Color and Communication in *Habronattus* Jumping Spiders:

Tests of Sexual and Ecological Selection

by

Lisa Anne Taylor

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Graduate Supervisory Committee:

Kevin McGraw, Chair
Ronald Rutowski
David Clark
James Johnson
John Alcock

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ABSTRACT

Differences between males and females can evolve through a variety of mechanisms, including sexual and ecological selection. Because coloration is evolutionarily labile, sexually dichromatic species are good models for understanding the evolution of sex differences. While many jumping spiders exhibit diverse and brilliant coloration, they have been notably absent from such studies. In the genus *Habronattus*, females are drab and cryptic while males are brilliantly colored, displaying some of these colors to females during elaborate courtship dances. Here I test multiple hypotheses for the control and function of male color.

In the field, I found that *Habronattus* males indiscriminately court any female they encounter (including other species), so I first examined the role that colors play in species recognition. I manipulated male colors in *H. pyrrithrix* and found that while they are not required for species recognition, the presence of red facial coloration improves courtship success, but only if males are courting in the sun. Because light environment affects transmission of color signals, the multi-colored displays of males may facilitate communication in variable and unpredictable environments. Because these colors can be costly to produce and maintain, they also have the potential to signal reliable information about male quality to potential female mates. I found that both red facial and green leg coloration is condition dependent in *H. pyrrithrix* and thus has the potential to signal quality. Yet, surprisingly, this variation in male color does not appear to be important to females.

Males of many *Habronattus* species also exhibit conspicuous markings on the dorsal surface of their abdomens that are not present in females and are oriented away from females during courtship. In the field, I found that these markings are paired with increased leg-waving behavior in a way that resembles the pattern and behavior of wasps; this may provide protection by exploiting the aversions of predators. My data also suggest that different activity levels between the sexes have placed different selection pressures on their dorsal color patterns. Overall, these findings challenge some of the traditional ways that we think about color signaling and provide novel insights into the evolution of animal coloration.

DEDICATION

To my parents, who have always encouraged my curiosity in creepy, crawly things

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PREFACE

Sexual dimorphism in animals can evolve for a variety of reasons, which can be broadly divided into either sexual selection (Andersson 1994) or ecological selection (Slatkin 1984). Sexual selection drives the evolution of traits via male-male combat over access to females, such as the antlers of deer or the horns of rhinoceros beetles (reviewed in Emlen 2008), or via female choice for exaggerated male ornaments such as the large elaborate trains of peacocks (Petrie & Williams 1993) or the complex mating calls of túngara frogs (Ryan & Rand 1990). In contrast, ecological selection promotes sex differences in traits due to life-history factors other than mating; in mosquitoes and many species of moths, males have reduced or modified mouthparts compared to females, which have likely been shaped by sex differences in diet (reviewed in Shine 1989).

Color has been studied extensively as a trait that can be shaped differently in males and females as a result of both ecological and sexual selection pressures. Particularly in invertebrates, sex differences in color (i.e., sexual dichromatism) are often so drastic that the males and females were originally described as different species (e.g., Pilgrim & Pitts 2006; Zhang & Weirauch 2011). Recent advances in spectrophotometry have improved our ability to objectively quantify and analyze differences in such colors (e.g., birds: Andersson & Prager 2006; Montgomerie 2006), allowing us to tackle important questions about their functions and evolution (e.g., Hill & McGraw 2006b, a). Animal color patterns are often evolutionarily labile (e.g., Prum 1997; Hofmann et al. 2006), making color an ideal system in which to examine the selection pressures that drive sexual dichromatism.

