage of local males in copulation probability across all female types was 13.4% (9.5% if the strongest case of discrimination by PLC females against P males [Figure B1a] is excluded). These results suggest female submission to copulation could represent a substantial source of divergent selection between local and foreign *H. americanus* males.

However, the trials also expose several other aspects of mating interactions that may contribute additional sources of variation among morphs. First, there was a consistently higher rank proportion of two-sided copulations during first mounts of virgin females by foreign compared to local males (Figure 3.2a; for individual population pairwise comparisons see Figure
Although individual comparisons were not significant (Table B.5), the significant pattern across all comparisons combined (Table 3.3) indicates local males may typically access both female spermatheca less frequently than foreign males do during copulation. This result is puzzling in light of previous studies on fertilization rates in spiders given that they all indicate spermatheca access tends to signify effective sperm transfer (Bukowski et al. 2001; Danielson-Francois & Bukowski 2005; Jones & Elgar 2008; Snow & Andrade 2005). If the same pattern holds true in *H. americanus* it implies, compared to foreign males, local males on average achieve lower fertilization success—more frequently transferring only half of their available sperm to virgin females, despite the relative advantage they experienced in probability of copulation. However, a feasible alternative is that in *H. americanus* two-sided matings in fact reflect inefficient sperm transfer, or occur more often among morphs that on average experience relatively less successful copulations. Testing the proximate effect of spermatheca access on male mating success awaits estimates of sperm transfer deriving from one- and two-sided matings by local and foreign males.

Second, the occurrence of multiple matings presents the opportunity for sperm competition (Birkhead & Møller 1998; Parker 1970) or cryptic female choice (Eberhard 1996; Thornhill 1983) to alter postcopulatory variation in male fertilization success. While only the latter process is likely to directly shape the evolution of male sexual displays, either mechanism might impact the degree of prezygotic sexual isolation among populations and hence the opportunity for divergence. Empirically verifying the unique action of these processes has proven challenging in other species due to the difficulty of isolating female from male effects on paternity rates (reviewed in Andersson & Simmons 2006; Birkhead 2000; Birkhead & Pizzari 2002; Simmons 2001), but Manier et al. (2010) recently provided strong evidence for the influence of both mechanisms on fertilization rates in fruit flies (*Drosophila melanogaster*). Importantly, results from diverse taxa suggest postcopulatory sexual selection can strongly shape mate competition—either complementing or opposing the direction of precopulatory mate choice (reviewed in Fedina 2007; Hunt et al. 2009). Assessing this possibility in the present system will require data comparing paternity rates among males of various morphs mated to individual females.

Other aspects of mating interactions differed among female types independently from the male morph they encountered, suggesting general female sexual strategies have diverged among populations. Although not targeting specific morphs, these relative shifts could influence natural *H. americanus* mating dynamics within populations, with subsequent effects on reproductive
isolation between them (reviewed in Andersson 1994). Compared to other females, virgin PL females exhibited a generally lower chance of two-sided matings (Figure 3.3a), while P females showed a greater decline in sexual receptivity following first copulations (Figure 3.2b). Changes in the probability or efficiency of copulation arising from either of these shifts may influence mate competition within populations if they alter mating opportunities for males. This, in turn, could constitute premating isolation among populations if it results in a mismatch between local female and foreign male breeding tactics (e.g., phenological isolation). On the other hand, such changes may weaken barriers if they happen to result in immigrants receiving a larger share of matings. This latter effect could also result from male display innovations that are universally attractive across populations—a significantly lower latency to copulate apparent in PLC males compared to other males across all female types (Figure B.3, Table B.3) presents a possible example of this scenario.

The impact of each of these factors on prezygotic sexual isolation depends on their individual contributions to total reproductive success. Coyne and Orr (1989; 2004) suggest that across sequential reproductive barriers this contribution is relatively greatest in those acting early in the sequence, due to the diminishing proportion of variation in success affected by each subsequent stage (see also Ramsey et al. 2003). However, I note that in cases where fitness effects oppose each other among stages, late acting effects may counteract or even reverse the effects of previous stages. In the present study, for example, the modest advantage in probability of copulation experienced by local males might be negated if foreign males that achieved copulation transfer more sperm or prevail during subsequent postcopulatory competition. This consideration underscores the need to express the outcome of different components of reproduction in a common currency for fitness (e.g., transmission of gametes or genotypes) if they are to be directly compared.

Test conditions in the present study may have masked sources of mate discrimination exhibited by H. americanus in nature, rendering the assessment of prezygotic isolation conservative. The setup and protocol closely resembles previous mate choice studies conducted on other Habronattus species that detected stark variation in female preferences for different natural (Hebets & Maddison 2005; Masta & Maddison 2002) or experimental (Elias et al. 2005) sexual displays. Nonetheless, variation in female choosiness may have been diminished as a result of the time they spent in social isolation prior to maturation, or due to the confined space in which mate trials occurred. Alternatively, the broad spectrum ambient light conditions and
simplified visual background in the test arena could have compromised any population differences in environment-dependent female sensory perception or male signal efficacy that may be present in nature. Finally, whereas male subjects were fed regularly throughout the present study, breeding males have been observe to exhibit limited hunting or feeding behavior in the wild (Chapter 1) and may frequently have unfilled abdomens while active on the ground surface (GSB, pers. obs.). The appeal of sexual displays likely depends in part on male body condition, either in terms of trait maintenance or behavioral vigor, and in nature this might be particularly diminished in foreign immigrants who have dispersed great distances. This source of variation was lacking from the study. Ultimately, only data from natural mating interactions can provide estimates of prezygotic isolation that are free of these potential limitations, while manipulative experiments might shed light on the magnitude of their individual effects.

3.5.2 Absence of strong postzygotic isolation

Critical to the role of sexual selection in speciation is whether it poses an initial source of divergent selection on population interactions or, alternatively, ensues only once other reproductive barriers are already in place (Panhuis et al. 2001). The results provide preliminary support for the former view. I detected no differences between mating combinations in the proportion of females successfully rearing eggs to hatch (Figure 3.4). Further, in all cases for which offspring survival was assayed (eight of the nine morph comparisons), I observed 100% survival of offspring through their second molt—a level of development at which juveniles are able to capture prey on their own. It is unknown if postmating reproductive barriers acting later in life or arising in a natural setting accompany phenotypic differentiation in this system—data on the lifetime reproductive output of females mated to heterotypic males, as well as survival and fecundity of hybrid offspring in nature, would be needed for a comprehensive assessment of this issue. I also cannot rule out the possibility that the low overall proportion of eggs produced by mated females in the present study reflects subdued reproduction in the lab, potentially obscuring fecundity differences between them. However, I can confirm that strong intrinsic reproductive barriers in the form of embryo inviability or early juvenile hybrid dysfunction are absent across all of the pairwise morph comparisons I examined. This result is consistent with the possibility that the stark phenotypic divergence observed among males has arisen prior to strong postzygotic reproductive barriers and that female mating preferences, to the extent they promote divergence, constitute an early source of reproductive isolation.
3.5.3 Divergence in mate preferences

The evolution of prezygotic sexual isolation among populations hinges on the differentiation of traits that mediate the outcome of mating interactions. Yet, while divergence in sexual displays is widely documented, in most species we have little understanding of which traits implement mate choice or how they vary across related lineages. Reciprocal mating interactions among closely related populations capture a snapshot of this variation, exposing potential behavioral sources of divergent selection between them as well as trends in patterns of differentiation.

Among the several components of mating interactions I assayed, the contrasting probabilities of copulation (Figure 3.2a) versus spermatheca access (Figure 3.3a) among local compared to foreign H. americanus males, as well as the universally lower latency to copulate in PLC males across all female types (Figure B5), suggest that sources of selection on displays in this species could be diverse and may even act in different directions. Although the fitness implications of these factors remain to be quantified, a practical lesson from the findings is that measurements of isolated behavioral components of mating interactions may misrepresent the complexity of mate choice behavior. This highlights the need to estimate the direct fitness effect of each component in order to determine its individual contribution to fitness, whereas only measures of long-term reproductive success are likely to accurately portray the net direction and strength of sexual selection on display morphs.

The differences I observed between local and foreign males in the probability of copulation and spermatheca access point to an evolutionary link between variation in mate choice and sexual displays within populations, as expected if mate preferences directly promote the differentiation of sexual displays. Moreover, the replication of this result across all three study populations suggests it might be common (Endler & Houde 1995). Candidate processes for this parallel outcome are numerous. Premating isolation might arise as a byproduct of co-evolution between sexual displays and mate preferences within individual populations (reviewed in Andersson 1994)—possible examples include selection favoring preferences for traits that identify heritable paternal benefits for offspring ("good genes", "sexy sons", Kirkpatrick & Ryan 1991; Zahavi 1975) or direct paternal benefits to females (Kirkpatrick 1987). Alternatively, intrapopulation processes might result from strictly male-female mating interactions ("Fisherian runaway", Fisher 1915; Fisher 1930; "chase-away", Holland & Rice 1998; Lande 1981). Any
contact among populations could generate additional sources of selection. For example, direct
selection for divergence of mating traits to enhance species recognition could arise if
hybridization among alternate forms results in low fecundity or unfit offspring—a process that
might commence following secondary contact of partially differentiated populations
("reinforcement", Coyne & Orr 1989; Liou & Price 1994), or even in sympatry from the outset of
divergence (Dieckmann & Doebeli 1999). Conversely, either of these geographic scenarios
could foster the buildup of premating isolation as a byproduct of selection favoring reduced
competition for resources among forms.

3.5.4 Prospects for long-term divergence

The impact of sexual selection on long-term population differentiation ultimately hinges on
its ability to counteract forces that oppose divergence. Gene flow can pose a major constraint in
this regard due to its tendency to homogenize genetic variation among populations (reviewed in
Lenormand 2002). Consequently, if migration rates between populations are high, strong
divergent selection might be required to prevent dilution of, or maintain linkage disequilibrium
between, local alleles that encode co-adapted male and female phenotypes. A survey to more
thoroughly document regional patterns of phenotypic divergence, coupled with estimates of gene
exchange among populations, is needed to assess this issue. Evidence for gene flow would imply
that the discrete male phenotypic differences observed among the populations, as well as the
divergent aspects of mating response behavior uncovered in the present study, are preserved by
divergent selection strong enough to oppose this effect. Conversely, the absence of this signal
would raise the possibility that populations have diverged in isolation—a scenario that would
permit population differentiation without any necessary buildup of prezygotic barriers.

The results uncover at least two aspects of mating strategies that I expect limit the scope for
gene flow between populations, irrespective of whether there has been strong divergence in
female mate preferences. First, females appear to exhibit only brief periods of sexual receptivity.
Whereas unfertilized field-matured females were highly receptive, fertilized females from this
sample rejected virtually all males (Figure 3.2c). Assuming the field-matured females
represented a range of ages and sexual experience, this pattern suggests female sexual receptivity
is governed strongly by their fertilization status. A similar decline, though less stark, was
apparent among the lab-reared females across their first and second mating encounters (Figure
3.2a,b). Assuming dispersal is male-biased, these results suggest the opportunity for foreign
males to achieve matings might strongly depend on how early in the breeding season they disperse. Males appear to survive as adults for only a single breeding season (GSB, unpublished data). If this defines the principle dispersal period among males, then immigrant males might typically miss the bulk of mating opportunities with virgin females at the outset of that season. Second, foreign males who do achieve matings are likely to face an additional mating disadvantage due to female acceptance of multiple sires—regardless of the net direction of postcopulatory sexual selection—given that paternity will typically be at least partially diluted by that of local males. In view of these mating disadvantages, effective migration by foreign males might be far lower than the frequency of dispersal among populations, potentially posing substantial reproductive barriers.

### 3.6 Acknowledgements

I would like to thank E. Leung and M. Salomon for assistance with field collections, and D. E. Irwin, J. Lee-Yaw, W. P. Maddison, S. P. Otto, and D. Schluter for comments on the manuscript. The U.S. National Park Service authourized spider collections in Yosemite National Park. This research was supported by an NSERC Canada Discovery Grant to W. P. Maddison.
Figure 3.1. *H. americanus* male sexual display morphs in the Sierra Nevada Mountains (USA). Morphs are defined in the present study by which traits are covered with red coloured bristles: (a) pedipalps (“P”) (b) pedipalps and anterior legs (“PL”) (c) pedipalps, anterior legs, and chelicera (“PLC”).
Figure 3.2. Proportion of local compared to foreign males achieving copulation during mate trials. (a) Local males (light gray) achieved significantly higher proportion of copulations than did foreign males (dark gray) across female type when encountering virgin lab-reared females (p-values indicate significance of Fisher’s exact test of differences between the bracketed bars). (b) Mated P females showed lower receptivity than mated PL females. (c) Fertilized field females were unreceptive to males. Numbers associated with bars are sample sizes. Error bars denote 95% Agresti-Coull confidence intervals.
**Figure 3.3.** Proportion of first mounts in which local and foreign male morphs accessed two spermatheca. (a) Foreign males accessed both spermatheca of virgin lab-reared females significantly more frequently than did local males (b) No difference was observed in spermatheca access between local and foreign males tested with mated lab-reared females. Error bars denote 95% Agresti-Coull confidence intervals. Colors are as in Figure 3.2.

**Figure 3.4.** Proportion of mated females that hatched eggs in 2007 when mated to local or foreign males. No difference was detected between local and foreign males or female types. Error bars denote 95% Agresti-Coull confidence intervals. Colors are as in Figure 3.2.
Table 3.1. Sample site locations (decimal degrees).

<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1a</td>
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<td>-120.003</td>
</tr>
<tr>
<td>P1b</td>
<td>38.248</td>
<td>-119.980</td>
</tr>
<tr>
<td>P2</td>
<td>38.325</td>
<td>-119.695</td>
</tr>
<tr>
<td>PL2</td>
<td>38.337</td>
<td>-119.660</td>
</tr>
<tr>
<td>PLC1</td>
<td>38.104</td>
<td>-119.483</td>
</tr>
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</table>

Table 3.2. Sample sizes for assessment of mating and postmating success.

<table>
<thead>
<tr>
<th>Female morph</th>
<th>Male morph</th>
<th>Mating combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>PL</td>
</tr>
<tr>
<td></td>
<td>P  PL PLC</td>
<td>P  PL PLC</td>
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<tr>
<td>Mating success</td>
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<td></td>
</tr>
<tr>
<td>Lab-reared, virgin</td>
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<td></td>
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<tr>
<td>Proportion copulate</td>
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<td>27 27</td>
</tr>
<tr>
<td>Latency to copulate</td>
<td>23</td>
<td>24 23</td>
</tr>
<tr>
<td>Spermatheca access</td>
<td>22</td>
<td>23 24</td>
</tr>
<tr>
<td>Copulation duration</td>
<td>22</td>
<td>21 20</td>
</tr>
<tr>
<td>Lab-reared, mated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion copulate</td>
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<td>17 19</td>
</tr>
<tr>
<td>Latency to copulate</td>
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<td>6 6</td>
</tr>
<tr>
<td>Spermatheca access</td>
<td>6</td>
<td>6 6</td>
</tr>
<tr>
<td>Copulation duration</td>
<td>6</td>
<td>5 5</td>
</tr>
<tr>
<td>Field, unmateda</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion copulate</td>
<td>0</td>
<td>0 0</td>
</tr>
<tr>
<td>Field, mated</td>
<td></td>
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<td>Proportion copulate</td>
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<td>7 5</td>
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<tr>
<td>Postmating success</td>
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<tr>
<td>Proportion hatched 2007</td>
<td>23</td>
<td>19 19</td>
</tr>
<tr>
<td>2nd instar offspring 2008</td>
<td>5</td>
<td>6 5</td>
</tr>
</tbody>
</table>

a. Mating status was inferred based on absence of sperm in spermatheca.
Table 3.3. GLM results for putative components of mating success. Mating interaction components represent: whether copulation occurred; the latency period from the start of the trial to copulation (seconds); access of one or both spermatheca during copulation; copulation duration (seconds). Postmating success is also examined in terms of the proportion of mated females to hatch eggs. Factors tested are female origin (P, PL, PLC), male general origin (local, foreign), a female × male interaction term, and Julian test date. The copulation duration model includes a term expressing the number of spermatheca accessed by males (see Results). Both p- and q-values are presented (see Methods), with significant values indicated in boldface type.

<table>
<thead>
<tr>
<th>Mating interaction component</th>
<th>Female sexual status</th>
<th>Factor</th>
<th>DF</th>
<th>Likelihood Ratio χ²</th>
<th>p</th>
<th>q</th>
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<tbody>
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<td>0.820</td>
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<td>0.663</td>
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<td><strong>0.010</strong></td>
</tr>
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<td></td>
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<td>2.863</td>
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<tr>
<td></td>
<td></td>
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<td>0.938</td>
<td>0.333</td>
<td>0.450</td>
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<tr>
<td></td>
<td>Mated</td>
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<td>9.077</td>
<td><strong>0.011</strong></td>
<td><strong>0.034</strong></td>
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<td>1.000</td>
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</tr>
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<td>Date</td>
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<td>9.915</td>
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<td>&lt; <strong>0.001</strong></td>
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Chapter 4: Evidence for selection promoting sexual display divergence in the presence of gene flow among conspecific jumping spider populations

4.1 Summary

Related populations often exist within close geographic proximity to each other, suggesting gene flow might frequently inhibit phenotypic divergence. A current aim in speciation research is to establish if selection can typically overcome this limitation, promoting population divergence despite the potential exchange of genes among populations. I investigate this question using a set of closely situated *Habronattus americanus* jumping spider populations in the Sierra Nevada Mountains, California (USA), that each appear to feature one of three distinct male sexual display morphs. Employing 210 AFLP markers and a 962 base pair region of the mitochondrial gene CO1, I find populations show relatively low genetic divergence. This result indicates the stark phenotypic differences between them have arisen rapidly, have persisted despite gene flow, or both. By any of these scenarios, selection is implicated in promoting display divergence. Isolation by distance (IBD) in among-morph population comparisons, despite a tendency for phenotypically similar populations scattered across the study area to be relatively more closely related, further suggests that selection has overcome the effects of gene flow. In sum, the results indicate selection correlated with sexual display expression poses an early source of divergence among populations that is strong enough to overcome migration between them.

4.2 Introduction

Identifying the factors that affect the buildup of reproductive isolation lies at the heart of speciation research (Coyne & Orr 2004; Nosil 2012; Nosil et al. 2009b; Rundle & Nosil 2005; Schluter 2000; Schluter 2001). One of the largest shifts in this field in recent years is an increased focus on how populations diverge in the presence of gene flow. This development has prompted a shifted emphasis away from speciation models that consider categorically discrete degrees of population contact (“geographic” models, e.g. Butlin et al. 2008) and toward identifying the factors that promote the evolution of reproductive barriers between interconnected populations (Rice & Hostert 1993; reviewed in Smadja & Butlin 2011). Challenges raised by this new approach include determining the frequency of divergence with
gene flow in nature, evaluating its occurrence at different stages of divergence, and identifying both the function and genetic architecture of traits that promote divergence (Nosil et al. 2009b; Smadja & Butlin 2011). Closely related, phenotypically divergent populations that are situated in close geographic proximity promise unique insights on these fronts, since they provide a means to assess the traits that initially overcome gene flow, and that might therefore play a crucial role in setting in motion the buildup of long-term reproductive isolation.

In the present study, I assess genetic evidence for selection promoting divergence in the face of gene flow among a set of closely situated but phenotypically distinct populations of *Habronattus americanus* jumping spiders (ARANAE: Salticidae). Adult males of this species possess red, blue, and white morphological features on their anterior cephalothorax and legs (Griswold 1987) that are prominently displayed to females during ritualized courtship dances. A striking aspect of phenotypic variation among males in this regard is the number of red-coloured traits comprising the display (Chapter 3; Figure 3.1): pedipalps (hereafter referred to as the “P” morph); pedipalps and anterior legs (“PL”); pedipalps, anterior legs, and chelicerae (“PLC”). In the northern Sierra Nevada Mountains, California (USA), all three forms are found, so far reported from a few populations that each features just one morph (Chapter 3). Several of these populations are separated by as little as a few kilometers.

The presence of alternative display morphs at high frequencies across populations suggests the action of divergent selection targeting either the display traits themselves or traits correlated with their expression. However, the same phenotypic pattern might arise via genetic drift. Indeed, considering the rugged topography and history of glaciations throughout the Sierra Nevada Mountains, the action of drift during population bottlenecks might at least periodically have exerted a powerful influence on trait divergence among local *H. americanus* populations. One way to distinguish between these two processes is by their expected effect on genomic structure. Drift on average affects variation at neutral loci equivalently across the genome. Hence, if it underlies the apparently stark differences in display traits among *H. americanus* populations then neutral genetic markers should exhibit comparable levels of divergence (e.g. Schemske & Bierzychudek 2007). Conversely, selection targeting individual traits is not expected to affect divergence at unlinked neutral loci.

I surveyed an area of the northern Sierra Nevada Mountains in the vicinity of previously discovered phenotypically contrasting *H. americanus* populations (Chapter 3) in order to further document the regional distribution of display morphs. I used nuclear amplified fragment length
polymorphism (AFLP) markers and sequence variation at mitochondrial gene CO1 to determine if the stark phenotypic differences observed among populations exceeds that of putatively neutral genetic divergence, as expected if display divergence is due to selection. I then sought evidence for gene flow among populations by assessing if there is a pattern of increasing genetic isolation with geographic distance (“IBD”) across the study area. IBD is the expected genetic pattern among populations arrayed across a landscape, due to the diminishing influence of dispersal on neutral genetic variation with increasing geographic distance between them (Kimura & Weiss 1964; Wright 1943). Evidence for the presence of gene flow between populations with distinct morphs would provide additional support for the action of divergent selection on male displays among populations, and would further suggest that this force is strong enough to maintain phenotypic divergence despite introgression among populations.

I took advantage of the presumably genome-wide distribution of the AFLP markers to identify FST “outliers”—markers exhibiting extreme FST values, and hence potentially representing loci affected by strong selection (Beaumont & Balding 2004; Beaumont & Nichols 1996; Foll et al. 2008; Foll & Gaggiotti 2008; Lewontin & Krakauer 1973; reviewed in Nielsen 2005; Storz 2005). I assessed if outlier occurrence is correlated with divergence in male display morphs. The outlier analysis also established a “non-outlier” marker class, representing a sample of putatively neutral nuclear genomic variation, which I employed for the analyses of population divergence and gene flow.

4.3 Methods

4.3.1 Evidence for a genetic basis of the different male display morphs

Phenotypic variation in H. americanus male sexual display traits probably stems in part from plastic responses to environmental variation, as has been directly demonstrated in male H. pyrrithrix (Taylor et al. 2011) and other salticid spiders (Lim & Li 2007). Although this aspect of display variation has not been quantified in H. americanus, several lines of indirect evidence suggest a strong genetic component underlies the discrete phenotypic differences considered in the present study. First, the continent-wide range of the PL morph covers a vast spectrum of climates, with populations varying from sandy bluffs along coastal beaches to alpine meadow. Second, P, PL, and PLC juvenile males have been found to develop the adult morph typical of their population of origin when they are reared under laboratory conditions on a common diet from at least two molts prior to sexual maturation (GSB, unpublished data). Lastly, an additional
morph, featuring red pedipalp and chelicera bristles only (i.e., a “PC” form), has recently been discovered to the south of the present study area (GSB, unpublished data), suggesting that red coloration of chelicera bristles and front legs are developmentally independent. While these observations by no means rule out the possibility that environmental effects influence variation in the expression of red display coloration, they argue against environmental determination of the discrete phenotypic differences considered in the present study.

4.3.2 Field collection

Two field assistants and I searched for new populations in the northern Sierra Nevada Mountains between June-July in 2010 at locations featuring suitable habitat. I attempted to collect specimens from across a broad area (at least approximately 150 m²) at each location where spiders were found and I assume this resulted in a random sample of specimens from each population. The specimens were hand-collected, sacrificed and preserved in 95% ethanol, stored on ice for up to 3 weeks in the field, and then stored permanently at -20°C.

4.3.3 AFLP genotyping

4.3.3.1 Primer choice

I initially screened 59 EcoRI/MseI primer pair combinations (Table C.1), which included several primer pairs previously employed in studies within the Arachnida (Jung et al. 2006; Weeks et al. 2000) or Insecta (Egan et al. 2008; Nosil et al. 2008). However, none of these produced sufficiently variable band profiles across the sample set to constitute informative AFLP markers. As a result, I screened 125 PstI/MseI primer combinations (Lambeets et al. 2010) (Table C.1) and from these I selected 15 combinations showing the greatest variation for further analyses (Table 4.1). PstI binding activity is sensitive to methylation (Barrett & Kidwell 1998; Knox & Ellis 2001), which has raised concern that it can produce AFLP band patterns reflecting epigenetic variation among samples (Bonin et al. 2005; Meudt & Clarke 2007). I note that the effects of methylation are low across most arthropod taxa tested to date (e.g., Diptera, Hymenoptera, Orthoptera), typically affecting 0-3% of the genome (for an exception in Lepidoptera [10% methylation] see Field et al. 2004; Regev et al. 1998; Tweedie et al. 1997; Walsh et al. 2010). In addition, documented methylated sites are usually clustered in the genome (Elango et al. 2009; Suzuki & Bird 2008), particularly within “housekeeping” genes (Elango et al. 2009; Tweedie et al. 1997). Thus, current evidence suggests that band profiles from markers
distributed throughout the genome markers are unlikely to be affected by methylation. The precautionary measure of processing only the legs of adult males further limits the scope for tissue-or age-specific gene methylation to affect variation in the data, although I acknowledge the potential for differences to exist among individuals in environment-induced methylation.

**4.3.3.2 Band scoring and marker selection**

Samples were processed in random order and blindly with respect to morph and population. Details of sample processing are summarized in Appendix C.2. AFLP bands were visualized using SAGA 2.0 AFLP software (LI-COR, Inc.). Samples producing AFLP profiles that were judged to be too dark or faint to accurately evaluate were re-run at least once—consistently low quality samples or gel regions were discarded from analyses. I then retained for further processing all fragment lengths (“markers”) for which at least 5% of the samples were also represented by replicate samples (range 5–18% across all markers; mean = 14.5%).

SAGA produces a peak reflectance profile for the bands of each sample, normalizing peak heights within each profile against the background reflectance intensity level and thus assuring that samples are comparable. However, the software does not report quantitative peak reflectance data, which presents a challenge for objectively defining a threshold for band presence when reflectance peaks vary in height. I therefore exported the reflectance profiles and quantified the height of the reflectance peaks in R (v. 2.12.2, R Foundation for Statistical Computing) using a new script (R. Fitzjohn). The script provided a systematic method to assign band presence as well as means to determine a cut-off error rate among replicated samples that could be used to systematically select which candidate markers to hand-check. The R script and a summary of the selection procedure are presented in Appendix C.3. Following hand checking, I discarded markers showing less than 90% repeatability between original and replicate samples (22% of 268 markers) as recommended by Bonin et al. (2004).

**4.3.4 Detection of outlier loci between populations**

I used BAYESCANN (v. 2.0, Foll & Gaggiotti 2008) to identify AFLP outliers. The software partitions the genetic diversity contributing to the FST coefficient at each locus into a locus-specific (“alpha”) and population-specific (“beta”) component. It then estimates the posterior probability that alpha contributes significantly more than other loci to the observed diversity. A large posterior probability associated with a positive alpha value constitutes evidence that
divergent selection may have acted on the locus in question (negative alpha values indicate potential balancing selection). Besides providing a currency for evaluating the potential effects of selection across loci, the posterior probabilities offer a means to quantify and control the false discovery rate when assessing multiple markers. Simulation studies indicate that BAYESCAN is effective at detecting signatures of divergent selection when applied to AFLP data sets, producing unbiased posterior probabilities even in cases where sample sizes are small (but with lower power, Foll & Gaggiotti 2008). The software is also particularly well-suited to systems showing relatively low differentiation between populations (Foll & Gaggiotti 2008), as is expected to be the case in the present study.

Populations were analyzed in all possible pairwise combinations in order to evaluate the potential effects of divergent selection both within and among displays (Egan et al. 2008; Nosil et al. 2008). I ran BAYESCAN using default settings, incorporating the beta prior approach to estimate $F_{IS}$ coefficients (Foll et al. 2008). Markers with a rare-allele frequency of less than 5% within each population pair were omitted from these analyses, in order to minimize potential biases that relatively uninformative loci can introduce to the posterior probability estimates (Foll 2010).

### 4.3.5 Mitochondrial sequencing

I obtained sequence data from the mitochondrial gene cytochrome oxidase 1 (CO1). Genomic DNA was extracted using a Puregene DNA Purification Kit (Gentra Systems, Inc.) DNA amplification and purification followed the protocol described in Hedin and Maddison (2001), using the primers C1-J-1718 5’ GGAGGATTTGGAAATTGATTAGTTCC 3’ (Simon et al. 1994) and C1-N-2776 5’ GGATAATCAGAATATCGTCGAGG 3’ (Hedin & Maddison 2001). Sequencing was conducted by the NAPS unit, Michael Smith Laboratories (UBC, Vancouver). Sequences were processed in the platform MESQUITE (v. 2.75, Maddison & Maddison 2006), with chromatograms accessed using PHRED (Ewing & Green 1998; Ewing et al. 1998; Green & Ewing 2002) and PHRAP (Green 1999) in CHROMASEQ (Maddison & Maddison 2011), and alignment performed using CLUSTALX (Thompson et al. 1994).

### 4.3.6 Genetic divergence

Genetic divergence among populations was estimated by $F_{ST}$. For AFLP markers, this was calculated in AFLP-SURV (v 1.0, Vekemans 2002) according to the method tailored for
dominant markers by Lynch and Milligan (1994). I used ARLEQUIN (v. 3.5.1.3, Excoffier & Lischer 2010) to generate $F_{ST}$ estimates for CO1 haplotypes.

In order to identify genomic divergence among populations, I used STRUCTURE (Falush et al. 2007; v. 2.3.1, Pritchard et al. 2000), which employs a Bayesian algorithm to partition genomic variation within each sample into a user-defined number of clusters (“K”) and then outputs the posterior probability of assignment of individuals to each cluster. In setting up STRUCTURE models I adopted the “admixture” approach, as recommended by Pritchard et al. (2000) for cases in which individuals may exhibit mixed ancestry from different populations. I also explicitly assumed that allele frequencies were correlated among populations due to the potential for both recent ancestry and ongoing gene flow among populations (Falush et al. 2003). Finally, I utilized populations in the estimation of prior probabilities; this approach has been shown to improve detection of true but subtle population structure, without misguiding the model when such structure is absent (Hubisz et al. 2009). Default parameter values were used within the above model framework.

To identify the K value receiving the highest statistical support I ran multiple models representing the full range of K values from one to ten. All model runs consisted of 150,000 rounds of burn-in to allow for parameter convergence, followed by 150,000 rounds of data collection. I replicated the model for each K value 20 times and then compiled the results for each value in STRUCTURE HARVESTER (v. 0.6.8, Earl and vonHoldt 2011), aligned the cluster proportions among samples in CLUMPP (v. 1.1.2, Jakobsson and Rosenberg 2007), and visualized the resulting plots with DISTRUCT (v. 1.1, Rosenberg 2004). I used STRUCTURE HARVESTER to calculate “$\Delta K$”, the change in the log likelihood of the data across all K values (Evanno et al. 2005). I defined optimal K to be the smallest of these values that maximized $\Delta K$. I also considered alternative K values if they produce study-wide structure that was coherent with populations or morphs, as recommended by Pritchard et al. (2000).

To assess divergence at the CO1 locus, I performed a locus-by-locus Molecular Analysis of Variance (AMOVA, Excoffier et al. 1992) in ARLEQUIN (v 3.5.1.2, Excoffier & Lischer 2010), which used a simulation-based approach to partition sources of genetic variation between individuals, populations, and morphs. The locus-by-locus approach is analogous to a regular AMOVA, but better suited to sequence data containing missing nucleotide calls (Excoffier 2009), as occasionally occurred in the present dataset. Distance matrix derivation for the analysis was based on pairwise nucleotide differences among haplotypes, and the test was
otherwise run on default settings. I also estimated a phylogenetic tree in order to visualize sequence variation at this gene. The tree search was conducted using the GARLI maximum likelihood (ML) method (Zwickl 2006), implemented in MESQUITE. *H. decorus* and *H. coecatus* were used as outgroups. I retained the best of one hundred trees and assigned bootstrap values to nodes based on one thousand permutations.

### 4.3.7 Gene flow

The specific aim of the IBD analysis was to compare genetic divergence to geographic distance between phenotypically contrasting populations. If populations featuring different display morphs have been in historical contact with each other then we might expect a positive relationship between genetic divergence and geographic distance, reflecting the diminishing effect of gene flow on genetic divergence between populations over increasing geographic distance.

I obtained geospatial coordinates for each population from Google Earth (v. 6.1.5; Google Inc.) and then used the R package AFLPDAT (Ehrich 2006) to calculate the straight-line geographic distances (km) between populations. I assessed IBD by first estimating a least squares linear function of non-outlier based genetic divergence ($F_{ST}$) versus straight-line geographic distance (km) for each of the three kinds of among-morph population comparisons (P-PL, P-PLC, and PL-PLC). I then used randomization tests to assess whether the calculated slope of each function departed significantly from a null expectation of zero. Slope was employed instead of a regression coefficient as the test statistic for this analysis because slope does not require that the data points generating the linear functions are independent. This property better suits it to the present analysis because the different pairwise population comparisons utilize each population multiple times.

The potential role of selection in holding apart populations with contrasting displays raises the possibility that gene flow, if it exists, occurs at a relatively higher rate between phenotypically similar populations. Such a process could result either in the preservation of shared historical genetic structure among phenotypically similar populations or the buildup of new structure between them. A potential outcome in either case is the existence of greater shared genetic structure in within- compared to between-morph population comparisons. To assess this issue while accounting for the spatial context in which the populations are found, I used a partial Mantel test (Legendre 2000; Legendre & Fortin 2010; Smouse et al. 1986) to examine the
relationship between phenotypic distance and non-outlier based genetic divergence, while statistically controlling for the effects of geographic distance. I employed a nonparametric Spearman rank version of this procedure, implemented in R using the NCF package, which was developed by O. N. Bjørnstad according to the method of Legendre and Legendre (1998). I also conducted a partial Mantel analysis that employed outlier-based estimates of genetic divergence in order to further examine the relationship between variation at outlier loci and phenotypic divergence.

In the absence of information on the genetic basis of red display coloration in *H. americanus*, I estimated phenotypic distance as the difference in the number of red traits possessed by each morph. This yielded values ranging from zero (i.e., similar morphs) to two (P versus PLC).

### 4.4 Results

I identified six new sample locations in the northern Sierra Nevada Mountains inhabited by *H. americanus*. Combining the new samples with previously collected samples (GSB, unpublished data), 190 adult male specimens from the ten locations across the study area contribute to the present genetic analyses (Table 4.2 presents summary statistics for all sites). I will use “population” as shorthand to refer to sample locations, although population boundaries are currently unknown. Population locations are indicated in Figure 4.1a, and each is named according to the morph it features (P, PL, or PLC) and its general location within the study area (labeled 1-3 from south to north).

Logistic constraints limited the sample size at five of the new populations (P3a, -b, -c, PL3, PLC3) to 4-11 adult males. More than 30 adult males were collected at the sixth new location (PLC2). Despite the modest sample sizes achieved at most of the new sites, each one so far appears to feature a single male morph, in accordance with the phenotypic pattern previously observed at more heavily sampled locations (P1, P2, PL2, PLC1; more than one hundred specimens collected from each location). For the AFLP analyses I combine three populations that feature a shared morph (P3a, -b, and -c; Figure 4.1a) as a single site (“P3a-c”; Table 4.2) in order to achieve a sample size of at least 10 individuals per collection location across the study area.

I obtained 210 reliable polymorphic AFLP markers, ranging in length from 123-562 base pairs, with an estimated average error rate of 4.2% per marker. The CO1 sequence data
produced 962 base pairs for analysis. Tajima’s D test (Tajima 1989) failed to reject a hypothesis of neutral evolution at CO1 in any of the populations (p > 0.20 in all cases).

4.4.1 Patterns of outlier occurrence

Details of the BAYESCAN results and $F_{ST}$ estimates are summarized in Table 4.3. I employed between 75-120 markers showing greater than 5% rare allele frequency within individual pairwise population comparisons, collectively representing 180 of the AFLP markers. All identified outliers exhibit positive alpha values, implying the potential effects of positive divergent selection. Negative alpha values did not achieve outlier status in any comparison, as expected given the low power for AFLPs to detect balancing selection among closely related populations (Foll & Gaggiotti 2008).

Three outliers surpass the widely adopted 95% posterior probabilities threshold for outlier status and two of these loci have posterior probabilities greater than 99% in at least one pairwise location comparison (Table 4.3, 4.4a). All outlier fall below the 5% false discovery rate advocated by Foll and Gaggiotti (2008). Collectively, these loci represent 1.7% of the 180 markers tested. This value is lower than the proportion of candidate loci revealed in genome scans using earlier detection methods (reviewed in Nosil et al. 2009a) but consistent with the relatively conservative results typical of BAYESCAN (Perez-Figueroa et al. 2010).

I further explored the potential contribution of the three detected outliers to divergence among populations by assessing their pattern of occurrence when an additional, lower, outlier threshold was used. I set the lower threshold at a classical boundary for identifying statistical outliers (Luikart et al. 2003; the value lying 1.5 interquartile ranges above the 3rd quartile of values, Zar 1999), estimated from the frequency distribution of all markers that exhibited positive alpha values within each pairwise population comparison. Each of the three originally detected outliers appear in several additional comparisons when this threshold is adopted (Table 4.4b) suggesting the outliers, or loci they are linked to, contribute to the estimates of population divergence across the study area. Variation in allele frequencies for the three loci among populations is illustrated in Figure 4.1b. The frequencies were estimated based on the observed frequency of the ‘band absent’ genotype (i.e., representing a homozygous state for band absence) under the assumption that alleles are in Hardy-Weinberg equilibrium within populations. The distribution of frequencies appears to exhibit an association with geography rather than morph, with neighboring populations showing similar frequencies: marker 17 in populations P1 and P2.
in the southwest; marker 141 in populations PLC1 and PLC2 in the southeast; marker 470 in the east, particularly in populations PLC2 and PL2.

4.4.2 Genetic divergence

4.4.2.1 Nuclear divergence and genomic structure

Differentiation among populations at the AFLP loci is generally low, with non-outlier based $F_{ST}$ estimates typically below 0.05 (Figure 4.2) across pairwise population comparisons (Table 4.3). Outliers produced high values of differentiation (0.115–0.814) with the exception of pairwise comparisons among the most northern populations (P3a-c, PL3, and PLC3: 0.000-0.035) and among PLC1 and PLC2 (0.012). In contrast to the discrete phenotypic differences among populations, few populations demonstrated fixed AFLP allele frequencies (e.g., the highest allele frequency estimates in the entire marker set are represented by the outliers in Figure 4.1b).

Genomic structure across the study area appears complex when visualized using STRUCTURE. Each of the marker classes reveals greatest $\Delta K$ support for 2-cluster partitioning of the data (Table C.3). Viewed at this cluster level, the full marker set reveals cluster membership that is only partially correlated with phenotypic variation (Figure 4.3a): P populations show high membership to the white-coloured cluster; PL populations belong only partially to the white-coloured cluster; two of the PLC populations belong almost entirely to the dark gray-coloured cluster. Population PLC3 poses an exception to this trend, exhibiting greatest membership to the white-coloured cluster in contrast to the other PLC sites. Further, location P1 stands apart from the other “P” locations in the degree of white-coloured cluster membership. Both of these exceptions further highlight the limited relationship between genetic structure and phenotypic differences among populations. Interestingly, results based solely on the three outliers do not exhibit any structure correlated with morph (Figure 4.3b; upper left panel), whereas the non-outlier marker class seems to exhibit the slight signal related to morphs that was observed in the full marker set (Figure 4.3b; lower left panel).

Within the outlier marker class, novel contrasts among populations emerge from the outlier marker class when genomic variation is instead partitioned into 3 clusters (Figure 4.3b: upper right panel), revealing geographically localized structure in the most northerly populations (PL2, P3a-c, PL3, and PLC3). This result accords with the patterns of outlier occurrence revealed by BAYESCAN (Table 4.3) and the outlier allele frequencies (Figure 4.1b). No such pattern was
observed among non-outliers (Figure 4.3b; lower right panel). Overall, the forces responsible for outlier based genetic variation appear unrelated to variation in male sexual display morphs.

4.4.2.2 Mitochondrial divergence at CO1

Contrary to the non-outlier based nuclear data, strong divergence is apparent among several populations based on FST estimates for CO1 (Table 4.3), due to the presence of a high frequency of shared haplotypes within but not across several of the populations as well as a relatively deep branch in the middle of the tree (Figure 4.4). Despite this result, haplotypes are frequently found in common among populations, including between display morphs (Figure 4.4). The AMOVA results support these patterns, showing a significant effect of among-population genetic variation but also significant variation within locations (Table 4.5). The significant among-morph effect further indicates that morphs show distinctive variation. Notably, haplotypes from the northern locations are found throughout the tree; strongly contradicting the idea that patterns of differentiation are correlated with morph.