Jumping spiders (family Salticidae) are an excellent group for such work because they are understudied, yet exhibit extreme color diversity between the sexes and across the family (Figure 1). Historically, there has been much interest in salticid color patterns (e.g.,Peckham & Peckham 1889, 1890; Crane 1949), yet only very recently have scientists begun to use modern spectrophotometric and statistical techniques to test hypotheses about the functions of salticid color variability. *Habronattus* is a particularly good study genus for this work because it is extremely diverse (Maddison & Hedin 2003); across the genus, males are highly ornamented, with a striking diversity of brilliant colors that they display to drab and cryptic females (Richman 1973; Richman 1982; Maddison & Hedin 2003). In addition to the brilliant colors used in courtship displays, males of many *Habronattus* also exhibit striking patterns of contrasting black and white chevrons on the dorsal surface of their bodies that are not present in females (e.g., Griswold 1987) and are oriented away from females during courtship (LAT, pers. obs.). Thus, within the same species, we can examine both the extent to which sexual selection shapes male display colors and the extent to which ecological selection shapes sexual dimorphism in dorsal colors. Of the more than 5000 species of jumping spiders described to date (Platnick 2011), only two other species have been the focus of coloration studies using modern spectrophotometric techniques: *Cosmophasis umbratica* (Lim & Li 2006b, a, 2007; Lim et al. 2007; Lim et al. 2008) and *Phintella vittata* (Li et al. 2008a; Li et al. 2008b). This previous work has focused entirely on sexual signaling functions of color rather than other potential ecological functions, and did not employ direct manipulations of color patterns in experiments, which are key for identifying causal relationships between specific ornaments and the benefits they convey.

The goal of this dissertation was to use a combination of field studies and laboratory experiments to address the following overarching question: Why has evolution shaped males to be more brilliant and conspicuously colored than females in *Habronattus* jumping spiders? Throughout, I consider multiple hypotheses that pertain to the costs, benefits, control, and functions of conspicuous male coloration including sexual selection hypotheses (e.g., species recognition, quality signaling) and ecological selection hypotheses (e.g., differential habitat use, different strategies of avoiding predation). Chapters 1 and 2 involve field studies on four sympatric species of *Habronattus* (*H. clypeatus, H. hallani, H. hirsutus,* and *H. pyrrithrix*), while the remaining chapters focus particularly on color and communication in *H. pyrrithrix*. I take a holistic approach throughout, weighing support for relevant alternative hypotheses simultaneously.

Dissertation outline

In Appendix A (Taylor & McGraw 2007), I briefly review the state of the field and highlight exciting advances made in the field of jumping spider coloration during the first year of my dissertation work. I review some of the reasons that salticids provide a promising system in which to examine color and communication.

In Chapter 1, I examine interactions between conspecifics and heterospecifics in four sympatric species of *Habronattus* (*H. clypeatus, H. hallani, H. hirsutus,* and *H. pyrrithrix)* in the field. First, by quantifying the interaction rates between male and female conspecifics, I lay the groundwork for studies of color and mate choice, which require and understanding of where, when, and how often conspecifics interact and court. In addition, I quantify the rates of heterospecific interaction and misdirected courtship to understand how these interactions might shape colorful signaling strategies. Together, these data elucidate the ecological context in which these spiders interact, allowing us to generate informed hypotheses about the different roles that color signals might play (e.g., species recognition, quality signaling).

In Chapter 2, I examine sex differences in dorsal color pattern in the same four species of *Habronattus*. In three of these species, males are more conspicuously patterned than females, with contrasting black and white stripes and chevrons on their dorsal carapace and abdomen. In this chapter, I use field behavioral data to test hypotheses for how ecological selection pressures (differences in habitat use and differences in movement rates) might result in different selection pressures on the color patterns of males and females (e.g., Slatkin 1984). Furthermore, I consider how male color pattern and behavior may interact to deceive potential predators through perceptual exploitation (e.g., Schaefer & Ruxton 2009; Ruxton & Schaefer 2011).

In Appendix B, I examine the condition dependence of male coloration in one particular species of jumping spider, *Habronattus pyrrithrix*, to weigh support for the many potential functional hypotheses for coloration. Generally, hypotheses about sexual signaling can be divided into content-based hypotheses (e.g., species recognition, quality signaling) or efficacy-based hypotheses (e.g., attention altering, sensory exploitation) (Guilford & Dawkins 1991). While these hypotheses are not mutually exclusive, each generates specific and testable predictions and only content-based, indicator models explicitly predict that colors should be condition-dependent (Hebets & Papaj 2005). Furthermore, content-based color signals are generally predicted to be more condition-dependent than colors that have evolved through ecological selection (Cotton et al. 2004). Thus, the goal of this study was to use both correlational and manipulative approaches to understand the condition dependence of male coloration to begin to tease apart potential signaling (or non-signaling) functions of color.

In Chapter 3, I examine ontogenetic color changes in *H. pyrrithrix*. In many animals, color patterns are not static throughout life, but change drastically during development, maturity, and senescence. In this chapter, I use scanning electron microscopy (SEM) to examine the scale and cuticle morphology of elaborately colored body regions in males. I then examine how the colors of these regions as well as dorsal color patterns change during development leading to sexual maturity and how male condition-dependent colors change as males age beyond sexual maturity. I present these results in the context of potential costs, constraints, and benefits of the production and maintenance of elaborate colors.

In Chapter 4, I test the hypothesis that a male's condition-dependent display colors (i.e., red faces and green legs) are required for or improve successful species recognition and/or mating. Furthermore, because the effectiveness of color signals depends on the lighting conditions in which they are sent (e.g., Endler 1993), I test the hypothesis that ambient light environment affects the role that male colors play in courtship. In a series of color-manipulation experiments under different lighting conditions, I assess the effect of the presence (vs. absence) of male color on his courtship success.

In Chapter 5, in light of consistent findings that a male's red facial coloration is condition-dependent in the field and dependent on juvenile diet in the lab, I test the hypothesis that natural variation in a male's red color signals aspects of male quality. Using both a correlational mate choice study and a color manipulation experiment, I examine how subtle, natural variation in male color affects courtship success.

While the study of animal colors is an active area of behavioral ecology, most work focuses on a few large, colorful, and traditionally well-studied taxa (e.g., birds: Hill & McGraw 2006b; Hill & McGraw 2006a; butterflies: Ingram & Parker 2008; fish: Amundsen 2003). Throughout this dissertation, I show that *Habronattus* jumping spiders have a unique biology that challenges some of the ways that we typically think about the costs, benefits, and functions of communicating with color, providing novel and exciting insights for the field of animal coloration.