4.4.3 Gene flow

For all analyses, I use geographic coordinates for P3a to represent the P3a-c populations (Table 4.2). Substituting either site P3b or P3c for this location produced qualitatively similar results (not shown), except in one instance noted below.

Genetic divergence varies positively with geographic distance in each of the three among-morph comparisons between populations (Figure 4.5). Randomization tests indicate the slopes of linear functions fit to each comparison type are significantly greater than expected from random pairings of genetic and geographic data (P-PLC: p = 0.034; P-PL: p = 0.004; PL-PLC: p = 0.019). A single exception to this result is observed in the ‘P-PLC’ comparison if P3c is used for the P3a-c geographic coordinate (p = 0.076). These patterns of isolation by distance (IBD) are consistent with the possibility that gene flow structures genetic relationships among phenotypically contrasting populations across the region.

The partial Mantel test reveals a significant relationship between non-outlier based genetic divergence and phenotypic distance across the study area (Table 4.6), implying variation at some or all of the non-outliers are correlated with the display differences among populations. The result is marginally non-significant when outlier markers are instead used to generate FST values. This might in part reflect greater error inherent in estimates of genetic divergence based on only
three available outlier loci. Nonetheless, it argues against the presence of a strong relationship between outlier based variation and phenotypic divergence. It also accords with the overall pattern of outlier occurrence and outlier based allele frequencies noted above (Table 4, Figure 4.1b). Analyses conducted while scoring phenotypic distance as 'same' versus 'different' (not shown) produced statistically similar results to those reported here.

4.5 Discussion

The current results support the idea that divergence in three *H. americanus* male sexual displays is promoted by selection in the face of gene flow. Only a single display morph was found at each of the ten locations sampled, despite collections ranging from several to more than one hundred males at each location. In contrast to this discrete phenotypic variation across populations, divergence between them at non-outlier AFLP loci is low (Figure 4.2, Table 4.3), suggesting displays have either diverged rapidly compared to putatively neutral loci, have persisted despite migration among populations, or a combination of the two processes. This pattern is not expected if displays and non-outliers have evolved in a similar manner, and instead implies selection has driven or maintains display divergence. The observed pattern of isolation by distance (“IBD”) across each type of among-morph pairwise population comparison is consistent with the presence of gene flow among phenotypically contrasting populations. Further support for the occurrence of gene flow comes from the close genetic relationship among phenotypically similar populations despite their geographically scattered distribution—a pattern that indicates either retention of shared historical genetic variation within morphs despite gene flow across the region, or repeated evolution of each form in separate locations followed by the build-up of shared genetic structure among phenotypically similar populations. Either scenario suggests ongoing selection maintains phenotypic differences among populations in the face of gene flow. Contrary to patterns of genetic divergence represented by the non-outliers, outlier loci do not closely match patterns of display variation, and instead appear to reflect geographically localized processes independent from phenotypic divergence.

4.5.1 Evidence for divergent selection on displays

The occurrence of only a single display morph within each population suggests that allele frequencies are extreme at loci encoding red sexual display traits. If genetic drift was responsible for these stark differences among populations, neutral alleles elsewhere in the
genome should vary in a similar manner to the display morphs. Contrary to this prediction, only two AFLP markers exhibit potential fixation of alternative alleles between locations (markers 17 and 470; see Figure 4.1b) and these cases are limited to a subset of the pairwise population comparisons. The remaining markers exhibit low divergence among even widely separated populations (Table 4.3, Figure 4.2). The average extent of this subtle differentiation across individual spiders is illustrated in the STRUCTURE plots which showed greatest support for a 2-cluster partitioning of global genetic variation across the three morphs and exhibit considerable admixture between these clusters in many of the samples (Figure 4.3a). Partially conflicting results are revealed by variation at CO1 among populations. Here, genetic divergence is high between several populations (Table 4.3) possibly reflecting faster lineage sorting owing to the smaller effective population size of this maternally inherited gene compared to nuclear loci. Alternatively, it could signify lower female compared to male dispersal rates and hence decreased gene flow at mitochondrial loci. Nonetheless, despite elevated genetic divergence at this gene, CO1 haplotypes are shared among morphs across the study area (Figure 4.4).

The discord between these genetic patterns and the discrete divergence in display morphs among populations is not expected if both types of trait are shaped by the same forces. Instead, the deterministic effect of selection appears to have driven genetic variation underlying display trait expression to extreme and contrasting frequencies between populations. Assuming the non-outlier AFLP markers are evolving neutrally, net divergence at these loci should primarily reflect the diversifying effects of lineage sorting owing to genetic drift within populations since they became geographically separated, countered by any gene flow that might occur between them. Hence, the fact that populations show little genetic differentiation implies the discrete divergence in display traits has occurred rapidly, has proceeded in the face of gene flow, or both. By any of these scenarios, divergent selection on display traits, or traits highly correlated with their expression, is implied.

4.5.2 Evidence for gene flow among populations

The strength and consistency of selection required to drive display divergence among populations depends heavily on the migration rate between them (Hendry et al. 2001; Yeaman & Guillaume 2009; Yeaman & Otto 2011; Yeaman & Whitlock 2011). Weak or sporadic selection might have prompted the observed discrete divergence among populations if they never exchanged migrants. Conversely, in the presence of gene flow, persistent selection would be
required that is sufficiently strong to preserve alleles for local morphs. The observed non-outlier based pattern of isolation by distance (“IBD”) across all the three types of among-morph comparison (Figure 4.5) supports the latter scenario—in each case potentially signifying the diminishing influence of gene flow on putatively neutral genetic divergence between populations over increasing geographic distances (Kimura & Weiss 1964; Slatkin 1993; Wright 1943).

Nevertheless, gene flow does not appear to have been equally effective among all populations, as indicated by the observed tendency for populations of the same morph to be more closely related to each other than phenotypically contrasting locations (Figures 4.2, 4.3a). This effect is evident in the significant correlation between phenotypic distance and non-outlier based genetic divergence, while controlling for geographic distance among populations (Table 4.6). The intermingled distribution of morphs across the landscape suggests two different processes might have produced this correlation in the presence of IBD. The first and simplest possibility, “divergence-then-range-expansion”, is that phenotypically similar populations share a common origin. By this scenario, each form evolves once prior to subdivision into its present geographic distribution. The shared genetic variation among phenotypically similar populations reflects their shared history. In order to explain the observed pattern of IBD among morphs (Figure 4.5), however, this scenario requires recent gene flow between neighbouring populations with different morphologies (i.e., gene flow following range expansion).

In the second possible scenario, “range-expansion-then-divergence”, the present geographical distribution of populations is established prior to (or during) phenotypic differentiation. Here, the populations are colonized by a single form, followed (or accompanied) by the repeated evolution of different morphs in parallel. Notably, in this scenario IBD could reflect gene flow among populations but might also arise merely due to sequential colonization of new populations during range expansion (i.e., with neighbouring populations exchanging no migrants yet being closely related due to their relatively recent common history, Slatkin 1993). Nonetheless, in this scenario at least some gene flow occurs across the region following phenotypic divergence and it involves disproportionately greater introgression within compared to between morphs. It is this differential introgression that produces the observed correlation between genetic and phenotypic divergence.

A role for divergent selection among phenotypically contrasting populations is inherent in both of these hypothetical scenarios. In the “divergence-then-range-expansion” scenario, selection preserves divergent morphs as IBD develops, despite migration among neighboring and
often phenotypically contrasting populations. It might also preserve historical non-outlier based genetic structure that is correlated with display divergence (i.e. posing a “general barrier” to gene flow among morphs, Michel et al. 2010; Nadeau et al. 2012; Nosil et al. 2008; Thibert-Plante & Hendry 2011). In the “range-expansion-then-divergence” scenario, divergent selection during gene flow among populations results in the build-up of genetic structure correlated with morph by permitting disproportionately greater introgression among phenotypically similar populations. The strength of selection required to produce either outcome is difficult to gauge, since each one might occur under a range of migration rates provided that selection is powerful enough to inhibit the introgression of foreign alleles correlated with display expression between phenotypically contrasting populations. Nonetheless, if gene flow indeed occurs among phenotypically contrasting populations as suggested by the present data, it implies that the observed discrete variation in displays among populations is maintained by persistent selection that counteracts this process, rather than merely reflecting historical processes or selection within isolated demes.

4.5.3 Lack of outlier-based evidence for divergent selection on displays

Although the results suggest selection underlies divergence correlated with variation in display morphs, none of the outlier markers are correlated with this variation (Table 4.4, Figure 4.1b). In principle, gene flow may have eroded formerly distinctive signatures of divergent selection near these loci, obscuring the relationship between variation in outliers and morphs. This could explain the relatively similar outlier-based genomic variation among the closely situated northern populations. However, such a process is likely to be inefficient due to the slow expected pace of introgression in regions affected by selective sweeps—a product of the immigration of foreign alleles and recombination at genetic sites between selected and outlier loci. By contrast, gene flow should homogenize non-outlier based genomic variation relatively rapidly, since in general it is not opposed by strong selection at these loci. However, contrary to these predictions, the study-wide correlation between phenotypic and genetic variation is stronger in non-outliers than in outliers (Table 4.6). This result argues against a close relationship between phenotypic and outlier based variation. Instead, it suggests that the observed geographically localized patterns of genetic variation in outliers reflect processes unrelated to the evolution of male display traits.
The absence of outliers that are highly correlated with phenotypic variation, despite the evidence for potentially strong selection underlying phenotypic divergence, is unsurprising. In the presence of only 180 markers, even wide chromosomal regions exhibiting reduced genetic variation due to selective sweeps might go undetected. Compounding this issue is the prospect that such regions may in fact be quite narrow, restricted to the immediate vicinity of target loci. This might be the case if recombination or mutation has renewed genetic variation near target loci since a selective sweep occurred, as is particularly likely following multiple generations of recombination in the presence of gene flow. Conversely, signatures of selection might be narrow from the outset of divergence if the original ratio of divergent selection to recombination in the vicinity of selected loci is relatively low, or if selection acts on standing genetic variation rather than new mutations ("soft sweeps", Hermisson & Pennings 2005; Radwan & Babik 2012; Strasburg et al. 2012). Under these conditions, a dense sample of markers might be required to detect loci linked to divergent selection, even if that force is strong. The alternative evidence I present for selection affecting variation in *H. americanus* displays, namely, stark phenotypic differentiation in the absence of comparable variation in putatively neutral genetic markers, highlights the fact that genome scans may often miss signatures of selection.

### 4.5.4 Sources of selection on divergent traits

The sources of selection responsible for divergence among morphs remain to be identified. Sexual selection represents the most obvious force shaping male display traits. Partial support for sexual selection promoting display divergence previously surfaced from laboratory-based mate trials conducted on spiders from three of the presently sampled phenotypically contrasting populations. In that study, females showed a slightly higher tendency to mate with local males (Chapter 3). However, the net direction and strength of sexual selection on local compared to foreign males were not resolved in that study. Clarifying this issue thus calls for measures of lifetime reproductive success, as well as measures of offspring fitness, resulting from both types of mating interaction.

Even if sexual selection does contribute to population divergence in this system, a larger task is to identify the selective forces prompting divergence in female and male mating traits. Theory suggests sources of selection in many candidate mechanisms of mating trait evolution have an ecological basis (Andersson 1994; Schluter 2000; Schluter 2001). Hence, a survey of ecological differences among phenotypically contrasting populations is needed. This might profitably
include accounts of natural mating interactions, with attention to how differences in environmental conditions among populations affect display efficacy or female preferences (Boughman 2002; Endler 1992; Endler & Basolo 1998). Outside of the immediate mating context, documentation of microhabitat or life history attributes could also reveal factors that directly shape mating traits (e.g., Chapter 2), or traits closely correlated with their expression.

The evolutionary significance of the outlier loci also warrants further investigation. Extremely differentiated loci are typically considered to signify the effects of divergent selection on closely linked traits that have a functional role in population differentiation. Numerous studies have reported associations between outlier-based genetic divergence and traits that may determine ecological boundaries among closely related populations (reviewed in Nosil et al. 2009a; Strasburg et al. 2012). Viewed in this light, the outliers detected here may reflect adaptation to geographically localized selection pressures, such as environmental gradients. The challenge posed by such candidate loci is to identify the functional and adaptive significance of the potential targets of selection that they are linked to, a step that is still relatively rare in non-model organisms (Strasburg et al. 2012) but ultimately necessary to forge a direct link between selection and phenotypic divergence (Barrett & Hoekstra 2011; Thornton et al. 2007). Even where outlier expression varies closely with specific ecological factors, such correlations could simply represent selection on unidentified traits that have pleiotropic or epistatic effects on a divergent trait of interest, or that are merely in linkage disequilibrium with loci encoding that trait (Pavlidis et al. 2012).

More generally, outliers need not signify selection at all if genetic drift, acting rapidly during population bottlenecks (Barton 1998; Foll & Gaggiotti 2008; Przeworski 2002; Thornton & Jensen 2007) or at the frontier of expanding populations ("surfing", Currat et al. 2006; Edmons et al. 2004; Excoffier & Ray 2008; Hallatschek et al. 2007; Klopstein et al. 2006; Wegmann et al. 2006), instead drives contrasting allele frequencies among populations. In the present system, the outliers may have arbitrarily drifted toward fixation and then subsequently spread among neighboring populations by gene flow, with or without the help of selection. Finally, similar patterns could also arise through the immigration of foreign alleles that exist in high frequency in unidentified neighboring populations. This might include undiscovered populations of H. americanus, or phenotypic variants of the congener H. sansoni that are also known to occur in the region and that show evidence of hybridization with H. americanus in some areas (GSB, unpublished data). Separating these alternatives is not a trivial task but a productive start would
be to determine whether outlier occurrence correlates with environmental variables and, if correlations occur, to assess the relationship between these variables and traits closely linked to the outliers.

4.5.5 Prospects for long-term divergence

Long-term divergence of populations that exchange migrants requires the establishment of reproductive barriers that are strong enough to impede gene flow, either through their direct effects on reproductive isolation or by sufficiently diminishing introgression to enable the establishment of additional barriers. The evolution of assortative mating can play a pivotal role in this process, since it directly limits gene exchange between populations (Felsenstein 1981; Smadja & Butlin 2011). In this respect, the divergence of mating traits is crucial, due to their role in mediating mating interactions (Coyne & Orr 2004; Gavrilets 2004; Panhuis et al. 2001), and hence might signify an advanced stage of differentiation among H. americanus populations. Assessing this possibility will entail identifying the source and quantifying the strength of divergent selection correlated with phenotypic divergence in relation to migration rates.

Empirical accounts of genomic divergence involving sexual selection are rare but accumulating. Chamberlain et al. (2009) report a recent apparent example of sympatric divergence of Heliconius butterfly wings (in both patterning and color), as well as heterotypic mating discrimination in one of the divergent forms. Other studies conducted within that genus provide examples of more advanced states of divergence in mate preferences, all stemming from the same source of divergent selection (Müllerian mimicry, Jiggins et al. 2001; Kronforst et al. 2006). A similar pattern is seen in cichlid fish of Lake Victoria, where divergent mate preferences of females adapted to differing light environments have repeatedly driven divergence in male display (Seehausen et al. 2008). These accounts suggest sexual selection might frequently act near the outset of population divergence, and despite migration between forms (reviewed in Chamberlain et al. 2009). The Habronattus jumping spiders could present new examples of this process. Masta and Maddison (2002) previously reported evidence for selection driving sexual display divergence between geographically isolated populations of H. pugillis, by showing that male display traits have diverged more rapidly than neutral mitochondrial variation. The results presented here supply an additional case in this genus and further suggest that selection enables differences in sexual display to persist or build in the face of gene flow. The presence of discrete differences in H. americanus sexual display traits among closely related
populations suggests selection on these traits is currently among the earliest forces promoting population divergence in this system. Moreover, the fact that this pattern exists over multiple geographic locations suggests the evolutionary processes involved are robust to ecological and demographic variation across the region.

4.6 Acknowledgements

I would like to thank N. Barrett, P. Halychuk, E. Leung, B. McGinty, M. Salomon, K. Seguin, and S. Vibert for assistance in the field; R. Andrew, M. Bodner, A. Miscampbell, L. Rieseberg, C. Ritland, H. Yueh, and J. Zhang for guidance on genetic data acquisition or analyses, and; D. E. Irwin, J. Lee-Yaw, W. P. Maddison, S. P. Otto, and D. Schluter for comments on the manuscript. The U.S. National Park Service and California Department of Fish and Game authorized spider collections. This research was supported by an NSERC Canada Discovery Grant to W. P. Maddison.
Figure 4.1. Map of the study area. (a) Populations are coloured according to which local adult male display traits feature red bristles: blue (P); orange (PL); red (PLC). (b) Estimated allele frequencies of AFLP outlier loci at individual populations, indicating an apparent association of allele frequencies with geography but not display morph. White denotes the ‘band present’ and green denotes the ‘band absent’ allele. Allele frequencies for P3a-c are pooled.
Figure 4.2. Genetic divergence (F\textsubscript{ST}) at non-outlier loci versus geographic distance (km) for all pairwise population comparisons. Divergence is relatively low across the study area. Coloured dots represent the three kinds of within-morph comparisons. Crosses represent all among-morph comparisons.

Figure 4.3. STRUCTURE output indicating the proportion of genomic nuclear variation belonging to two or three clusters across AFLP marker types. Each vertical line represents and individual. (a) The analysis based on all loci revealed greatest support for a two-cluster partitioning of the data, and exhibits much admixture between clusters within several populations. (b) Genomic structure of outlier (upper panels) and non-outlier loci (lower panels) treated separately. Weak morph-associated structure at the two-cluster level (left panels) appears limited mainly to the non-outlier markers. Imposing a three-cluster partitioning of the data (right panels) reveals some outlier-based genomic variation is shared among the most northerly sample sites. Headings follow the same color scheme as described in Figure 4.1.
Figure 4.4. Best fit maximum likelihood tree for CO1 sequence data. The tree reveals sequence variation is shared by samples from the same populations, but also among populations featuring different morphs. Nodes receiving greater than 75% bootstrap support are labeled. Branches are scaled to reflect proportion of sequence divergence.

Figure 4.5. Genetic divergence ($F_{ST}$) at non-outlier loci versus straight-line geographic distance (km) for among-morph pairwise population comparisons. A least squares linear function is depicted for each type of comparison, indicating a significant pattern of isolation by distance in each case.
Table 4.1. Primer pairs chosen for selective amplification of AFLP markers.

<table>
<thead>
<tr>
<th>Primer</th>
<th>MseI</th>
<th>PstI</th>
</tr>
</thead>
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<tr>
<td></td>
<td>GATGAGTCCTGA</td>
<td>CACGACGTTGTA</td>
</tr>
<tr>
<td></td>
<td>GTAA+</td>
<td>AAACGACTGCGT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ACATGCAGC+</td>
</tr>
<tr>
<td>Primer extensions</td>
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<td>AG</td>
</tr>
<tr>
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<td>ACAG</td>
<td>AGA</td>
</tr>
<tr>
<td></td>
<td>ACAG</td>
<td>AGA</td>
</tr>
<tr>
<td></td>
<td>ACCA</td>
<td>AGG</td>
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<tr>
<td></td>
<td>ACCC</td>
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<td></td>
<td>ACTC</td>
<td>AGT</td>
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<td>AGA</td>
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<td></td>
<td>AGCG</td>
<td>AGG</td>
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Table 4.2. Summary data for individual populations. All samples are comprised of adult males.