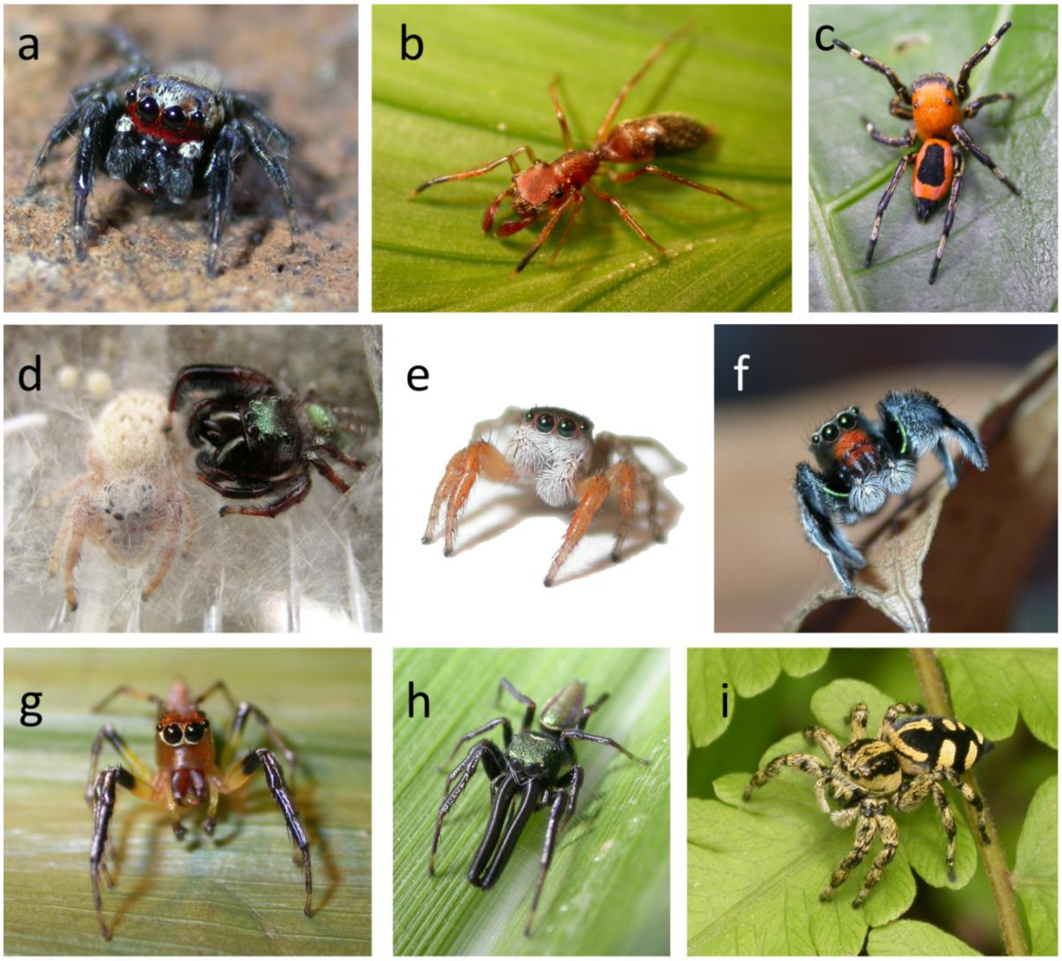

Figure 1. Diversity of color patterns in jumping spiders. (a) male *Evarcha culicivora*, Kenya, (b) female *Myrmarachne* sp., Costa Rica, (c) female *Phiale mimica*, Costa Rica, (d) female and male *Paraphidippus* sp., Costa Rica, (e) male *Habronattus icenoglei*, USA, (f) red-faced morph of male *Habronattus hirsutus*, USA, (g) male *Hypaeus benignus*, Costa Rica, (h) unidentified male salticid, Costa Rica, (i) female *Phiale guttata*, Costa Rica

Chapter 1

MISDIRECTED COURTSHIP IN A NATURAL COMMUNITY OF COLORFUL *HABRONATTUS* JUMPING SPIDERS

Abstract

Dramatic and costly male courtship display is common in many animals. In some cases, males engage in courtship indiscriminately, spending significant time and energy courting heterospecifics with whom they have no chance of mating or producing viable offspring. Due to high costs and no clear benefits, we might expect mechanisms to evolve to reduce such misdirected courtship (or 'reproductive interference'). In *Habronattus* jumping spiders, males frequently court heterospecifics with whom they do not mate or hybridize; females are larger and are voracious predators, posing a severe risk to males who court indiscriminately. In this study, we examine how misdirected courtship plays out in a natural community of four sympatric species of *Habronattus* (*H. clypeatus, H. hallani, H. hirsutus,* and *H. pyrrithrix).* Using direct observations of spiders in the field, we weigh support for two potential hypotheses to explain how these species co-exist and how they reduce the costs associated with misdirected courtship. Our first hypothesis is that heterospecific interaction rates are effectively reduced by differential use of the microhabitat (i.e., substrate, light environment) by the four different species. Our second hypothesis is that these species are not segregated in the microhabitat, but rather, they interact frequently and must rely solely on communication with every individual that they encounter to identify appropriate mates and to mitigate the costs associated with misdirected courtship. Our data show that, while the four species of *Habronattus* do show some differences in microhabitat use, all four species still overlap substantially. As a result, for three of the four species, individuals were just as likely to encounter a heterospecific as they were to encounter a conspecific. Males courted females at every opportunity, regardless of the species of the female. In some cases, this heterospecific courtship led to aggression and predation by the female. These results suggest that, while differences in microhabitat use might reduce misdirected courtship to some extent, co-existence of these four species is possible due to complex communication between both conspecifics and heterospecifics. To our knowledge, this is the first study to examine misdirected courtship in a system where such behavior presents the severe risk of predation from the female being courted. To date, studies of misdirected courtship and its consequences in the field are limited and may broaden our understanding of how biodiversity is maintained within a community.