<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude (DD)</th>
<th>Longitude (DD)</th>
<th>n</th>
<th>H (% polymorphic)</th>
<th>AFLP</th>
<th>CO1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AFLP</td>
<td></td>
<td>all markers</td>
<td>outliers</td>
</tr>
<tr>
<td>P1</td>
<td>38.248</td>
<td>-119.980</td>
<td>30</td>
<td>0.111 (45.5)</td>
<td>0.111 (45.5)</td>
<td>0.134 (45.9)</td>
</tr>
<tr>
<td>P2</td>
<td>38.325</td>
<td>-119.695</td>
<td>29</td>
<td>0.138 (65.3)</td>
<td>0.087 (33.3)</td>
<td>0.149 (46.3)</td>
</tr>
<tr>
<td>P3</td>
<td>38.514 b</td>
<td>-119.802 b</td>
<td>16 c</td>
<td>0.130 (66.3)</td>
<td>0.130 (66.3)</td>
<td>0.167 (66.3)</td>
</tr>
<tr>
<td>P3a</td>
<td>38.516</td>
<td>-119.914</td>
<td>7</td>
<td>0.166 (67.3)</td>
<td>0.166 (67.3)</td>
<td>0.177 (66.3)</td>
</tr>
<tr>
<td>P3b</td>
<td>38.494</td>
<td>-119.981</td>
<td>4</td>
<td>0.152 (64.4)</td>
<td>0.152 (64.4)</td>
<td>0.167 (64.3)</td>
</tr>
<tr>
<td>P3c</td>
<td>38.533</td>
<td>-119.660</td>
<td>5</td>
<td>0.151 (64.4)</td>
<td>0.151 (64.4)</td>
<td>0.204 (64.3)</td>
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<td>P3a</td>
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<td>-119.483</td>
<td>30</td>
<td>0.166 (67.3)</td>
<td>0.166 (67.3)</td>
<td>0.177 (66.3)</td>
</tr>
<tr>
<td>PLC2</td>
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<td>-119.617</td>
<td>31</td>
<td>0.152 (64.4)</td>
<td>0.152 (64.4)</td>
<td>0.167 (64.3)</td>
</tr>
<tr>
<td>PLC3</td>
<td>38.545</td>
<td>-119.834</td>
<td>10</td>
<td>0.161 (78.2)</td>
<td>0.161 (78.2)</td>
<td>0.216 (77.6)</td>
</tr>
</tbody>
</table>

a. Names in common with those in Chapter 3 represent the same locations except for “P1”, which represents “P1b” in that study.
b. Listed are coordinates for P3a. Additional populations represented by this sample are: 38.516, -119.914 (P3b); 38.494, -119.981 (P3c).
c. Sample sizes from contributing populations are: 7 (P3a); 4 (P3b); 5 (P3c).
d. All individuals are from population P3a.
e. Calculated out of all loci (AFLP) or chromosomal sites (CO1).
Table 4.3. Summary data for pairwise population comparisons.

<table>
<thead>
<tr>
<th>Pheno. pairing</th>
<th>Pop. pair</th>
<th>Geogr. dist. (km)</th>
<th># loci</th>
<th>AFLP outlier descriptive statistics</th>
<th>Genetic divergence (FST )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Marker</td>
<td>Post. prob.</td>
</tr>
<tr>
<td>P–P</td>
<td>P1–P2</td>
<td>26.2</td>
<td>85</td>
<td>—</td>
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<tr>
<td></td>
<td>P1–P3</td>
<td>33.4</td>
<td>78</td>
<td>17</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>P2–P3</td>
<td>23.0</td>
<td>91</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PL–PL</td>
<td>PL2–PL3</td>
<td>25.7</td>
<td>95</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PLC–PLC</td>
<td>PLC1–PLC2</td>
<td>22.5</td>
<td>101</td>
<td>—</td>
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<tr>
<td></td>
<td>PLC1–PLC3</td>
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<td>120</td>
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<tr>
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<td>17, 470</td>
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<td></td>
<td>P3–PL3</td>
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<td>75</td>
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a. False Discovery Rate among outlier loci.

b. False Non-Discovery Rate among outlier loci.
Table 4.3 …continued

<table>
<thead>
<tr>
<th>Pheno. pairing</th>
<th>Pop. pair</th>
<th>Geogr. dist. (km)</th>
<th># loci</th>
<th>AFLP outlier descriptive statistics</th>
<th>Genetic divergence (FST)</th>
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<td>Marker</td>
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</tr>
<tr>
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<td>0.992</td>
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<td></td>
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<td>95</td>
<td>470</td>
<td>0.983</td>
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<tr>
<td></td>
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<td>93</td>
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<td>—</td>
</tr>
<tr>
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<td>108</td>
<td>—</td>
<td>—</td>
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<tr>
<td></td>
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<td>105</td>
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<td>—</td>
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<tr>
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<td>99</td>
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<td>P3–PLC1</td>
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<td>95</td>
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<td>33.4</td>
<td>94</td>
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<td>—</td>
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<tr>
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<td>PL3–PLC3</td>
<td>2.0</td>
<td>89</td>
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\(a\) False Discovery Rate among outlier loci.

\(b\) False Non-Discovery Rate among outlier loci.
Table 4.4. Outliers detected across all pairwise population comparisons. (a) AFLP outliers revealed by pairwise population comparisons at a 95% posterior probability outlier threshold. Outlier occurrence reveals a lack of association with morph differences among populations. (b) The same outlier loci, showing their pattern of occurrence when a lower, quartile-based, outlier threshold is employed. This threshold indicates the three outlier loci contribute at least moderately to population divergence across the study area. Asterisks denote loci surpassing a 99% posterior probability.

(a)

<table>
<thead>
<tr>
<th></th>
<th>P1</th>
<th>P2</th>
<th>P3a-c</th>
<th>PL2</th>
<th>PL3</th>
<th>PLC1</th>
<th>PLC2</th>
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</tr>
<tr>
<td>P3a-c</td>
<td>17*</td>
<td>—</td>
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<td>17, 470*</td>
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<td></td>
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<td>17*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
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<tr>
<td>PLC1</td>
<td>470*</td>
<td>—</td>
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<td>PLC2</td>
<td>470</td>
<td>—</td>
<td>141</td>
<td>141</td>
<td>—</td>
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<tr>
<td>PLC3</td>
<td>—</td>
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</tr>
</tbody>
</table>

(b)

<table>
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<tr>
<th></th>
<th>P1</th>
<th>P2</th>
<th>P3a-c</th>
<th>PL2</th>
<th>PL3</th>
<th>PLC1</th>
<th>PLC2</th>
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<td>P2</td>
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<td>P3a-c</td>
<td>17*, 470</td>
<td>17</td>
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<td>PL2</td>
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<td>17, 470</td>
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<td>17, 141, 470</td>
<td>17, 141, 470</td>
<td>141, 470</td>
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<tr>
<td>PLC3</td>
<td>17, 470</td>
<td>17</td>
<td>—</td>
<td>470</td>
<td>—</td>
<td>141, 470</td>
<td>141, 470</td>
</tr>
</tbody>
</table>
**Table 4.5.** Locus-by-locus AMOVA results based on sequence variation at **CO1.** A significant contributions of individuals, locations, and morphs to sequence variation at this locus is apparent.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>F statistics</th>
<th>Sum of squares</th>
<th>Variance components</th>
<th>Percentage variation</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among morphs</td>
<td>0.112</td>
<td>164.124</td>
<td>1.151</td>
<td>11.217</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Among locations, within morphs</td>
<td>0.220</td>
<td>139.606</td>
<td>2.002</td>
<td>19.515</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Within locations</td>
<td>0.307</td>
<td>684.828</td>
<td>7.107</td>
<td>69.268</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total</td>
<td>0.307</td>
<td>988.559</td>
<td>10.26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 4.6.** Summary of partial Mantel tests. Correlations and partial correlations are shown for genetic distance (FST), phenotypic distance (difference in the number of types of red trait possessed), and geographic distance (km) across different marker classes. Of specific interest is the significant correlation between genetic divergence of AFLP non-outlier loci and phenotypic distance when controlling for geographic distance among populations.

<table>
<thead>
<tr>
<th>Marker class</th>
<th>Genetic × phenotypic</th>
<th>Genetic × geographic</th>
<th>Genetic × phenotypic</th>
<th>Genetic × geographic</th>
<th>Genetic × phenotypic</th>
<th>Genetic × geographic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r        p</td>
<td>r        p</td>
<td>r        p</td>
<td>r        p</td>
<td>r        p</td>
<td>r        p</td>
</tr>
<tr>
<td>All markers</td>
<td>0.382    0.031</td>
<td>0.649    0.003</td>
<td>0.427    0.010</td>
<td>0.667    0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outliers</td>
<td>0.231    0.079</td>
<td>0.568    0.018</td>
<td>0.219    0.101</td>
<td>0.564    0.018</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-outliers</td>
<td>0.518    0.004</td>
<td>0.644    0.003</td>
<td>0.604    0.002</td>
<td>0.701    0.002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter 5: The evolution of sex ratio adjustment in the presence of intralocus sexual conflict

5.1 Summary

Sex ratio adjustment (SRA) of broods has received widespread interest as a means for optimizing parental investment in offspring. Classical explanations for the evolution of SRA focus on improving offspring fitness in light of resource availability or mate attractiveness. Here, we use genetic models to demonstrate that SRA can evolve to alleviate sexual antagonism by improving the chance that the alleles of a sexually antagonistic trait are transmitted to the sex they benefit. In cases where the trait is autosomally inherited, this result is obtained regardless of whether SRA is based on the mother’s or father’s genotype, and irrespective of the recombination rate between the trait and SRA loci. SRA also evolves in this manner when the trait is sex-linked, provided SRA decisions are based on the homogametic genotype (XX mothers or ZZ fathers). By contrast, when based on traits in the heterogametic sex, SRA promotes fixation of the allele that is detrimental to that sex, preventing the evolution of substantial levels of SRA. Our models indicate that the evolution of SRA in nature should be strongly influenced by the genetic architecture of the traits on which it is based and the form of selection affecting them.

5.2 Introduction

Sex ratio adjustment (SRA) of broods has been documented in numerous taxa and can result in brood sex ratio bias ranging from a few (e.g. Desfor et al. 2007; Servanty et al. 2007) to nearly one hundred percent (reviewed in Cockburn 2002; Davison & Ward 1998; Komdeur et al. 1997; West 2009; West et al. 2002). SRA has gained both theoretical and empirical attention as an avenue for the evolution of optimal sex allocation (investment in each sex), based on the idea that parents who can predict the differential fitness prospects for sons and daughters might favorably bias the sex ratio of their broods toward the fitter sex. Previous hypotheses for the adaptive value of SRA have focused on improving offspring fitness in light of a mother’s condition and resource availability (Trivers & Willard 1973; West & Sheldon 2002), and on biasing brood sex ratios toward sons when mates possess attractive male-specific traits (Burley 1981; Burley 1986; Fawcett et al. 2007). As a result, SRA has been linked to factors including
social rank (e.g. Clutton-Brock et al. 1984; Clutton-Brock et al. 1986), population density (e.g. Kruuk et al. 1999), mate quality (Long & Pischedda 2005), and local competition for mates (e.g. Flanagan et al. 1998; Herre 1985) or other resources (e.g. Aars et al. 1995; Gowaty 1993) (reviewed in Hardy 2002; West 2009). However, the degree of SRA observed and the factors governing SRA decisions vary widely across species (Clutton-Brock & Iason 1986; Cockburn 2002; Sheldon 1998), and several authors have suggested that we require a better understanding of the types of trait that prompt SRA and the mechanistic constraints on its evolution (Cockburn 2002; Krackow 1995; Uller et al. 2007; West 2009; West et al. 2002; West & Sheldon 2002; West et al. 2005).

With this aim in mind, we evaluate the potential for SRA to alleviate sexual antagonism, an issue that has received growing empirical support (Calsbeek & Bonneauad 2008; Calsbeek & Sinervo 2004; Connallon & Jakubowski 2009; Roulin et al. 2010) but little theoretical attention (but see Alonzo & Sinervo 2007), despite recognition of its potential importance (Fawcett et al. 2007; Patten & Haig 2009). We focus on intralocus sexual conflict (IASC), a form of sexual antagonism that arises when selection at a locus favors different alleles in males versus females (Lande 1980; Rice & Chippindale 2001). Under the appropriate conditions, IASC can stably maintain genetic polymorphisms (Albert & Otto 2005; Lande 1980; Patten & Haig 2009; Rice 1987; Rice 1984), preventing either sex from reaching its optimal phenotypic state and lowering mean population fitness (Lande 1980). The possibility that SRA diminishes this effect is of broad importance given the widespread occurrence of this source of sexually antagonistic selection in nature and present efforts to identify mechanisms by which it can be resolved (Bonduriansky & Chenoweth 2009; van Doorn 2009).

We consider a trait that has opposing fitness effects in the two sexes and develop a diploid population genetic model to track the evolution of a modifier allele at a separate SRA locus. We predict that selection will favor the evolution of the modifier allele such that females bias the sex ratio of their broods according to which sexually antagonistic alleles their offspring are likely to receive, in a manner that increases the match between offspring sex and the alleles that benefit that sex. We supplement our analytical model with simulations that allow continual mutations to occur at the SRA modifier allele, in order to follow the long-term evolution of SRA and its effect on the trait. While sexually antagonistic traits are expected to arise throughout the genome, they are more likely to remain polymorphic on sex chromosomes due to the imbalanced manner in which sex-linked genes are inherited and expressed among sexes (Gibson et al. 2002; Mank
We therefore compare results for autosomal and sex-linked traits.

5.3 Methods

5.3.1 Analytical model

A single locus (T) governs a trait that is under sexually antagonistic selection, where one allele T is favored in females while the alternative allele t is favored in males. Such opposing selection pressures are expected for physiological, morphological, and behavioral traits that have different optima between sexes owing to differences in their ecology or reproductive biology (Frayer & Wolpoff 1985; Glucksmann 1974; Lande 1980; Rice 1984; Shine 1989). Sexual antagonism can also be driven by natural and sexual selection acting in different directions; our models accommodate this scenario, provided we assume that female preferences are fixed.

Sexually antagonistic selection is described by sex-specific fitness values (Table 5.1), with selection coefficients against the deleterious allele in females and males given by $s_f$ and $s_m$, respectively ($0 \leq s_i \leq 1$), and dominance coefficients in females and males given by $h_f$ and $h_m$ (with $0 \leq h_i \leq 1$). A second locus, M, is a modifier that allows a female to adjust the sex ratio of her brood (hereafter referred to as “SRA”; sex ratio adjustment). Initially, the population is fixed for one allele (M), which may code for no SRA or some initial level of SRA. We then introduce a second modifier allele, m, and determine its fate. We assume throughout that SRA is subject only to maternal control; if paternal control were instead considered, we expect that equivalent results would be obtained (but with the sexes reversed with respect to selection and XY/ZW sex determination).

The proximate mechanisms of SRA are not well understood. Here, we assume it can be implemented via any of the potential behavioral or physiological mechanisms of SRA such as hormonal effects on sperm selection and gestation, and post-parturition maternal effects or external environmental factors (e.g., ambient temperature) (Krackow 1995; Uller et al. 2007). In addition, we impose no direct costs of SRA. Recombination between T and M occurs at rate r, and mutations are ignored. Standard two-locus recursions were developed and analyzed using Mathematica 6.0 (see Appendix D.1).

In general, a female might adjust her sex ratio strategy in response to either her own state or that of her environment, including her social and mating environment (Alonzo & Sinervo 2007; Burton-Chellew et al. 2008; Flanagan et al. 1998). In our models, we allow a female to adjust
her SRA based on her own trait genotype (self-SRA) or the trait genotype of her mate (mate-SRA), assuming trait evaluation occurs during reproduction via any feasible cognitive or physiological mechanism. Specifically, the probability that a mother produces a daughter is given by $D_{ij}$, where $i$ represents the number of $m$ alleles in her modifier genotype and $j$ represents the number of $t$ alleles either in her trait genotype (in the case of self-SRA) or her partner’s genotype (in the case of mate-SRA) (Table 5.2). Although plausible values of $D_{ij}$ range from 0 to 1, any errors in a female’s assessment of her own or her mate’s genotype would reduce the expression of SRA (bringing the $D_{ij}$ for a female closer to her mean SRA across trait genotypes) and so would constrain the evolution of SRA. Given that the nature of these constraints is unknown, we assume females have perfect information regarding the trait genotype, although assessment errors could easily be incorporated by appropriate choices of $D_{ij}$ and would not alter the structure of the model.

### 5.3.2 Simulating the long-term evolution of SRA

We next built an individual-based simulation that allowed multiple alleles to arise at the modifier locus, enabling us to track the long-term evolution of SRA in the presence of sexual antagonism. Only self-SRA was simulated, because the results for mate-SRA are expected to be similar. To reduce the number of parameters, we assumed complete additivity at the modifier locus ($D_{i1} = (D_{i0} + D_{i2})/2$; $D_{1j} = (D_{0j} + D_{2j})/2$), so that each modifier allele could be fully characterized by its action in homozygotes. Specifically, we represented the SRA strategy of a homozygous modifier genotype, say $MM$, as $\{D_{00}, D_{02}\}$, where the two elements of this vector give the offspring sex ratio when the mother is $TT$ and $tt$, respectively.

To facilitate comparison with previous work, we followed the form of the Model 1 simulations in Fawcett et al. (2007), used to explore the evolution of SRA in the presence of sexual selection (in that study, the trait was expressed only in males). In particular, mutations occurred at the modifier at a rate of $\mu = 0.05$ per allele per generation, with each mutation causing a $+1/250$ or a $-1/250$ change in one of the pair of strategies characterizing an allele (e.g., a modifier allele with strategies $\{124/250, 121/250\}$ might mutate to $\{123/250, 121/250\}$). In the standard set of simulations, selection coefficients were set to $s_f = s_m = 1/6$, and the dominance coefficients to $h_f = h_m = 1/10$ (dominance was not present in the haploid model considered by Fawcett et al.). The population was held fixed at a size of 4000 diploid individuals (Fawcett et
al. modeled the same number of alleles, but in 8000 haploid individuals, and also allowed slight variation over time in the population size depending on the sex ratio). The trait and modifier loci were assumed to be unlinked \((r = 1/2)\). We placed no intrinsic limit on the degree of SRA control females can possess, in order to let SRA evolve freely within the context of sexually antagonistic selection.

5.4 Results
5.4.1 Evolution of sex ratio adjustment at autosomal loci
5.4.1.1 Analytical model

We first identify the conditions under which a stable polymorphic equilibrium exists when the population is fixed for the \(M\) allele and then determine whether the \(m\) allele could invade if it alters SRA. Here we present only a summary of the analyses in order to provide the reader with an intuitive understanding of the evolutionary dynamics; full analytical details are available in the Mathematica 6.0 file at http://www.zoology.ubc.ca/~otto/Research/BlackburnEtAl2010.nb. For tractability in the present analysis, we assume that selection and the effect of the modifier are weak. Specifically, we assume that \(s_f, s_m\) and the differences among the \(D_{ij}\) are all on the order of a small term, \(\xi\). In the next section, we perform simulations that relax these assumptions.

The analytical results for autosomal loci are identical under self-SRA and mate-SRA, so they are not distinguished in the present section. With the \(M\) allele fixed, opposing selection pressures in males and females can, under appropriate conditions, maintain variation in a trait, with the equilibrium frequency of allele \(T\) equal to:

\[
\hat{p}_T = \frac{(1 - h_f) s_f - h_m s_m - H\theta}{(1 - 2h_f)s_f + (1 - 2h_m)s_m + (1 - 2H)\theta} + O(\xi),
\]

where \(O(\xi)\) represents terms that are of order \(\xi\) and hence smaller than the leading term. The term \(H\) measures the dominance of the SRA strategy in \(Tt\) females; \(H = (D_{01} - D_{02})/(D_{00} - D_{02})\). The term \(\theta\) represents the effect of Fisherian sex ratio selection, which acts to maintain a balanced population-wide sex ratio (Bodmer & Edwards 1960; Fisher 1930; Kolman 1960), and takes the form:

\[
\theta = \left(\sqrt{D} - \frac{1}{2}\right) \frac{\Delta D}{D(1-D)},
\]

87
where \( \bar{D} \) is the mean sex ratio at birth in the population (proportion females) and \( \Delta D \) is the difference in SRA in response to \( TT \) versus \( tt \) genotypes (\( \Delta D = D_{00} - D_{02} \)). The term \( \theta \) is non-zero whenever the population-wide sex ratio departs from 1/2 and the \( T \) and \( t \) alleles are associated with different offspring sex ratios. For example, if the population is female-biased (\( \bar{D} > 1/2 \)) and \( T \)-bearing females are more likely to produce daughters (\( \Delta D > 0 \)), \( \theta \) has a positive value and drives down the equilibrium frequency of the \( T \) allele.

A stability analysis indicated that the polymorphic equilibrium is stable only when the denominator of equation (1) is positive. Polymorphism is likely when the dominance coefficients are sufficiently low that \( Tt \) heterozygotes are more fit than the homozygotes when averaged across the sexes. We expect to see the persistence of high levels of sexually antagonistic fitness variation only at autosomal loci that allow a stable polymorphism, and we therefore focus exclusively on such loci.

Next, a new SRA modifier allele, \( m \), was introduced to the polymorphic equilibrium represented by equation (1). Our goal was to assess which conditions would permit the modifier to spread in the population while preserving this equilibrium. The leading eigenvalue (\( \lambda \)) within the characteristic polynomial of the local stability matrix is useful in this regard, because this value describes when the equilibrium remains stable in the presence of \( m \). Specifically, the modifier can invade whenever \( \lambda \) has a value greater than 1, and selection will thus favor any \( m \) allele that places \( \lambda \) within this range of values. We determined how the parameters in our model affect \( \lambda \) by representing it as a power series, \( \lambda = \lambda_0 + \lambda_1 \xi + \lambda_2 \xi^2 + \ldots \), where \( \xi \) represents the order of terms assumed to be small (the selection coefficients and the differences in \( D_{ij} \)). Solving for the successive terms in this series, we obtain:

\[
\lambda = 1 - \frac{1}{2} \left( \frac{\bar{D} - \frac{1}{2}}{\bar{D}(1 - \bar{D})} \right) \left\{ \hat{p}_T^2 (D_{10} - D_{00}) + 2 \hat{p}_T \hat{p}_t (D_{11} - D_{01}) + \hat{p}_t^2 (D_{12} - D_{02}) \right\} \\
+ \hat{p}_T \hat{p}_t \hat{s}_m \left[ (1 - h_m) \hat{p}_T + h_m \hat{p}_t \right] \left\{ \hat{p}_T (D_{10} - D_{00}) + (\hat{p}_t - \hat{p}_T) (D_{11} - D_{01}) - \hat{p}_t (D_{12} - D_{02}) \right\},
\]

(3)

While equation (3) appears not to depend on selection in females, this is because we have used equation (1) to rewrite \( s_f \) in terms of \( s_m \) and \( \hat{p}_t \) to simplify the presentation (see Mathematica 6.0 file).
We can see that the first line of equation (3) dominates the value of $\lambda$ whenever the population-wide sex ratio departs substantially from 1/2, exerting Fisherian sex ratio selection in favor of strategies that equalize the sex ratio. For example, if the population is female-biased ($\bar{D} > 1/2$), selection favors any $m$ allele that increases the production of sons, causing the term in braces on the first line to be negative (i.e., resulting in a positive $\lambda$). If the population-wide sex ratio is nearly 1/2, then the term $\bar{D} - 1/2$ is small (of order $\xi$) and the second line of (3) also contributes to the fate of the new modifier allele. The sign of the second line depends on the sign of the term in braces, and it is this term that drives the evolution of SRA (Figure 5.1).

Specifically, the term $\hat{p}_t (D_{10} - D_{00})$ indicates that the system will evolve to produce more daughters when in $TT$ females (self-SRA) or when mated to $TT$ males (mate-SRA). The term $- \hat{p}_t (D_{12} - D_{02})$ indicates that the system will evolve to produce fewer daughters when in $tt$ females (self-SRA) or when mated to $tt$ males (mate-SRA). Finally, the evolution of the SRA strategy in heterozygotes, represented by the term $(\hat{p}_t - \hat{p}_s)(D_{11} - D_{01})$, favors producing daughters when $T$ is rare and sons when $t$ is rare. To understand this latter result, consider the case when $T$ is rare. Mothers that are $Tt$ (self-SRA) or have mated with $Tt$ males (mate-SRA) will produce offspring that are more likely to carry the $T$ allele than the rest of the population (who are predominantly $tt$). Because the $T$ allele benefits females, female offspring will be more fit relative to the rest of the population while male offspring will be less fit, thus explaining why SRA among $Tt$ individuals evolves to favor daughters when $T$ is rare. Reversing this logic explains why SRA among $Tt$ individuals evolves to favor sons when $t$ is rare.

This local stability analysis demonstrates that SRA evolves as expected; favoring daughters when parents carry the allele advantageous in females ($T$) and favoring sons when parents carry the allele advantageous in males ($t$). In fact, as long as the population sex ratio remains nearly equal and assessment errors are low, stronger and stronger SRA is expected to evolve, and the equilibrium (1) will remain valid and stable throughout this process. The evolution of SRA can be rapid, with selection on the modifier on the order of the strength of selection at the $T$ locus times the extent to which SRA is altered by the new modifier allele (equation 3). The fact that $r$ does not enter into the leading order terms of $\lambda$ implies that SRA evolution should be insensitive to the recombination rate between the trait and the modifier.
5.4.1.2 Simulation

Over 50000 generations, strong sex ratio adjustment occurred in the predicted direction (Figure 5.2), increasing the mean fitness of each sex in the population (Figure 5.3). Mean male fitness (blue curve) reached a slightly higher value than mean female fitness (red curve), in accordance with the final frequency of the female-benefit $T$ allele being slightly below 0.5 in Figure 5.2. Both the pattern and speed of SRA evolution were comparable to that observed by Fawcett et al. (2007) in simulations of Fisherian sexual selection with recurrent deleterious mutations or sexual selection on traits indicating male condition. Note, however, that the simulations of Fawcett et al. included two loci contributing to sex ratio adjustment (doubling the SRA mutation rate) and assumed individuals were haploid (effectively increasing the strength of selection relative to the diploid model used here), which suggests that sexual antagonism is at least as effective as sexual selection in generating SRA.

In contrast to the predictions of the analytical model, the speed of SRA evolution was relatively slow in the simulations, taking tens of thousands of generations, as emphasized also by Fawcett et al. (2007). We explored two potential explanations for this result. First, it is possible that most mutations were prevented from spreading to high frequency because they perturbed the sex ratio from 1/2. To test this explanation, we ran the simulations again while allowing each mutation to have equal and opposite effect in $TT$ and $tt$ mothers while keeping the magnitude of the mutation at 1/250 (that is, the mutational effect was either $\{+1/500,-1/500\}$ or $\{-1/500,+1/500\}$). Under the default parameter settings, it took on average 31116 generations (SE = 2643) for an SRA difference of 0.5 to evolve, which was not significantly faster than when mutations affected the response in $TT$ or in $tt$ mothers separately. Second, the speed of SRA evolution might have been limited by the amount of genetic variance in SRA, which is expected to be proportional to the mutation rate at the modifier locus times the square of the effect size of modifier mutations (Dieckmann & Law 1996). Simulation results for a range of effect sizes are shown in Figure 5.4 for both the default mutation rate, $\mu = 0.05$, and $\mu = 0.005$. The time required to produce an SRA strategy differing by more than 0.5 between $TT$ and $tt$ mothers declined dramatically as the mutation rate and effect size of the modifier increased. We thus conclude that the most important factor limiting the speed of SRA evolution in these simulations was the amount of genetic variation for SRA.
The simulations considered above involved symmetrical selection in males and females. Because the trait allele then equilibrated at a frequency of 0.5, strongly biased SRA could evolve where $TT$ and $tt$ genotypes nearly always resulted in the production of daughters and sons, respectively. When the frequency of $T$ departs from 1/2 because of sex differences in the strength of selection, the evolution of strong SRA is hampered because it would cause the population sex ratio to depart from 1/2. As an extreme example, if the frequency of $t$ were 0.9 and if $tt$ genotypes resulted in the exclusive production of sons, then the sex ratio would be extremely biased (>80% male). Consistent with this interpretation, we found that when selection was weaker in females ($s_f = 1/12$ and $s_m = 1/16$, leading to $\hat{p}_f = 0.3$), SRA evolved to a lower level in response to the more common $tt$ genotypes than the rarer $TT$ genotypes, ensuring that the sex ratio remained near 1/2 (black curves) (Figure 5.2, top side panel). The exact opposite was seen when selection was weaker in males ($s_f = 1/6$ and $s_m = 1/12$, leading to $\hat{p}_f = 0.7$; Figure 5.2, bottom side panel). We expect this constraint to be weaker if SRA in $Tt$ mothers were free to evolve, rather than being fixed at the average of $TT$ and $tt$ mothers. Nevertheless, these examples illustrate the main restriction on the evolution of SRA: Fisherian sex ratio selection prevents the evolution of substantial SRA in any case that leads to a large departure from a balanced sex ratio within the population.

5.4.2 Evolution of sex ratio adjustment at sex-linked loci

5.4.2.1 Analytical model

Here, we summarize the outcomes for X- and Z linkage; detailed quantitative results are presented in Appendix D.2 and D.3, and the entire analysis is available in the Mathematica 6.0 file noted above. Assuming a sex ratio of 1/2 ($\theta = 0$), stability requires only that the female-benefit allele $T$ be partially recessive in females (with X linkage) or that the male-benefit allele $t$ be partially recessive in males (with Z linkage) (Table D.2). In the case of X linkage, we consider both autosomal and sex-linked modifiers when assessing whether a modifier allele altering offspring sex ratios could spread in a population that is polymorphic for a sex-linked trait. With a Z-linked modifier and maternal sex ratio control, however, modifier alleles that increase the proportion of sons are always favored, regardless of the trait carried, because the Z chromosome possessed by mothers passes only from mothers to sons. This induces strong meiotic drive that can lead to “extraordinary” sex ratio imbalances and potentially even
population extinction (Hamilton 1967). Thus, we only consider autosomal modifiers of SRA in the presence of a Z-linked trait.

The results differ between self-SRA and mate-SRA when there is sex linkage, so we must now treat these scenarios separately. In the case of a sex-linked trait and SRA based on the homogametic sex (self-SRA based on XX females or mate-SRA based on ZZ males), the evolution of SRA is qualitatively the same as in the autosomal model. However, dramatically different results are obtained when SRA occurs in response to the hemizygous genotype (mate-SRA based on XY males or self-SRA based on ZW females); SRA then directly affects the allele frequency dynamics at the trait locus. The reason is that SRA induces transmission distortion when traits in the heterogametic sex are used to adjust offspring sex ratios. Consider mate-based SRA involving $T$-bearing and $t$-bearing X chromosomes in XY males. Because $T$ is favored in females, SRA is expected to evolve such that females mated to $T$-bearing males produce more daughters, which causes the X chromosome carrying the $T$ allele ($X^T$) to be inherited by more offspring than the Y chromosome. In contrast, females mated to $t$-bearing males evolve to produce more sons, which causes the Y chromosome to be inherited and reduces the transmission of the $t$ allele to offspring. The opposite tendency develops in the case of self-SRA with ZW sex determination; $T$-bearing females evolve a daughter-biased sex ratio, which results in an increased inheritance of W instead of $Z^T$, reducing transmission of the $T$ allele. Thus, when traits in the heterogametic sex are used to adjust offspring sex ratios, SRA evolves in a manner that induces a transmission disadvantage against the allele that is fitter in the heterogametic sex. As a result, if SRA becomes too extreme, the trait polymorphism can be lost, with allele $T$ fixed in XY systems and $t$ fixed in ZW systems. In addition, if SRA is based on the hemizygous sex and the modifier is autosomal, it is also possible for SRA evolution to halt at a low level ($\Delta D_{\text{hemi}} = s_m/4$ for mate-SRA in XY systems and $\Delta D_{\text{hemi}} = s_f/4$ for self-SRA in ZW systems, where $\Delta D_{\text{hemi}} = D_{00} - D_{01}$, as defined in Table D.2). This occurs because of genetic associations that develop between the modifier and trait loci, inhibiting further evolution of SRA even though the trait locus remains polymorphic (see Appendix D).

In summary, while sex linkage can make it easier for sexually antagonistic selection to maintain polymorphism for a trait in the first place, it can also constrain the evolution of offspring SRA in response to this trait. Specifically, whenever the genotype of the heterogametic sex is used to adjust the offspring sex ratio (mate-SRA with X-linked traits and self-SRA with Z-
linked traits), SRA evolves only up to a point at which either the trait polymorphism is lost or selection on the modifier changes sign, preventing the further evolution of SRA.

5.4.2.2 Simulation

We explored the evolution of SRA over longer periods of time using simulations with an X-linked trait. We were particularly interested in testing the predictions made by our analyses concerning the role of transmission distortion. The simulations were built as described for the case of an autosomal trait and run for equivalent parameter values. We first simulated self-SRA with an X-linked trait and an X-linked modifier. The analysis predicted that SRA should evolve in a manner similar to the autosomal case but be faster by a factor of approximately 4/3 (see Appendix D.3). The simulation results in Figure 5.5a confirm this prediction; SRA in response to TT genotypes and in tt genotypes evolved at nearly the rate predicted from a regression based on the autosomal case (Figure 5.2), with slopes multiplied by 4/3.

Under mate-based SRA, a different picture emerged. Mothers evolved to alter their brood sex ratio such that X'TY males produced more daughters and X'Y males produced more sons, resulting in transmission distortion that caused T alleles to increase rapidly from the frequency of 1/2 predicted by natural selection alone (green curves in Figures 5.5b, c). As expected with an X-linked trait and an X-linked modifier (see equation C1), SRA evolved only to the point where the trait polymorphism was lost. As the T allele rose in frequency, the sex ratio when mated to T-bearing males became more and more constrained to remain near 1/2 (red curve, see inset in Figure 5.5b) to ensure that the population-wide sex ratio was even. Meanwhile, the sex ratio when mated to t-bearing males declined (blue curves), until the frequency of T reached one. At this point, the sex ratio strategy when mated to t-bearing males was free to drift around neutrally.

Finally, we simulated the case of mate-SRA with an X-linked trait locus and an autosomal modifier locus. For the parameters used in Figure 5.5, SRA was predicted to evolve only up to an intermediate level, with \( \Delta D_{hemi} = s_m/4 \) (equation C2). Assuming a population at equilibrium (Table D.2) with an even population-wide sex ratio, the analysis predicts that SRA should have stopped evolving once mothers produce 51% daughters when mated to T-bearing males and 47% daughters when mated to t-bearing males, with an equilibrium frequency of T of \( \hat{p}_r = 0.8 \). While the simulation results (Figure 5.5c) suggest that the system briefly hovered near this point, the system exhibited substantial fluctuations and ultimately the T allele fixed in all ten replicates. The same result was obtained in simulations initiated using the exact modifier strategy and T-
allele frequency at the point where SRA evolution is expected to halt (results not shown), indicating the $T$ allele fixed due to fluctuations about the equilibrium value. We suspect that these fluctuations were caused either by random genetic drift (given the finite population size of 4000 diploids) or by the stochastic sequence of modifier mutations that happened to arise during the simulations, causing the sex ratio to depart too far from 1/2 or causing too much transmission bias on the $T$ allele.

Overall, the simulations with sex linkage confirm one of our main results: basing offspring sex ratios on the trait of a hemizygous individual (mate-based SRA in the case of male heterogamety) generates a transmission bias that is generally too strong to permit the evolution of high levels of SRA. Instead, only a low sex ratio bias evolves, followed by fixation of the allele that benefits the homogametic sex. While we had predicted an intermediate evolutionarily stable strategy (ESS) in the case of an autosomal modifier, the simulations indicated that this ESS is not robust to departures from the assumptions of the analytical model (infinite population size, weak selection, weak SRA), and again we observed fixation of the allele that benefits the homogametic sex.

5.5 Discussion

5.5.1 Alleviation of sexual antagonism via SRA

Our results indicate that sex ratio adjustment (SRA) can evolve in the presence of intralocus sexual conflict (IASC), diminishing sexually antagonistic fitness effects by increasing the match between offspring sex and the alleles benefitting that sex (e.g., Figure 5.2). Thus, SRA can be viewed as a mechanism by which sexual antagonism can be resolved (Alonzo & Sinervo 2007; Fawcett et al. 2007; Patten & Haig 2009), improving the fitness of both sexes (Figure 5.3). In this respect, our models corroborate a previous game theory analysis on side-blotched lizards, *Uta stansburiana*, by Alonzo and Sinervo (2007). We extend these findings by establishing an explicit genetic framework for the evolutionary dynamics and exploring the evolution of SRA under different modes of inheritance. The analytical models revealed that sexually antagonistic selection on SRA can be strong, proportional in strength to the product of the effect size of the modifier and the strength of selection on the trait. Our simulations corroborated these results; provided sufficient genetic variation exists at the SRA locus (Figure 5.4), SRA for autosomal traits can evolve rapidly, increasing the mean fitness in each sex (Figure 5.3). However, we also find that differences in genetic architecture strongly influence SRA evolution. In particular, SRA
relieving sex-linked sexual antagonism is only likely to persist when based on the homogametic sex (female characteristics in XY systems and male characteristics in ZW systems)(Figure 5.5a). The reciprocal pattern is likely to be transient (Figure 5.5b,c); systems lacking SRA may include cases where sex-linked polymorphisms have been lost due to the evolution of SRA based on traits in the heterogametic sex.

SRA alters gene expression at the level of the individual, manipulating whether an allele is expressed in a daughter or a son. While we have considered only a single gene, it is likely that multiple loci throughout the genome experience intralocus sexual antagonism, and future work would do well to examine how this affects the evolution of SRA. If a mother adjusts her brood sex ratio in response to only one of several sexually antagonistic traits, we expect that genetic variation for the other traits would act in a similar way to errors in genotype assessment: both, at least occasionally, would cause mothers to produce more of the less fit sex (due to a mistaken assessment of the genome-wide advantage of producing daughters versus sons or to an incorrect assessment of the T-locus genotype). On the other hand, if multiple polymorphic genes contribute to the same trait, then SRA could potentially evolve to alleviate sexual antagonism at more than one gene at the same time, strengthening selection for SRA. In any case, we expect that selection will continue to favor the evolution of SRA in the presence of multiple genes, as long as there remains a net sexually antagonistic fitness effect. Numerous empirical examples confirm the presence of net sexually antagonistic fitness effects with respect to individual traits (e.g. Long & Rice 2007; Merila et al. 1997; Rice 1996) or genome-wide fitness differences (reviewed in Bonduriansky & Chenoweth 2009; e.g. Chippindale et al. 2001; Gibson et al. 2002; Prasad et al. 2007). Provided females can detect and respond to this variation, we expect SRA to evolve as in our models.

### 5.5.2 Identifying a role for SRA in alleviating sexual antagonism

Variation in patterns of SRA in nature might stem in part from the action of several different mechanisms and distinguishing among alternatives remains a major empirical and theoretical challenge (Cockburn 2002; West 2009). Toward this goal, our results indicate that the effects of sexual antagonism need to be considered in addition to classic conditional sex allocation hypotheses. Few studies have measured the sex-specific fitness effects of traits subject to SRA, so it is unclear whether SRA commonly helps to alleviate sexually antagonistic selection in nature. However, several recent cases support the prediction that broods with skewed sex ratios
will exhibit diminished sexually antagonistic fitness effects. Connallon and Jakubowski (2009) reported that, in the fruit fly *Drosophila melanogaster*, females produced female-biased broods when mated to males who carried alleles with positive fitness effects in daughters. Similarly, in barn owls, *Tyto alba*, parents possessing relatively small spots on their plumage are more likely to produce sons, mitigating the sexually antagonistic fitness effects associated with this trait (Roulin et al. 2010). A related pattern occurs in side-blotched lizards, *Uta stansburiana* (Calsbeek & Sinervo 2004), and brown anoles, *Anolis sagrei* (Calsbeek & Bonneaud 2008); although sex ratios within broods are typically balanced in both species, females have been shown to reduce sexual conflict with respect to body size by mating multiple times and then cryptically assigning sperm from relatively large sires to sons and small sires to daughters (Calsbeek & Bonneaud 2008; Calsbeek & Sinervo 2002; Calsbeek & Sinervo 2004).

While we have focused on sex ratio adjustment only in response to traits exhibiting sexually antagonistic selection, different forms of selection may act simultaneously, driving SRA in similar or opposite directions. For example, cases in which SRA facilitates the transmission of an allele conferring attractiveness to sons (Burley 1981) should be strengthened if that trait also has sexually antagonistic effects as depicted in our mate-SRA models. By contrast, mothers in good condition are expected to evolve increased investment in sons when additional resources disproportionately increase the fitness of sons relative to daughters (Trivers & Willard 1973), whereas our self-SRA models suggest this effect would be counteracted if female condition is based on sexually antagonistic selection. On the other hand, the selection pressures in this latter example would act in the same direction if additional resource investment disproportionately benefits daughters (see also Silk 1983; West 2009).

Disentangling the effects of multiple selection pressures on SRA evolution in an experimental context could entail varying the strength of individual selective forces and then tracking the evolution of SRA. In systems where this is not feasible, the issue could instead be approached empirically by measuring the relevant parameters (e.g., the strength of sexual selection on males, the impact of parental condition on offspring fitness, and selection coefficients in each sex) and determining the relative strength of selection on an SRA modifier arising from the different forces. Theoretical analyses like ours assist these approaches by supplying estimates of the direction and strength of selection on SRA modifiers resulting from a given form of selection (e.g., as given by equation 3). Similar estimates from models investigating other forms of selection are needed for a comprehensive theoretical framework in
this respect. The sum total of the SRA changes expected under each force considered in isolation would provide an estimate of the rate and direction of SRA evolution, supplemented by simulations to assess departures from this additive expectation.