Introduction

In many animals, dramatic and costly courtship displays have evolved to facilitate successful mating, often by providing information about a potential mate's location, sex, species, or quality as a mate (reviewed in Andersson 1994). Yet, courtship often incurs costs, such as increased energy expenditure (e.g., crickets: Hoback & Wagner 1997; frogs: Wells & Taigen 1989), decreased longevity (e.g., mosquitoes: Cordts & Partridge 1996; fruit flies: South et al.

2009), and increased predation risk (e.g., flashing fireflies: Woods et al. 2007). We would thus expect that selection should favor individuals that reduce their courtship efforts in situations where these costs outweigh potential reproductive benefits. Yet, many animals in a variety of taxa invest time and energy courting heterospecifics with which they never mate or are unable to produce viable offspring (e.g., ground-hoppers, moths, flies, ticks, lizards, fish , reviewed in Groening & Hochkirch 2008) or even attempt mating with inanimate objects (e.g., buprestid beetles frequently attempting to copulate with beer bottles: Gwynne & Rentz 1983). In addition to simply wasting energy that could be invested in other activities, such misdirected courtship (or 'reproductive interference') can also reduce or prevent viable mating opportunities for both sexes (e.g., Andrews et al. 1982). For example, in reptile ticks, males attempt to mate with and guard heterospecifics, physically blocking the female genitalia and preventing conspecific matings (Andrews et al. 1982). Given such costs, we might expect selection to favor mechanisms that prevent or reduce misdirected courtship. However, this topic has been given little attention in the ecological literature and is strongly biased towards laboratory rather than field studies where the ecological relevance is sometimes unclear (see Groening & Hochkirch 2008).

Jumping spiders (family Salticidae) are an excellent group in which to examine mechanisms that reduce heterospecific courtship because, for male jumping spiders, the consequences of courting a female of the wrong species are particularly severe. Females of most species are generalist predators and thus courting males, even of the same species, can become either a potential mate or a potential prey item (e.g., Jackson & Pollard 1997). As such, even *conspecific* courtship is risky; if heterospecific courtship never results in offspring, we might expect strong selection on males to avoid it. Despite approximately 44 studies of misdirected courtship in animals, to our knowledge, none of these have considered the potential cost of predation from the female that is being courted; this cost is unique to voracious and cannibalistic predators such as spiders (see Groening & Hochkirch 2008 for a review of misdirected courtship).

Across the jumping spider genus *Habronattus*, adult males are not very discriminating in courtship; in the lab, they will readily court dead conspecific female specimens as well as live heterospecific females (LAT, pers. obs.). Here we examine how this phenomenon plays out under natural conditions where multiple *Habronattus* co-occur. Specifically, our study examined this phenomenon in a riparian area in which four sympatric species of *Habronattus* exist in high abundance and overlap in the timing of sexual maturity and mating. These species are all from different species groups (Maddison & Hedin 2003) and do not hybridize (LAT, pers. obs.), yet males from all four species have been observed to readily court females of any of the other species in the lab, even though both conspecific and heterospecific adult females are voracious and cannibalistic predators (see Chapter 2, Table 3). In addition to the risks of predation from females, courtship for *Habronattus* males is likely to be energetically costly; males engage in dramatic dances consisting of coordinated combinations of color, motion, and seismic cues (e.g., Richman 1973; Cutler 1988; Maddison & Stratton 1988; Elias et al. 2003; Maddison & Hedin 2003;

Elias et al. 2006b) and will court continuously for hours in the lab, even if females are unreceptive or aggressive (LAT, pers. obs). In addition to the energetic expense of dancing, the conspicuously colored ornaments that males display to females (Taylor et al. 2011, Appendix B) may increase predation risk by visual predators.

In this study, we aim to address the following question: under natural conditions, how do males avoid the high costs associated with misdirected heterospecific courtship? Here we weigh support for two potential hypotheses using direct observations of spiders in the field. Our first hypothesis is that heterospecific interaction rates are effectively reduced by differential use of the microhabitat (e.g., substrate, light environment) by the four species. Reproductive interference in *Tetrix* ground-hoppers may be reduced in this way; despite significant overlap, subtle differences in microhabitat preferences and dispersion patterns appear to reduce heterospecific interactions (Groening et al. 2007). In *Habronattus,* males typically spend more time actively moving through the habitat (presumably seeking out females), while females spend more time at rest (see Chapter 2). While little is known about jumping spider movement patterns, there is evidence from one other species that males move more than females, while females show higher site fidelity and feed more (e.g., *P. clarus* (Hoefler & Jakob 2006)). These data, combined with field observations, suggest that females seek out particular microhabitats (for feeding, nesting, etc.) and that males actively seek out females and court them whenever and wherever they encounter them (LAT, pers. obs.). Thus, if the *Habronattus* species in our study are utilizing the habitat differently, we would expect it to be females that are partitioned in space and we would expect males to preferentially search for females in areas where they would be most likely to find conspecifics. In each of the four species in our study, males have very different colorful display ornaments, ranging in color from solid black, to black and white striped, to bright red, to iridescent green and pink (see Figure 2, see also Griswold 1987). Because the transmission of colored visual signals is strongly affected by both the visual background and the light environment where courtship takes place (e.g., Endler 1991, 1992, 1993; Endler & Thery 1996), it is reasonable to expect differences in substrate preferences of females from these differently colored species. If the four species are indeed partitioned in space with little overlap, this may explain why males have adopted the strategy of indiscriminately courting every female they encounter; such a strategy may be beneficial if heterospecific interactions are relatively rare compared with conspecific interactions.

An alternative is that habitat differentiation does little to reduce heterospecific interaction rates between species and, as a result, heterospecific interactions are just as common as conspecific interactions. Rather than being partitioned in the habitat, high interaction rates mean that males and females of the different species rely solely on communication with one another to identify appropriate mates. If these four species are not partitioned in the available habitat, and if heterospecific interaction rates are high, this may help to explain why *Habronattus* males have evolved such a rich diversity in display traits, as these may be necessary to communicate species identity to females from a safe distance.

In this study, we use focal behavioral observations on free-ranging spiders to weigh support for these two hypotheses. While we present them as discrete alternatives, both generating clear and testable predictions, we anticipate that subtleties in the data will allow us to weigh the relative support for each, ultimately allowing us to place each species pair along a hypothetical continuum ranging from complete isolation from one another (i.e., heterospecifics do not interact in the field) to complete overlap (i.e., heterospecific interaction rates are just as common as conspecific interaction rates). A better understanding of how these species overlap and interact under natural conditions will help us understand how similar species can co-exist, allowing for the maintenance of biodiversity within a habitat.

Methods

Study species

The genus *Habronattus* is the most diverse jumping spider genus, with approximately 100 species (Maddison & Hedin 2003). Males are highly ornamented with a striking diversity of brilliantly colored faces and legs that they display to drab, cryptic females during elaborate and often multimodal courtship dances (e.g., Richman 1973; Richman 1982; Maddison & Stratton 1988; Elias et al. 2003; Maddison & Hedin 2003; Elias et al. 2006b). Evidence from geographically isolated sky island populations of *Habronattus pugilis* suggests that sexual selection is responsible for driving such striking male diversification (Maddison & McMahon 2000; Masta & Maddison 2002). Our study focused on four sympatric *Habronattus* species, described in more detail below. Geographic variation in coloration is common within the genus (see Griswold 1987) and thus some subtleties of color pattern described here might be typical of this population in Phoenix, Arizona, USA.

Habronattus clypeatus (Banks). Adult male *H. clypeatus* have white faces with contrasting dark vertical bands beneath their anterior median eyes and the undersides of their first pair of legs used in display are gray and covered with white spatulate scales (Figure 2a). Females are a drab gray and brown with white faces (Figure 2b). *H. clypeatus* is found in northern Mexico and the southwestern USA and as far north as Wyoming, USA (Griswold 1987).