5.5.3 Alternative mechanisms to alleviate sexual antagonism

Sexual antagonism is widespread (Chippindale et al. 2001; Foerster et al. 2007) and may affect diverse evolutionary processes (Bonduriansky & Chenoweth 2009; Parker & Partridge 1998; van Doorn 2009), highlighting the need to identify mechanisms that affect its strength and persistence (Bonduriansky & Chenoweth 2009). Sexually dimorphic trait expression is considered the principle means to resolve IASC (Cox & Calsbeek 2009; Lande 1980; Rice 1984; van Doorn 2009), arising via the evolution of genomic imprinting or modifiers that result in sex-limited expression (reviewed in Bonduriansky & Chenoweth 2009; Ellegren & Parsch 2007). SRA represents an alternative potential strategy toward this end, biasing the inheritance of sexually antagonistic traits such that daughters are more likely to inherit alleles that are favored in females, and vice versa for sons. SRA might be particularly important in this regard if the evolution of sexual dimorphism is constrained, for example if the genes underlying the trait are highly correlated between sexes (Lande 1980) or have pleiotropic functions (Badyaev 2002; Ellegren & Parsch 2007; van Doorn 2009). Disparities in nature between the observed degree of sexually antagonistic selection and sexual dimorphism suggest that such constraints might be common (Cox & Calsbeek 2009). For example, male yellow-pine chipmunks, Tamias amoenus, are close to their optimal body size but females are displaced from their optimum (Schulte-Hostedde et al. 2002) whereas, in house finches, Carpodacus mexicanus, neither sex expresses optimal body size (Badyaev & Martin 2000). Numerous other examples exist (Bjorklund & Senar 2001; reviewed in Cox & Calsbeek 2009; Forsman 1995) and include cases in which sexual dimorphism is absent despite the presence of sexual antagonism (Merila et al. 1997). Indeed, Cox and Calsbeek (2009) provide evidence that residual sexual antagonism is widespread even among species exhibiting strong sexual dimorphism, suggesting that traits may frequently be displaced from their optimal value in each sex (see also Roulin et al. 2010). Hence, SRA might often have the opportunity to evolve in conjunction with sexual dimorphism, mitigating sexually antagonistic selection to a greater degree than either mechanism alone.
5.5.4 Conclusions

Our study indicates that sexually antagonistic selection poses a potent source of selection for differential sex ratio allocation and that, in turn, SRA evolution can help to diminish sexual conflict. The impact of SRA on sexual antagonism likely varies among populations according to the genetic variation available at potential modifier loci, the mode of inheritance of sexually antagonistic traits, the action of other putative selective pressures on SRA, and the potential for alternative strategies to mediate the conflicting fitness pressures on daughters and sons (e.g., sex-limited gene expression, genomic imprinting). We expect that consideration of these factors will help to better explain variation in patterns of SRA in nature.

5.6 Acknowledgements

We would like to thank T. W. Fawcett for sharing simulation details, and D. E. Irwin, W. P. Maddison, L. K. M’Gonigle, and three anonymous reviewers for helpful comments on the manuscript. We are also grateful to R. G. FitzJohn, W. P. Maddison, and L. K. M’Gonigle for computer support. Funding was provided by the Natural Sciences and Engineering Research Council of Canada through a Discovery Grant to W. P. Maddison (GSB), a post-doctoral fellowship (AYKA), and a Discovery Grant (SPO).
Figure 5.1. Selection for sex ratio adjustment. The heat map shows the strength of selection for a modifier altering sex ratios (second line of equation 3), assuming that the overall sex ratio remains at 1/2. Color ranges from white (selection strength of 0) to red when selection favors the modifier (selection strength of $2s_m / 27$), and from white to blue when selection acts against the modifier (selection strength of $-2s_m / 27$). A modifier that increases investment in daughters (by an amount specified by the y-axis) is always favored in $TT$ mothers or when mated to a $TT$ father (panel “a”), is only favored in response to a $Tt$ individual when $\hat{p}_T < 1/2$ (panel “b”), and is always selected against in response to a $tt$ individual (panel “c”). The opposite results apply to a modifier that increases investment in sons. Selection is most effective when the modifier has a larger effect (higher values along y-axis) or when there is substantial variation in the trait (intermediate values along x-axis). Other parameters: $h_m = 1/2$. 
**Figure 5.2.** Evolution of sex ratio adjustment in response to sexually antagonistic selection. The average frequency of daughters born in response to \( TT \) genotypes increased (red curves), while the average frequency of daughters born in response to \( tt \) genotypes decreased (blue curves), maintaining the overall sex ratio near \( 1/2 \) (black curve). The frequency of the \( T \) allele is shown in green. Dotted curves show these data from each of ten replicate simulations, and solid curves plot the means. Box plots show the 25%-75% quantiles (with whiskers showing the range) for the entire population of strategies present in the first replicates at 50,000 generations. In the standard set of simulations (left panel), \( s_f = s_m = 1/6 \), \( h_f = h_m = 1/10 \), and the mutation rate was set to \( \mu = 0.05 \). The panels to the right explore changes to the strength of selection in one sex.

**Figure 5.3.** Change in mean population fitness over time as SRA evolves. The mean fitness of each sex increased as SRA evolved during the autosomal simulations. Mean male fitness (blue curve) reached a slightly higher value than mean female fitness (red curve), in accordance with the final frequency of the female-benefit \( T \) allele being slightly below 0.5 in Figure 5.2. Parameter values are as in the left panel of Figure 5.2.
Figure 5.4. The speed of SRA evolution in relation to mutation rate and effect size. The time until SRA differed by at least 0.5 between $TT$ and $tt$ genotypes decreased dramatically with increasing effect size of the mutations and with increasing mutation rate at the trait locus. Curves indicate the mean value among five replicates (dots). Remaining parameter values areas in Figure 5.2.
Figure 5.5. Evolution of sex ratio adjustment with sex linkage. (a) With an X-linked trait and modifier, self-SRA evolves in a similar manner to Figure 5.2, as expected. The response to selection is quicker by about 4/3 (sloped lines show a regression based on the autosomal case in Figure 5.2, with the slopes multiplied by 4/3). (b) In contrast, with an X-linked trait and modifier, mate-SRA induces a strong transmission bias favoring $T$, causing this allele to rise rapidly to fixation (green curve) and hampering the evolution of SRA (red and blue curves). (c) With an X-linked trait and an autosomal modifier, mate-SRA also induces a transmission bias favoring $T$. Insets show greater resolution near $p_r = 0.5$; remaining details as in Figure 5.2.
**Table 5.1.** Fitness of diploid sexually antagonistic trait genotypes.

<table>
<thead>
<tr>
<th>Trait genotype</th>
<th>Female fitness</th>
<th>Male fitness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$TT$</td>
<td>$Tt$</td>
</tr>
<tr>
<td>Female fitness</td>
<td>1</td>
<td>$1 - h_f s_f$</td>
</tr>
<tr>
<td>Male fitness</td>
<td>$1 - s_m$</td>
<td>$1 - h_m s_m$</td>
</tr>
</tbody>
</table>

**Table 5.2.** Sex ratio adjustment (SRA) in the case of a diploid trait and modifier.

<table>
<thead>
<tr>
<th>Female modifier</th>
<th>Female trait (self-SRA) or male trait (mate-SRA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$TT$</td>
</tr>
<tr>
<td>$MM$</td>
<td>$D_{00}$</td>
</tr>
<tr>
<td>$Mm$</td>
<td>$D_{10}$</td>
</tr>
<tr>
<td>$mm$</td>
<td>$D_{20}$</td>
</tr>
</tbody>
</table>
Chapter 6: General discussion and conclusions

6.1 Sexual selection and population divergence in *H. americanus*

6.1.1 Major findings and implications

My observations of *H. americanus* life history behavior suggest female mate choice may strongly mediate the outcome of mating interactions (Chapter 2) (Peckham & Peckham 1889; Richman 1982). Males appeared to prioritize mate search over prey capture. They rarely engaged in hunting behavior and were never observed feeding, yet travelled widely compared to females. Males vigorously courted each adult female they encountered with a ritualized behavior that displays the colorful morphology on the anterior part of the body. By contrast, females frequently hunted and spent approximately 10% of their time feeding. During mating interactions in nature, females observed male courtship behavior but usually rejected their suitors. The results argue against the presence of selection arising from male-male interactions, given that these appear to be rare, brief and non-ritualized. Male-male interactions were never observed to escalate to direct combat. Supplementing this finding, the ecological context of breeding, although only partially documented here, also gives no indication that males would benefit by attempting to guard or fight directly over individual females. First, females as well as the prey and shelter they depend on are apparently widely distributed across the landscape throughout at least the first several weeks of the breeding season, indicating males would be unable to monopolize mating opportunities. Second, males encountered females approximately every couple of hours of activity on the surface, implying those who roam widely will have frequent mating opportunities. Overall, these results suggest female choice for male during courtship interactions represents a strong source of sexual selection on male sexual displays in *H. americanus*.

It is unclear if female choice presents a reproductive barrier between phenotypically contrasting populations. When considered together, virgin females from all three of the populations exhibit partial discrimination against foreign males, on average permitting copulation less frequently with foreign compared to local males (Chapter 3). Although this effect is small in comparisons of individual pairwise mating crosses—significantly different in only a single comparison—the generally equal or higher rank proportion of local males achieving copulation compared to foreign males suggests female rejection of foreign males might represent a modest but common source of sexual isolation between populations (e.g., Endler & Houde.
1995; Gray & Cade 2000; Seehausen et al. 2008). However, patterns of latency to copulate and spermatheca access among foreign versus local males appear to contradict this finding, showing potentially higher success of foreign males in the lab setting. This observed variation across components of mating interactions suggests the way in which mate preferences are expressed is complex. The net effect of female mate choice on male mating success depends on which morph each component of the mating interactions favors, and how strongly each one influences male fertilization success (Coyne & Orr 1989; Coyne & Orr 2004).

The mate trials uncover aspects of mate competition that may hinder gene flow among populations, and hence facilitate phenotypic differentiation between them, irrespective of the direction of female mate preferences. First, the dwindling sexual receptivity of females across subsequent copulations, together with the potentially high rate at which they encounter prospective sires, suggests each female is likely to be sexually receptive for only a brief period of the breeding season. Variation among females in receptivity across mating encounters and in patterns of spermatheca access suggests populations also differ in aspects of female mating strategies. Either factor might particularly limit foreign male mating success if these males are more likely to be temporally out of synch with female receptivity than are local males. Second, female acceptance of multiple mates likely further limits the mating success of foreign males, given that any foreign male achieving copulation will typically experience diminished paternity due to postcopulatory competition from local males. Ultimately, quantifying the degree to which these factors contribute to sexual isolation will require detailed observations of breeding ecology and dispersal rates across populations.

Although direct evidence for divergent sexual selection among phenotypically contrasting populations is presently lacking, the genetic results (Chapter 4) provide indirect evidence suggesting selection on displays or traits highly correlated with them are contributing to divergence across the study area. The population survey established several locations in the region that each appear to feature a single display morph. Despite the stark phenotypic contrast among populations, genetic divergence between them is low. None of the AFLP markers show repeated fixation of different alleles among phenotypically contrasting populations, and F:\text{ST} estimates suggest minimal divergence across putatively neutral non-outlier loci. Accordingly, the STRUCTURE analyses indicate substantial admixture among populations. These results indicate the phenotypic differences observed among populations have either arisen rapidly compared to neutral differentiation, have persisted in the face of gene flow, or both (Coyne &
Orr 1989; Gray & Cade 2000). Each of these scenarios implies the action of divergent selection correlated with sexual displays.

The genetic data also indicate that selection is likely acting in the face of gene flow, and hence is strong enough to maintain phenotypic differences among populations despite the exchange of genes between them. Support for the action of gene flow comes from significant isolation by distance (IBD) in each type of among-morph comparison across the study area—a predicted outcome of the diminishing effect of gene flow between populations distributed across a landscape compared to genetic drift within them as the geographic distance between populations increases (Kimura & Weiss 1964; Wright 1943). I also detected a tendency for phenotypically similar populations to be more closely genetically related, suggesting either retention of historically shared genetic variation between them (i.e., from a shared origin of phenotypically similar populations) or the buildup of this association following the separate origin of each population. Considering the presently intermingled distribution of phenotypically contrasting populations throughout the region, this result combined with the pattern of IBD suggests that differentially greater gene flow has occurred between phenotypically similar populations.

6.1.2 Future research directions

The results presented here are consistent with the idea that selection promotes divergence in _H. americanus_ sexual displays. However, the main sources of selection affecting population differentiation remain to be identified. Field observations and mate trials suggest female choice could be a strong source of prezygotic isolation, but data on sperm transfer rates and paternity will be required to determine if mating interactions ultimately translate into a local or foreign male reproductive advantage.

A more comprehensive assay of female fecundity following sexual encounters with local versus foreign males is also needed in order to assess whether postzygotic barriers exist among populations. Interestingly, although postzygotic isolation might stem from intrinsic reproductive barriers, the close genetic relationships that I observed among collection locations as well as high early offspring survival rates across most pairwise crosses suggest that any existing postzygotic barriers to hybridization are most likely to manifest in adult hybrids, possibly via a mating disadvantage—this form of hybrid disadvantage would represent a second means by which sexual selection might promote prezygotic isolation (Coyne & Orr 1989). Mate trials conducted
on hybrid offspring will shed light on this issue, and offspring from these matings could be used to further test for long-term intrinsic fitness costs of among-morph matings in the form of lowered hybrid fecundity or F2 hybrid fitness disadvantages. Finally, extrinsic limits to hybridization of phenotypically contrasting populations might also exist, underscoring the need to verify fitness differences in a natural setting, or at least to identify and experimentally test the effects of ecological differences among phenotypically divergent populations.

6.2 Sex ratio adjustment in the presence of sexually antagonistic selection

6.2.1 Major findings and implications

The models indicate sex ratio adjustment (SRA) can evolve in the presence of intralocus sexual conflict (IASC) by directing fitness benefits toward the sex that will profit from them, thereby helping to alleviate intralocus sexual conflict. Further, this sex allocation strategy can evolve under autosomal or sex-linked inheritance of the sexually antagonistic trait.

Two of the model results confirm previous theory regarding constraints on the evolution of SRA. First is the conjecture of Fisher (1930) that selection should favor a 1:1 population-wide sex ratio. This result dominated the outcome of most of the models, with SRA evolving only if females and males remained at roughly equal frequencies—that is, provided the sex ratio of broods were skewed toward males and females in equal frequency across all parents in the population. The second finding concerns the only exception to the maintenance of Fisherian sex ratios that was detected, exposing a major genetic constraint on the stability of SRA that was first identified by Hamilton (1967). This occurs when the SRA modifier locus is sex-linked (located on the X- or Z chromosome) and SRA is under the control of the heterogametic sex (XY males or ZW females). In this case, selection favoring transmission of the SRA modifier itself results in the meiotic drive of strong SRA favoring the homogametic sex (e.g., favoring ZZ sons if ZW females control SRA), leading to highly skewed sex ratios and potential population extinction regardless of the degree of sexual antagonism.

The models also expose a previously unknown genetic constraint on SRA evolution. In the case of sex-linked inheritance of the sexually antagonistic trait and an autosomal sex ratio modifier, the evolution of substantial SRA is limited to scenarios in which the genotype of the homogametic sex (XX females or ZZ males) is used as the basis for SRA decisions. The explanation for this outcome is that reference to the heterogametic sex (i.e., to the father’s genotype in XY species or the mother’s genotype in ZW species) introduces transmission
distortion in favor of the sexually antagonistic allele that benefits the homogametic sex. This occurs because the modifier allele stimulates transmission of the Y- or W- chromosome, instead of the sexually antagonistic allele itself, whenever the reference type is considered fit.

The predictive ability of traditional sex allocation theory has in general been variable (Charnov 1982; Clutton-Brock & Iason 1986; Cockburn 2002; Sheldon 1998), raising the possibility that additional forces or constraints on the evolution of SRA have yet to be identified (Cockburn 2002; Krackow 1995; West 2009; West et al. 2002). Our results suggest that intralocus sexual conflict might account for some of the previously unexplained variation in sex allocation strategies in nature, a claim that is corroborated by recent examples from nature in which SRA appears to diminish sexually antagonistic fitness effects (lizards, Calsbeek & Bonneaud 2008; fruit flies, Connallon & Jakubowski 2009; beetles, Harano et al. 2010; owls, Roulin et al. 2010). These findings also advance SRA as a novel means to alleviate IASC, supplementing or replacing the evolution of sexual dimorphism.

6.2.2 Future research directions

The prevalence of SRA in nature owing to IASC is currently unknown. With numerous feasible models of SRA evolution in existence, experiments that alter factors specific to individual models and track SRA evolution could in principle separate the effects of each one. However, in cases where observing evolution in action is impractical, an alternative approach could be to quantify fitness effects of the factors and determine the strength of SRA promoted by each. It is possible that some cases where SRA has been confirmed to occur, as well as cases where SRA was expected but lacking, might be better explained by the presence or absence, respectively, of IASC. Further, species exhibiting clear examples of IASC might provide useful systems to assess the presence of SRA, particularly where closely related groups lacking sexually antagonistic effects are available for comparative purposes.
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Appendices

Appendix A Supplementary material for Chapter 2

A.1 Estimating population densities within habitat patches

During May, 2006, I divided the study grids established in 2005 (see Methods) into 2m × 100m transects. I also re-oriented the *H. americanus* grid in order to ensure it encompassed optimal habitat, as judged from spider surface activity in 2005. The re-oriented grid area covered habitat that was contained within the 2005 searchable area (see Methods); thus, it improved the accuracy of survey estimates without compromising their relevance to the 2005 data. A transect from each grid was randomly chosen for survey once every hour between 08:00 and 12:00 on relatively clear, calm days. The survey procedure consisted of walking at a slow, standardized pace along a transect and recording the age category (adult, subadult, or immature) and sex (adults and subadults only) of all observed jumping spiders. Temperature (degrees Celsius) and humidity (percent) were recorded at approximately 2 cm height above the ground surface, as well as an additional temperature reading 2 cm below the ground surface, immediately prior to the start of each transect survey at a single location next to each grid. Qualitative wind and sky condition estimates were also made at this time. Wind condition categories were: zero (completely calm); light (short grasses move slightly); moderate (short grasses tremble and tree boughs move); strong (short grasses shake and tree trunks sway). Sky condition categories were: clear (unobstructed sunlight); haze (thin haze in front of sun); and overcast (cloud in front of sun). Adult female and male population sizes for each habitat patch were estimated by multiplying the number of adult males and females observed in each survey by the number of transects within each study grid and the area of each habitat patch relative to its respective survey grid. To estimate the area of each habitat patches relative to the survey grid it contained I traced the perimeter of the patches and grids onto a digital aerial photograph obtained from Google Earth (v. 6.1.5; Google Inc.). I imported the traces into Adobe Photoshop (v. 6.0, Adobe Systems, San Jose, CA), used the “Histogram” function to determine the area (in pixels) of each trace, and divided each habitat patch area by its respective study grid area.

The estimated habitat patch areas are 6500 m² and 900 m² for *H. americanus* and *H. ophrys*, respectively. Surveys were conducted on May 9, 15, 16, and 19, 2006, producing 15 population estimates within four days on each grid (Table A.1). Weather conditions during the surveys were approximately the same as those occurring during the 2005 study (see Results). In our view, the maximum adult population density estimates deriving from this survey (*H. americanus*: 163...
females, 98 males; *H. ophrys*: 14 females, 37 males) represent minimum actual population sizes, given that I likely overlooked some spiders that were active on the surface during surveys and also potentially missed periods of peak surface activity.
Table A.1. Jumping spider study grid survey results at Iona Beach during May, 2006. Indicated are the number of spiders observed per square meter and weather conditions associated with each transect sample. (a) *Habronattus americanus* (b) *H. ophrys.*

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Adult</th>
<th>Subadult</th>
<th>Immature</th>
<th>Sky conditions</th>
<th>Wind speed</th>
<th>Humidity (%)</th>
<th>Air temperature (ºC)</th>
<th>Ground temperature (ºC)</th>
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<td>Subadult</td>
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<td>Wind speed</td>
<td>Humidity (%)</td>
<td>Air temperature (ºC)</td>
<td>Ground temperature (ºC)</td>
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<td>Clear Zero</td>
<td>Haze</td>
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<td>19</td>
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<td></td>
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<td>23</td>
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</tbody>
</table>
A.2 Description of male courtship behavior

Male courtship behavior qualitatively resembled that which has been observed during staged lab trials (GSB, unpublished data; see video in Maddison 1995; Maddison & Stratton 1988). Courtship details differ among *H. americanus* and *H. ophrys* but the basic movements are similar and can be divided into four phases. In the first phase, the male faces the female, holds his first legs out in front of him, and exposes his chelicerae to her by spreading his pedipalps apart. In the second phase, the male sidles from side to side while waving his pedipalps, moving closer to the female as he sidles. When the two are about one body length apart the male enters a third phase—he adopts a stationary stance and flicks his first legs several times toward the female while rhythmically moving his abdomen up and down. The leg and abdominal movements may produce sonic signals, as has been observed in other *Habronattus* species (Elias et al. 2006; Elias 2003; Maddison & Stratton 1988). If the female remains stationary, the male mounts (fourth phase) by climbing over her head and onto her back. The remaining mating interaction details derive from lab observations of *H. americanus* (GSB; unpublished data), and accord with the single copulation observed in *H. ophrys* in the present study. The mounted male uses his anterior pair of legs to sweep one of the female’s posterior legs upward and to pivot the ventral surface of her abdomen toward him. He then copulates by inserting the tip of one pedipalp into one side of her epigynum. After about 10-30 sec he withdraws his pedipalp and usually attempts to copulate again by releasing her abdomen, sweeping the female’s other posterior leg upward, and inserting his second pedipalp into the other side of her epigynum. At least in *H. americanus*, the male’s two posterior legs jitter during the course of phase four, possibly representing additional signaling. A female can apparently curtail or entirely prevent copulation by keeping her posterior legs on the substrate and refusing to allow her abdomen to pivot or, alternatively, by wriggling until the male dismounts.
Appendix B  Supplementary material for Chapter 3

B.1  Geographic distances between populations.

Table B.1. Approximate distance (km) among populations.

<table>
<thead>
<tr>
<th></th>
<th>P1a</th>
<th>P1b</th>
<th>P2</th>
<th>PL2</th>
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<td></td>
<td></td>
</tr>
<tr>
<td>P1b</td>
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<td></td>
</tr>
<tr>
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<td>30</td>
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B.2 Additional analyses of mating success

Figure B.1. Proportion of males of each morph achieving copulation. (a) Virgin lab-reared females (b) mated lab-reared females (c) fertilized field females. Error bars denote 95% Agresti-Coull confidence intervals. Colors signify the type of male morph occurring at the population where male and female spiders were collected: P (blue), PL (orange), PLC (red). Numbers associated with bars are sample sizes.
Table B.2. Fisher’s exact tests comparing the proportion of males to copulate across pairs of morphs. All pairwise combinations of male morphs are compared within each female type. The p-value in bold is at the Bonferroni corrected alpha level for nine tests (α = 0.006).

<table>
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<th>Male morph comparison</th>
<th>p</th>
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<tr>
<td>P</td>
<td>P-PL</td>
<td>0.115</td>
</tr>
<tr>
<td></td>
<td>P-PLC</td>
<td>0.240</td>
</tr>
<tr>
<td></td>
<td>PL-PLC</td>
<td>1.000</td>
</tr>
<tr>
<td>PL</td>
<td>P-PL</td>
<td>0.407</td>
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<tr>
<td></td>
<td>P-PLC</td>
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<td></td>
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<td><strong>0.006</strong></td>
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<tr>
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<td>PL-PLC</td>
<td>0.484</td>
</tr>
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Figure B.2. Least-squares linear regression of latency to copulate (sec) with virgin females versus Julian test date. A significant positive relationship exists between these factors and was statistically controlled in the GLM model of latency to copulate (see Table 3.3). Colors denote the type of male morph occurring at each female’s population of origin: P (blue), PL (orange), PLC (red).
Figure B.3. Boxplots of latency to copulate across male morphs. (a) There is no significant difference between local and foreign males with respect to female type for virgin lab-reared females. (b) Mated lab-reared females were not formally analyzed due to low sample sizes. Whiskers denote 1.5 interquartile ranges above and below the third and first quartiles, respectively. Colors are as in Figure B.1. Numbers associated with boxplots are sample sizes.
Table B.3. Marginal means from a GLM of male latency (seconds) to copulate with virgin lab-reared females. Different letters in the right-hand column signify non-overlapping Wald 95% confidence intervals among pairwise type comparisons.

<table>
<thead>
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<th>Female morph</th>
<th>Male morph</th>
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<th>Confidence interval</th>
<th>Levels of overlap</th>
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<tr>
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<td>P</td>
<td>4.068 (0.079)</td>
<td>3.912 - 4.224</td>
<td>b</td>
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<tr>
<td>PLC</td>
<td>P</td>
<td>3.682 (0.078)</td>
<td>3.529 - 3.834</td>
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<td>P</td>
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<td>3.770 - 4.112</td>
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<td>P</td>
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<td>PL</td>
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Figure B.4. Proportion of first mounts in which males of each morph accessed two spermatheca. Error bars denote 95% Agresti-Coull confidence intervals. (a) Virgin lab-reared females (b) mated lab-reared females. Colors are as in Figure B.1. Numbers associated with bars are sample sizes.

Table B.4. Fisher’s exact tests of proportion of local versus foreign males to access both spermatheca. Results from mate trials with each female type are shown.

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B.3 Additional analyses of 2007 postmating success

Summary egg lay statistics and criteria for female inclusion in hatch success model

Mean latency to lay eggs following copulation was 30 ±10 (SD) days (n = 37) across all lab-reared females (range: 15-52 days). The different maturation dates of lab-reared females resulted in a wide range of time spent in laboratory simulated ‘summer’ environmental conditions between the date of their final mate trial and that of the imposed winter diapause (range: 4-188 days). As a result, I assessed egg production of each female within the 60 day period following her final mate trial, a time span which encompasses three standard deviations above the mean latency to lay across all females. Twenty-four females who either died or entered diapause prior to 60 days following their mate trial were excluded from the analyses. Out of the remaining 162 females who copulated during mate trials, 23.4% laid eggs and 16.7% reared eggs successfully to hatch, and these included representatives from all pairwise mating combinations (Figure 3.4). Mean clutch size across all females was 4.6 ±2.4 (SD) eggs (range: 1-7).

![Figure B.5](image)

**Figure B.5.** Logistic plot of female tendency to lay and hatch eggs versus Julian test date in 2007. The plot partitions the probability of each hatch state (no: below line; yes: above line) at each Julian date. A positive relationship is apparent between these factors. Colors are as in Figure B.2.
Appendix C Supplementary material for Chapter 4

C.1 Primer pair search

Table C.1. Primer pairs tested but not chosen for selective amplification. Each pair was judged to exhibit insufficient band variation among samples to be informative in population genetic analyses. (a) All combinations using EcoRI (CACGACTTTGTTAAAACGACGACTGCGTACCAATTC+) (b) most combinations using PstI (see Methods).

(a)

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C.2 AFLP template preparation and reactions

All samples were processed in random order and blind with respect to morph and population. A subset of the samples was processed twice from original tissue to permit an assessment of the reproducibility of AFLP bands within samples across the entire protocol. These replicate samples represented individuals from each field population and were processed together with the regular samples.

Genomic DNA was extracted from 2-4 adult male legs per specimen using a Puregene DNA Purification Kit (Gentra Systems, Inc.) and visualized on 1.5 % agarose gels. Only samples showing clean extraction profiles were retained. Template was processed using a protocol adapted by C. E. Ritland from Remington et al. (1999).

Digestion was performed for 3 hours at 37°C in 30.0 µl volumes consisting of 6.0 µl of 5X R/L buffer, 1.0 µl of Pst1 (12 U/µl), 2.0ul of Mse1 (4 U/ µl), 5.0-20.0 µl of DNA (25 ng/ µl), and distilled water. Adapter ligation was performed for 3 hours at 37°C in 25.0 µl volumes consisting of 0.5 µl of Pst1 adapter (5 pmol/µl), 0.5 µl of Mse1 adapter (50 pmol/µl), 0.5 µl of ATP (10 mM), 1.0 µl of 5X R/L buffer, 2.0 µl of distilled water, 20.0 µl of digested DNA, and 0.5 µl of T4 DNA ligase (0.5 Weiss U). This was diluted to 10 % concentration with distilled water.

Pre-amplification was performed for 3 hours at 37°C in 30.0 µl volumes consisting of 6.0 µl of 5X R/L buffer, 1.0 µl of Pst1 (12 U/µl), 2.0ul of Mse1 (4 U/ µl), 5.0-20.0 µl of DNA (25 ng/ µl), and distilled water. Adapter ligation was performed for 3 hours at 37°C in 25.0 µl volumes consisting of 0.5 µl of Pst1 adapter (5 pmol/µl), 0.5 µl of Mse1 adapter (50 pmol/µl), 0.5 µl of ATP (10 mM), 1.0 µl of 5X R/L buffer, 2.0 µl of distilled water, 20.0 µl of digested DNA, and 0.5 µl of T4 DNA ligase (0.5 Weiss U). This was diluted to 10 % concentration with distilled water.

Pre-amplification was performed to create Pst1+A and Mse1+A adapter extensions by combining 5.0 µl of ligation mixture with 0.6 µl of Pst1 primer with an M13 tail (50 ng/µl), 0.6 µl of Mse1 primer (50 ng/µl), 0.8 µl of 10X dNTP mixture, 2.0 µl of 10X PCR buffer (Roche), 0.24 µl of Taq polymerase (5 U/µl) and 10.76 µl of distilled water. Denaturation was run at 94°C for 1 min. Annealing was run for 28 cycles of 94 °C (0.5 min.), 60 °C (0.5 min.), and 72 °C (1.0 min.). Extension was run at 72 °C for 5.0 min.

Selective amplification added 2- or 3-base extension to the Pst1 adapter and a 4-base extension to the Mse1 adapter by combining 2.5 µl of pre-amplified template with 0.42 µl of M13-labelled primer (1 pmol/µl), 0.42 µl of TPst1+###(#) primer (6 ng/µl), 0.42 µl of Mse1+#### primer (6 ng/µl), 0.4 µl of 10X dNTP mixture, 1.0 µl of 10X PCR buffer, 4.72 µl of distilled water, and 0.12 µl of Taq polymerase (5 U/µl). Denaturation was run at 94 °C for 1 min. and 5 cycles of 94 °C (0.5 min.), 65 °C (0.5 min.), 72 °C (1 min.). Annealing was run for 12 cycles of 94 °C (0.5 min.), 65C (0.5 min.) - 0.7 °C per cycle, and 72 °C (1.0 min.), followed
by 23 cycles of 94 °C (0.5 min.), 56 °C (0.5 min.), 72 °C (1 min.). Extension was run at 72 °C for 5.0 min.

Following final amplification, 4.0 µl of formamide loading buffer was added to each reaction product. Samples were run on 66-lane 7% Long Ranger acrylimide gels on LiCor 4200 autosequencers for 4.5 hours under the following parameter settings: speed = 4; voltage = 2000 V; current = 35 mA; power = 70 W; temperature = 50 °C. Samples were arranged on the gels in random combinations of up to three sets. I included in each gel run a negative control (distilled water processed in the same manner as the actual DNA samples) in order to monitor for potential gel contamination, a positive control (a single, arbitrarily chosen DNA sample, processed in the same manner as the other DNA samples) to help assess signal quality variation among gels, and a fluorescent ladder in at least two lanes to assign marker sizes.

C.3 AFLP band scoring protocol and marker selection

All gels were visualized and SAGA with “sample image width” set at 5 and “pixels per base pair” set at 1. Band peaks signify the reflection intensity of bands above background gel intensity levels. The relationship between peak size and band intensity is constant at all regions of the gel. Peak intensities are therefore directly comparable at different marker sizes, as well as among different samples and gels.

Peaks ranged in intensity approximately across the full 20-pixel range within each lane. Because band intensity was difficult to systematically judge relative to background reflectance levels within each sample, I used the peak intensity profiles for the bands to score peaks as present or absent. The peaks are highly correlated with band intensity, except for bands producing a saturated signal (i.e., clearly “present”). To minimize measurement error in this procedure I partly automated it by performing an initial score using an original script written in R (below). I subsequently hand-checked all potentially useful markers.

Gel image acquisition

Gel images for all samples, including repeated runs, of a given primer pair were generated and aligned vertically according to fragment length in SAGA. Vertical “bin” lines were superimposed over AFLP bands across the entire range of the gel wherever bands were present. The function of the bin lines at this stage was to facilitate accurate gel alignment. The composite
image was then exported as a jpeg file in two formats (Figure C.1): one showing the gel image together with its associated peak height profiles; one showing only the peak height profiles.

**Figure C.1.** Partial gel images showing two alternative views of the complete gel range of a single primer pair. The upper panel shows band reflectance intensities on a gel with overlain peak reflectance profiles. The lower panel shows peak reflectance profiles with coloured bars over automatically scored reflectance peaks. Numbers in the left margin indicate sample identification codes and those above each image indicate the fragment length (in base pairs) of each marker. Vertical green lines were used to align all gel images for a given primer pair and markers greater than 100bp in length were subsequently used to initially score profiles for potentially informative AFLP markers. Coloured bars indicate peak height scores (in pixels) assigned to the peak profiles during automated scoring: red (2), green (3), blue (4), pink (5 or more).
**R script for automated gel scoring**

The following R program, written by Rich Fitzjohn, was used to assign initial scores to band peaks that occurred along bin lines:

```r
library(rimage)
library(png)

## Reads in the matrix as rows x cols x rgb matrix
img <- read.jpeg(file.choose())

## Identify which vertical columns of pixels correspond to "bins":
bins <- which(apply(img[40,,], 1, min) > 0.1)

## Gracefully subset an image (e.g., horizontal lanes), preserving
## attributes.
sub <- function(x, r, c=1:dim(x)[2]) {
  tmp <- x[r, c, ]
  class(tmp) <- class(x)
  attr(tmp, "type") <- "rgb"
  tmp
}

## These are the different horizontal band beginnings.
## The first is the first row (from the top) containing "data" (not
## heading). The second is the approximate end of the data
## region.
## The third is the height of each band of data. This splits the
## image into horizontal lanes (img.sub), then computes the y
## position of the
## greatest difference between the blue and red channel (y).
i.from <- seq(81, which(img[,1,1] > 0.5)[1], 20)
img.sub <- lapply(i.from, function(i) sub(img, i:(i+19)))
f <- function(x)
  apply(x[,3] - x[,1], 2, which.max)
y <- 20 - t(sapply(img.sub, f))

## This scores the peaks. Look dx.look pixels around the bin for the
## true top of a peak. From this position, compute the height of the
## line dx.cmp pixels forward and backwards. Rules are applied to
## these numbers.
```
score <- function(bin, y, dx.look=2, dx.cmp=6) {
    ## First, identify all x positions around the bin worth looking in.
    x <- (bin-dx.look):(bin+dx.look)

    ## Find the x position within the region around the bin where the y value is maximised. In some cases there are multiple trace pixels at the maximum
    ## height so we include instructions to choose the pixel the closest
    ## to the true bin in that case
    f <- function(z) {
        i <- x[which(z == max(z))]
        i[which.min(abs(i - bin))]
    }
    at <- apply(y[,x], 1, f)

    ## The cbind() makes a "array index"; each row will be one element
    ## extracted from 'y'. The first element is the row index, the second is the column.
    peak.height <- numeric(nrow(y))
    for (i in seq_len(nrow(y)))
        peak.height[i] <- y[i, at[i]]
    peak.height <- y[cbind(1:nrow(y), at)]

    ## This repeats the above logic, but looking ahead and behind from
    ## the peak value.
    drop.l <- peak.height - y[cbind(1:nrow(y), at - dx.cmp)]
    drop.r <- peak.height - y[cbind(1:nrow(y), at + dx.cmp)]
    drop.min <- pmin(drop.l, drop.r)
    drop.max <- pmax(drop.l, drop.r)

    ## Rules for assigning a peak score. Two-pixel height (2), Three (3), Four (4)
    ## Five+ (5)
    ret <- rep("0", nrow(y)) # Assume "0" unless
    ret[drop.max == 2 & drop.min == 2] <- "2" # in which case call it "2", unless
    ret[drop.max >= 3 & drop.min >= 0] <- "3" # in which case call it "3", unless
    ret[drop.max >= 4 & drop.min >= 0] <- "4" # in which case call it "4", unless
    ret[drop.max >= 5 & drop.min >= 0] <- "5" # in which case call it "5".
}
```r

## This is a height(horizontal lanes) x length(bins) matrix of scores.
res <- lapply(bins, score, y)
res.score <- sapply(res, function(x) x$score)
res.height <- sapply(res, function(x) x$score)
res.at <- sapply(res, function(x) x$at)

img2 <- img
pos <- which(res.score == "2", TRUE) ## red
for ( i in 1:nrow(pos) ) {
  img2[(pos[i,1]+5):(pos[i,1]+15),pos[i,2],1:3] <- 0
  img2[(pos[i,1]+5):(pos[i,1]+15),pos[i,2],1] <- 1
}

pos <- which(res.score == "3", TRUE) ## green
for ( i in 1:nrow(pos) ) {
  img2[(pos[i,1]+5):(pos[i,1]+15),pos[i,2],1:3] <- 0
  img2[(pos[i,1]+5):(pos[i,1]+15),pos[i,2],1] <- 1
}

pos <- which(res.score == "4", TRUE) ## dark blue
for ( i in 1:nrow(pos) ) {
  img2[(pos[i,1]+5):(pos[i,1]+15),pos[i,2],1:2] <- 0
  img2[(pos[i,1]+5):(pos[i,1]+15),pos[i,2],3] <- 1
}

pos <- which(res.score == "5", TRUE) ## pink
for ( i in 1:nrow(pos) ) {
  img2[(pos[i,1]+5):(pos[i,1]+15),pos[i,2],2:3] <- 0
  img2[(pos[i,1]+5):(pos[i,1]+15),pos[i,2],3] <- 1
}

writePNG(img2, "scored_image_out.png")
write.csv(res, "scored_data_out.txt")
```

The program scores gel images (upper panel in Figure C.1) by scanning along the top (black) region of the image until it encounters a bin line, at which point it proceeds vertically downwards, scoring each encountered peak line according to a set of pre-defined rules, until it reaches the bottom of the image. It then returns to the top of the image and proceeds to the next bin line. Scores are indicated as coloured bars over the peak profiles in a new image file, and are also output numerically to a text file.

The user-defined rules consist of the range (in pixels) to search on either side of the bin line for a maximum peak height (tip), and a distance on either side of the tip from which to estimate the peak height. Peak height was measured by calculating the vertical drop from the maximum peak height to the profile baseline at an established distance on either side of peak. The precise search values chosen for this distance are not critical, so long as they remain consistent across all samples and generally capture peak heights for all individual markers. In the present study, hand-checking of test runs indicated that a 2-pixel search range on either side of the bin line for maximum peak height, followed by a 6-pixel peak height measurement distance detected and measured peaks satisfactorily.

We expressed peak score in terms of their height (in pixels) above baseline reflectance levels. Admittedly, this scale is of lower than optimal resolution for precisely judging peak heights but pixels were the smallest available unit of measure in the gel image files. I note that while this scale is likely to diminish the precision of the peak height estimates, it is not expected to bias peak height estimates.

We were particularly interested in objectively scoring the smaller peaks to determine if they carried true signal about AFLP band presence, signified by repeatable peaks across replicated samples. To do this I assigned all peaks a score between 2 to 5, representing peaks ranging in height from 2 to 5 or more pixels, respectively (approximately 10% to 25% or more, respectively, of the 20-pixel lane width of each sample). I limited the scores to this range of pixel heights because I judged any peak with a height lower than 2 pixels above baseline to represent noise and with a height greater than 5 pixels as being clearly present (i.e., not requiring quantification more accurate than the score “5”). This set of rules limited the coloured peak score bars to an easily manageable set for hand-checking.
**Marker selection for hand checking**

Overall AFLP marker quality was assessed according to gel brightness and to whether or not bands could be easily distinguished from nearby markers. Gel regions that were too dark (e.g., all markers below marker 218 in the upper panel of Figure C.1) or too faint (e.g., marker 561) to evaluate were ignored, as were markers with peaks that were difficult to distinguish (e.g., marker 478).

To determine a band presence threshold I inspected the frequency distribution of peak heights for each marker. I established the threshold for “present” status to be the peak height above which there was a visible increase in frequency across the samples for each marker; peak heights below this threshold were scored as “absent” (Bonin et al. 2004). This threshold typically occurred abruptly at a height of 15% of the peak profile range (i.e., 3 pixels or greater; see examples in Figure C.2a), which I adopted as the peak height threshold. Markers with greater than 10% of peaks within the 2-pixel category or lacking a mode of peak frequencies visibly distinct from zero (Figure C.2b) were discarded (20% of 339 markers). The mean proportion of peak scores per marker adjusted to absent (i.e., lying below the threshold) across the retained 268 markers was 2.6%.