Habronattus hallani (Richman). In adult male *H. hallani*, the faces and first two pairs of legs are adorned with iridescent scales that change in hue from green to pink, depending on viewing angle (Figure 2c). Females are a drab gray and brown with white faces and characteristic dark, curved bands below their anterior median eyes (Figure 2d). *H. hallani* is distributed through the southwest USA to northern Mexico.

Habronattus hirsutus (Peckham and Peckham). Adult male *H. hirsutus* have dark gray/black front legs, the underside of which exhibit a narrow greenish band, and are further adorned with dense hairs (Figure 2e). Most adult males in our focal population have completely black faces (Figure 2e), yet occasionally we have observed males with bright red facial patches (LAT, pers. obs.). This degree of variation in facial coloration is typical on a geographic scale but is not well understood (see Griswold 1987). More than 95% of males in our focal population were of the black-faced form, including all of those that were the subjects of focal observations. Females are a drab gray and brown with white faces that have subtle dark markings just below and just above the anterior median eyes (Figure 2f). *H. hirsutus* is broadly distributed across western North America, from southern Canada to Mexico (Griswold 1987).

Habronattus pyrrithrix (Chamberlin). Adult male *H. pyrrithrix* have bright red faces and green front legs (Figure 2g), both of which are condition-dependent (Taylor et al. 2011, Appendix B). Male red facial coloration is important for courtship success in certain light environments (Chapter 4). Females are drab gray and brown with white faces (Figure 2h). *H. pyrrithrix* is distributed from the southwest USA to Sinaloa, Mexico (Griswold 1987).

Study site

All behavioral observations were made at the Rio Salado Habitat Restoration Area in Phoenix, Arizona, (Maricopa County, 33.42°N, 112.07°W), USA. The goal of this organization is to reestablish native wetland and riparian habitats that were historically associated with the Salt River (Rio Salado), which used to flow year-round (City of Phoenix 2011). *Habronattus* were generally concentrated in the leaf litter and vegetation within the gallery forests dominated by cottonwood (*Populus fremontii*) and desert willow (*Chilopsis linearis*).

Data collection

Behavioral observations were carried out between 900 and 1500 hrs. from March to November in 2009 and 2010. We located *Habronattus* by visually scanning the leaf litter and vegetation in the field. When we located a spider, we conducted a 15-minute behavioral observation in which we followed that spider from approximately 1m away and recorded behavior using voice recorders. Our sample sizes vary due to differences in abundance among species (*H. clypeatus*: n= 12 (5 females, 7 males), *H. hallani*: n=14 (8 females, 6 males), *H. pyrrithrix*: n=34 (20 females, 14 males), *H. hirsutus*: n=27 (10 females, 17 males)).

We quantified the amount of time spent in the sun versus the shade as well as the amount of time spent on different substrate types (cottonwood leaf litter, desert willow leaf litter, cottonwood vegetation, desert willow vegetation, grass, or dirt/rock). We recorded all interactions between the focal spider and either conspecific or heterospecific *Habronattus*. We defined an ‘interaction’ as any case in which both spiders responded to the presence of the other by orienting their anterior median eyes at the other individual (e.g., Jackson 1982). We recorded all instances of courtship, aggression (attacks), predation/cannibalism, and copulation. Because we frequently saw other individuals in the vicinity that did not interact with the focal spider, but that still provided valuable information about the local abundance and activity of the community, we recorded the number of other non-interacting *Habronattus* that we saw within 0.5m of the focal spider during the observation. Because our attention was focused on the behavior of the focal individual, our estimates of other *Habronattus* in the vicinity are likely to be more conservative than the actual abundance of spiders inhabiting the area. If spiders were found feeding, or captured prey during the focal observation, we recorded the identity of the prey item (identified to family, where possible).