Many of the bin lines used to align the gel images (Figure C.1) clearly either represented monomorphic markers, or markers that would be difficult to score. Many of these were ignored and I hand-checked only those markers that produced error rates from the automated scoring procedure of less than 15% across replicated samples as these generally showed clear bands that appeared appropriate for scoring (a few candidate markers that showed greater than 15% error apparently due to image misalignment or that produced very clear bands were also hand checked).
Figure C.2. Examples of sample peak height frequency distributions for different AFLP markers. (a) Two distributions showing a mode distinct from zero and indicating a “presence” threshold at peak score of 3 pixels above baseline. The upper distribution shows high observed band presence frequencies, while the lower distribution shows among the lowest observed band presence frequencies; (b) a distribution lacking a mode distinct from zero and subsequently discarded.
C.4 **STRUCTURE** analysis results.

**Table C.2.** Summary of ΔK analyses for different marker sets. Maximum ΔK values within each set signify the cluster number with greatest support.

| AFLP marker class | K   | Mean LnP(K) | Standard Deviation LnP(K) | Ln'(K) | |Ln''(K)| |ΔK   |
|-------------------|-----|-------------|---------------------------|--------|--------|--------|-------|
| All markers       | 1   | -7972.42    | 0.04                      | —      | —      | —      | —     |
|                   | 2   | -7606.88    | 5.71                      | 365.55 | 293.07 | 51.30  |       |
|                   | 3   | -7534.40    | 9.27                      | 72.48  | 55.68  | 6.01   |       |
|                   | 4   | -7517.61    | 22.71                     | 16.80  | 8.24   | 0.36   |       |
|                   | 5   | -7509.05    | 24.56                     | 8.56   | 49.56  | 2.02   |       |
|                   | 6   | -7550.05    | 59.50                     | -41.00 | 57.41  | 0.96   |       |
|                   | 7   | -7533.64    | 33.66                     | 16.41  | 132.58 | 3.94   |       |
|                   | 8   | -7649.81    | 23.83                     | -116.17| 185.76 | 7.80   |       |
|                   | 9   | -7951.74    | 202.45                    | -301.93| 260.35 | 1.29   |       |
|                   | 10  | -7993.32    | 333.05                    | -41.58 | —      | —      | —     |
| Outliers          | 1   | -495.74     | 0.08                      | —      | —      | —      | —     |
|                   | 2   | -352.73     | 0.97                      | 143.02 | 102.32 | 105.01 |       |
|                   | 3   | -312.03     | 8.94                      | 40.70  | 37.60  | 4.21   |       |
|                   | 4   | -308.93     | 14.73                     | 3.10   | 8.50   | 0.58   |       |
|                   | 5   | -314.33     | 12.25                     | -5.40  | 10.27  | 0.84   |       |
|                   | 6   | -309.46     | 11.63                     | 4.87   | 4.15   | 0.36   |       |
|                   | 7   | -308.74     | 2.63                      | 0.72   | 0.49   | 0.19   |       |
|                   | 8   | -307.53     | 3.92                      | 1.21   | 0.94   | 0.24   |       |
|                   | 9   | -307.25     | 8.19                      | 0.28   | 10.39  | 1.27   |       |
|                   | 10  | -317.36     | 11.42                     | -10.11 | —      | —      | —     |
| Non-outliers      | 1   | -7476.82    | 0.13                      | —      | —      | —      | —     |
|                   | 2   | -7266.33    | 2.21                      | 210.50 | 177.97 | 80.60  |       |
|                   | 3   | -7233.80    | 14.18                     | 32.53  | 32.16  | 2.27   |       |
|                   | 4   | -7233.43    | 12.95                     | 0.37   | 1.69   | 0.13   |       |
|                   | 5   | -7231.38    | 47.54                     | 2.06   | 11.74  | 0.25   |       |
|                   | 6   | -7217.59    | 24.30                     | 13.79  | 293.61 | 12.08  |       |
|                   | 7   | -7497.40    | 158.95                    | -279.82| 262.69 | 1.65   |       |
|                   | 8   | -7514.53    | 120.84                    | -17.13 | 118.86 | 0.98   |       |
|                   | 9   | -7650.51    | 306.66                    | -135.99| 246.99 | 0.81   |       |
|                   | 10  | -8033.49    | 184.15                    | -382.98| —      | —      | —     |
Appendix D  Supplementary material for Chapter 5

D.1 Developing recursion equations

To illustrate how the recursions were developed for an SRA modifier in the face of sexually antagonistic selection, we focus on the case of X linkage, which is simpler to display than the autosomal case. Table D.1 lists the available genotypes in the population, in terms of the SRA modifier (M) and sexually antagonistic trait (T), when loci are X-linked.

Table D.1. Female and male genotypes with X-linked trait and modifier loci.

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<th>Female genotype</th>
<th>Male genotype</th>
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<td>$x_1 = TM/TM$</td>
<td>$y_1 = TM$</td>
</tr>
<tr>
<td>$x_2 = TM/tM$</td>
<td>$y_2 = tM$</td>
</tr>
<tr>
<td>$x_3 = tM/tM$</td>
<td>$y_3 = Tm$</td>
</tr>
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<td>$x_4 = TM/Tm$</td>
<td>$y_4 = tm$</td>
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</tr>
<tr>
<td>$x_8 = Tm/Tm$</td>
<td></td>
</tr>
<tr>
<td>$x_9 = Tm/tm$</td>
<td></td>
</tr>
<tr>
<td>$x_{10} = tm/tm$</td>
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</tr>
</tbody>
</table>

We assume natural selection or sexual selection acts only at $T$. The frequency of each female genotype after selection and before mating is determined by its relative fitness

$$x_n^s = x_n W_n / \overline{W}_f,$$

where $W_n$ is the fitness of genotype $x_n$ and

$$\overline{W}_f = x_1 + x_2 (1 - h_f s_f) + x_3 (1 - s_f) + x_4 + x_5 (1 - h_f s_f) + x_6 (1 - h_f s_f) + x_7 (1 - s_f) + x_8 + x_9 (1 - h_f s_f) + x_{10} (1 - s_f)$$

is the mean fitness across all female genotypes. For example, the frequency of $TM/tM$ after selection becomes $x_2^s = x_2 (1 - h_f s_f) / \overline{W}_f$. The frequency after selection of each male genotype is similarly given by $y_n^s = y_n W_n / \overline{W}_m$, with a mean male fitness of

$$\overline{W}_m = y_1 (1 - s_m) + y_2 + y_3 (1 - s_m) + y_4.$$

We assume that, once selection has acted on the genotypes, parents mate randomly, produce offspring, and die. Thus, recursion equations representing the frequency of each genotype in
females following one generation of reproduction ($x'_1$) consist of the sum of all pairwise parental gametic combinations that can produce that genotype, subject to the rate of recombination ($r$) between $\mathbf{M}$ and $\mathbf{T}$ in mothers during gametogenesis and the probability $D_{ij}$ that mothers with genotype $i$ and referring to genotype $j$ (either her own [self-SRA] or her mate’s [mate-SRA] genotype) produce a daughter (Table 5.2). For example, in the case of mate-SRA, $\text{TM/TM}$ daughters are produced by the adult mating combinations $\text{TM/TM} \times \text{TM}$, $\text{TM/tM} \times \text{TM}$, $\text{TM/TM} \times \text{TM}$, $\text{TM/TM} \times \text{TM}$ and $\text{Tm/tM} \times \text{TM}$ at a total frequency of

$$x'_1 = x'_1 y'_1 D_{00} + \frac{1}{2} x'_2 y'_1 D_{00} + \frac{1}{2} x'_1 y'_1 D_{10} + \frac{1}{2} x'_2 (1 - r) y'_1 D_{10} + \frac{1}{2} (x'_d r) y'_1 D_{10}$$

Similarly, $\text{TM/tM}$ daughters occur at a frequency of

$$x'_2 = x'_3 y'_1 D_{00} + x'_1 y'_2 D_{01} + \frac{1}{2} x'_2 y'_1 D_{00} + \frac{1}{2} x'_3 y'_1 D_{10} + \frac{1}{2} x'_2 y'_2 D_{10} + \frac{1}{2} y'_1 (1 - r) D_{10} + \frac{1}{2} (x'_d r) y'_1 D_{11}$$

The same logic applies to males. For example, $\text{TM}$ sons occur at a frequency of

$$y'_1 = x'_1 y'_1 (1 - D_{00}) + y'_2 (1 - D_{01}) + y'_3 (1 - D_{02}) + y'_4 (1 - D_{03}) + \frac{1}{2} x'_5 (1 - D_{00})$$

$$+ y'_2 (1 - D_{01}) + y'_3 (1 - D_{02}) + y'_4 (1 - D_{03}) + \frac{1}{2} x'_5 (1 - D_{00}) + y'_2 (1 - D_{01}) + y'_3 (1 - D_{02}) + y'_4 (1 - D_{03})$$

The same method is used to generate recursion equations for the remaining eight female genotypes and 3 male genotypes listed in Table D.1. Analogous methods are used to construct recursion equations for $\text{Z}$-linked $\mathbf{M}$ and $\mathbf{T}$ loci, but in this case the genotypes listed in Table D.1 are switched between sexes. Finally, in the case of autosomal loci, both male and female genotypes resemble those listed for females in Table D.1 and recombination occurs in both parents. All recursion equations are presented and analyzed in the accompanying Mathematica 6.0 file (http://www.zoology.ubc.ca/~otto/Research/BlackburnEtAl2010.nb).

D.2 Polymorphic equilibria for sex-linked traits

The equilibrium frequency of $T$, $\hat{p}_x$, when the $\mathbf{T}$ locus is sex-linked is given in Table D.2 for different forms of SRA, assuming that both SRA and selection are weak (derivations are

**Table D.2.** Equilibrium frequency, \( \hat{p}_T \), of a sex-linked trait in the presence of SRA.

<table>
<thead>
<tr>
<th></th>
<th>Self-SRA</th>
<th>Mate-SRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-linked</td>
<td>( \frac{(1-h_f)s_f - \frac{1}{2}s_m - H\theta}{(1-2h_f)s_f + (1-2H)\theta} )</td>
<td>( \frac{(1-h_f)s_f - \frac{1}{2}s_m + \frac{\Delta D_{\text{hemi}}}{2\bar{D}}}{(1-2h_f)s_f} )</td>
</tr>
<tr>
<td>Z-linked</td>
<td>( \frac{1}{2}s_f - h_ms_m - \frac{\Delta D_{\text{hemi}}}{2(1-\bar{D})} )</td>
<td>( \frac{1}{2}s_f - h_ms_m - H\theta )</td>
</tr>
</tbody>
</table>

The appropriate values of \( D_{ij} \) are given by Table 5.2 for cases in which SRA depends on the homogametic individual’s genotype (self-SRA with X linkage; mate-SRA with Z linkage). The formulae for these cases depend on \( H \), which represents the dominance of the SRA strategy in response to a \( Ti \) genotype \( (H = (D_{01} - D_{02})/(D_{00} - D_{02})) \), and \( \theta \) (see equation 2), which describes the relationship between the mean sex ratio in the population \( (\bar{D}) \) and the difference in SRA in response to a \( TT \) versus \( tt \) genotype \( (\Delta D = D_{00} - D_{02}) \). Table D.3 should be consulted for cases in which SRA is based on the heterogametic sex (mate-SRA with X-linkage and self-SRA with Z-linkage). The formulae in these cases depend on the difference in SRA in response to a \( T \) versus \( t \) genotype in a hemizygous individual \( (\Delta D_{\text{hemi}} = D_{00} - D_{01}) \). Notice that when sex ratios are altered in response to the heterogametic sex, the equilibrium frequency of \( T \) depends strongly on the SRA strategy via the term \( \Delta D_{\text{hemi}} \), even if the population sex ratio remains balanced \( (\theta = 0) \). The effect of SRA on the equilibrium trait frequency reflects transmission distortion, which is biased against the allele favored in the heterogametic sex.

**Table D.3.** SRA in the presence of a hemizygous trait and diploid modifier.

<table>
<thead>
<tr>
<th>Female modifier</th>
<th>Female Trait (self-SRA) or Male Trait (mate-SRA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( MM )</td>
<td>( D_{00} ) \hspace{1cm} ( D_{01} )</td>
</tr>
<tr>
<td>( Mm )</td>
<td>( D_{10} ) \hspace{1cm} ( D_{11} )</td>
</tr>
<tr>
<td>( mm )</td>
<td>( D_{20} ) \hspace{1cm} ( D_{21} )</td>
</tr>
</tbody>
</table>
A stability analysis was performed for each polymorphic equilibrium, assuming the \( M \) allele is fixed in the population; in each case the equilibrium is stable only if its denominator is positive.

**D.3 Invasion of an SRA modifier at sex-linked loci**

Here, we summarize results for sex-linked traits in the same format as presented for autosomal linked traits in the main text. Complete analyses can be found in the accompanying Mathematica 6.0 file (http://www.zoology.ubc.ca/~otto/Research/BlackburnEtAl2010.nb).

**X-linked loci**

In the case of an X-linked trait and SRA based on the homogametic sex (self-SRA), sex ratio allocation evolves in a qualitatively similar manner to the autosomal model, with the following slight changes:

- **Self-SRA with an X-linked trait and an X-linked modifier:**
  The leading eigenvalue, \( \lambda \), is given by equation (3) but with \( h_m \) set to 1/2 and with the terms in braces on the first and second lines multiplied by 4/3.

- **Self-SRA with an X-linked trait and an autosomal modifier:**
  The leading eigenvalue, \( \lambda \), is given by equation (3) but with \( h_m \) set to 1/2 and the term in braces on the second line multiplied by 6/5.

Thus, we again expect offspring sex ratios to be adjusted in favor of daughters among \( TT \) mothers and in favor of sons among \( tt \) mothers. As long as the population sex ratio remains near 1/2, stronger and stronger SRA is expected to evolve, and the equilibrium (Table D.2) will remain valid and stable throughout this process.

In contrast, with mate-SRA and an X-linked trait, selection on the modifier changes form because of the transmission distortion caused by adjusting offspring sex ratios based on the trait of the heterogametic sex.
• Mate-SRA with an X-linked trait and an X-linked modifier:

The leading eigenvalue becomes:

\[
\lambda = 1 - \frac{2}{3} \frac{\left( D - \frac{1}{2} \right)}{D(1-D)} \left\{ \hat{p}_T (D_{10} - D_{00}) + \hat{p}_i (D_{11} - D_{01}) \right\} \\
+ \frac{1}{3} \hat{p}_T \hat{p}_i \left( s_m - \frac{\Delta D_{hemi}}{D} \right) \left\{ (D_{10} - D_{00}) - (D_{11} - D_{01}) \right\}, \quad \text{(C1)}
\]

\[+ \mathcal{O}(\xi^3)\]

Initially, if the population sex ratio is balanced (\( D = 1/2 \)) and there is no SRA (\( \Delta D_{hemi} = 0 \)), the second line dominates equation (C1), and SRA should evolve in the expected direction. Specifically, modifiers can invade if they cause mothers to produce more daughters when mated with \( T \)-bearing males \((D_{10} - D_{00} > 0)\) and more sons when mated with \( t \)-bearing males \((D_{11} - D_{01} < 0)\), as defined in Table D.3. As stronger SRA evolves, it can be shown that the term, \( s_m - \frac{\Delta D_{hemi}}{D} \), remains positive as long as the trait polymorphism is maintained. Thus, equation (C1) indicates that more and more extreme SRA should evolve (assuming that the population-wide sex ratio remains near 1/2). At some point, however, the evolution of SRA causes \( \Delta D_{hemi} \) to become so large that the trait polymorphism is lost because of the transmission distortion favoring \( T \)-bearing X chromosomes. This occurs when the equation for \( \hat{p}_T \) given in Table D.2 for mate-SRA with X linkage reaches one.

• Mate-SRA with an X-linked trait and an autosomal modifier:

The leading eigenvalue becomes:

\[
\lambda = 1 - \frac{1}{2} \frac{\left( D - \frac{1}{2} \right)}{D(1-D)} \left\{ \hat{p}_T (D_{10} - D_{00}) + \hat{p}_i (D_{11} - D_{01}) \right\} \\
+ \frac{1}{5} \hat{p}_T \hat{p}_i \left( s_m - 2 \frac{\Delta D_{hemi}}{D} \right) \left\{ (D_{10} - D_{00}) - (D_{11} - D_{01}) \right\}, \quad \text{(C2)}
\]

\[+ \mathcal{O}(\xi^3)\]
If the population sex ratio is balanced ($\bar{D} = 1/2$) and there is no SRA ($\Delta D_{\text{hemi}} = 0$), SRA evolves as in equation (C1). Again, as stronger SRA evolves, the transmission distortion can become so strong that the trait polymorphism is lost. It is also possible, however, that as stronger SRA evolves, the term, $s_m - 2\Delta D_{\text{hemi}} / \bar{D}$, can switch signs, even under conditions that maintain the trait polymorphism. Thus, more extreme SRA should evolve only up to an intermediate level, given by $\Delta D_{\text{hemi}} - s_m/4$ (assuming $\bar{D} = 1/2$), at which point further increases in SRA are selected against, even though the trait remains polymorphic. A numerical analysis suggests that when $\Delta D_{\text{hemi}} \approx s_m/4$, a new modifier allele that further increases SRA (producing more daughters when mated with $T$-bearing males and/or more sons when mated with $t$-bearing males) becomes genetically associated with $TT$ females, which experience only a modest selective advantage over $Tt$ females, and with $T$ males, which are strongly selected against. The association with $T$-bearing males occurs because the new modifier experiences a stronger transmission bias against $X^t$ when mated with $X^tY$ males. Thus, further increases in SRA become increasingly opposed by natural selection, halting the evolution of further SRA despite the fact that a sexually antagonistic polymorphism remains.

**Z-linked loci**

- Self-SRA with a Z-linked trait and an autosomal modifier:

  The leading eigenvalue governing the spread of a new modifier allele is:

  \[
  \lambda = 1 - \frac{1}{2} \left( \frac{\bar{D} - 1}{\bar{D}} \right) \left\{ \hat{p}_T (D_{10} - D_{00}) + \hat{p}_t (D_{11} - D_{01}) \right\} \\
  + \frac{1}{5} \hat{p}_T \hat{p}_t \left( s_f - 2 \frac{\Delta D_{\text{hemi}}}{(1 - \bar{D})} \right) \left\{ (D_{10} - D_{00}) - (D_{11} - D_{01}) \right\},
\]

  where all $D_{ij}$ are given by Table D.3 with self-SRA and $\Delta D_{\text{hemi}} = D_{00} - D_{01}$. As stronger SRA evolves, the term $s_m - 2\Delta D_{\text{hemi}} / (1 - \bar{D})$ can become negative, even under conditions that maintain the trait polymorphism. Thus, equation (C3) indicates that more extreme SRA should evolve up to an intermediate level, given by $\Delta D_{\text{hemi}} = s_f/4$ (assuming $\bar{D} = 1/2$), at which point further increases in SRA are selected against, even though the trait remains polymorphic. As
SRA evolves and $\Delta D_{hemi}$ becomes more positive, the system equilibrates at a point, $\hat{p}_T$, where the $t$ allele declines, on average across the sexes, due to natural selection but increases in frequency due to transmission distortion in its favor. A numerical analysis suggests that, when $\Delta D_{hemi} = s_f/4$, a new modifier allele that further increases SRA (producing more daughters amongst $T$-bearing mothers and/or more sons amongst $t$-bearing mothers) becomes genetically associated with $tt$ males, which experience only a modest selective advantage over $Tt$ males, and with $t$ females, which are strongly selected against. Thus, further increases in SRA become increasingly opposed by natural selection, halting the evolution of further SRA despite the fact that a sexually antagonistic polymorphism remains.

In contrast, with mate-SRA and a $Z$-linked trait, this transmission bias does not arise because the mate is ZZ and passes each Z chromosome equitably to both sons and daughters. In this case, the evolution of offspring sex ratios is qualitatively similar to the autosomal case, with the following slight changes:

- **Mate -SRA (Z-linked trait and autosomal modifier):**
  
  The leading eigenvalue, $\lambda$, is given by equation (3) but with $h_m$ set to 1/2 and the term in braces on the second line multiplied by $6s_f/5s_m$.

  Thus, we again expect offspring sex ratios to be adjusted in favor of daughters among $TT$ mothers and in favor of sons among $tt$ mothers. As long as the population sex ratio remains near a half, stronger and stronger SRA is expected to evolve, and the equilibrium (Table D.2) will remain valid and stable throughout this process.