After all data were collected, we temporarily captured each individual in a clear plastic vial. We confirmed the maturity of females by examining their epigynum; mature females can be distinguished from immatures by the presence of a sclerotized epigynum (Foelix 1996). To ensure that no individual was observed more than once, we marked spiders after observations with a small black dot (~1mm in diameter) on the underside of their abdomen using non-toxic liquid eyeliner (Urban Decay Cosmetics, Costa Mesa, CA, USA), which produced a permanent mark.

Data analysis

To determine if females of the four species utilized the available microhabitat differently, we compared substrate and light environment use among females of the four species using nonparametric Kruskal-Wallis tests with Steel-Dwass pairwise comparisons with an alpha level of 0.05. For substrate, we first compared each species' use of the three broad categories of microhabitat (leaf litter, vegetation, dirt/rock) and then we repeated the analysis on a finer scale that considered more subtle differences in microhabitat (cottonwood leaf litter, willow leaf litter, cottonwood vegetation, willow vegetation, grass, dirt/rock). We then compared the amount of time that females of each species spent in the sun (vs. the shade).

To examine if males were focusing their mate search in microhabitats where they would be most likely to encounter conspecific females, we then went on to determine if there was a correlation across species between female microhabitat use (i.e., substrate, light environment) and male microhabitat use using non-parametric Spearman rank correlations. Because there was a clear difference in broad patterns of habitat use, with *H. hirsutus* spending the majority of time in the vegetation and the other three species spending the majority of time in the leaf litter (see Results), we ran an additional analysis on just the litter-dwelling species to determine if males of these three species were preferentially searching for females in microhabitats where they would be most likely to find conspecifics.

To compare the mean number of conspecific interactions during focal observations with the number of heterospecific interactions for each species, we used non-parametric Wilcoxon signed rank tests. Because our study focused on the ecological importance of misdirected courtship, we conducted a second analysis, where we excluded interactions with juveniles and examined interactions between sexually mature adults.

We used non-parametric statistics because out data did not meet relevant assumptions. All statistical analyses were performed using SAS 9.2 and JMP 9.0.2 (SAS Institute, Cary, NC, USA).

Results

Use of substrate and light environment

Female *H. clypeatus, H. hallani,* and *H. pyrrithrix* all spent the majority of their time on the ground in the leaf litter (67%, 86%, and 80% of their time, respectively). In contrast, female *H. hirsutus* spent the majority of their time above the ground in the vegetation (72%). While there were significant differences among species in the females' use of the leaf litter and vegetation (leaf litter: X^2=15.29, P=0.0016, vegetation: X^2=16.85, P=0.0008; dirt/rock: X^2=2.69, P=0.441), there was still substantial overlap; females of all four species were found, at least occasionally, in both the leaf litter and the vegetation (Figure 3a). When we examined female substrate use on a finer scale, we again found significant differences between the species in their use of the substrate but, again, there was substantial overlap in substrate use among species (cottonwood leaf litter: X^2=14.86, P=0.0019, willow leaf litter: X^2=6.45, P=0.084, cottonwood vegetation: X^2=14.44, P=0.0024, willow vegetation: X^2=6.70, P=0.082; grass: X^2=4.66, P=0.198, dirt/rock: X^2=2.69, P=0.441, Figure 3b). Females of the four species differed in the amount of time spent in the sunlight, with *H. hallani* spending the least time in the sunlight and *H. hirsutus* and *H. pyrrithrix* spending the most (X^2=10.802, P=0.013; Figure 4).

When all four species were analyzed together, there were significant positive correlations between female substrate preference and the preference of conspecific males in some, but not all, substrate types (Table 1). Similarly, when the analysis was restricted to the three predominantly litter-dwelling species (*H. clypeatus, H. hallani,* and *H. pyrrithrix*), there were significant positive correlations between female preference and those of conspecific males in some, but not all, substrate types (Table 2). Female preferences for sunlight were not correlated with the preferences of conspecific males, either when all four species were analyzed together (Table 1) or when the analysis was restricted to the predominantly litter-dwelling species (Table 2).

Behavioral interactions

Densities of *Habronattus* were high; in 57 of 87 (66%) observations we spotted at least one other *Habronattus* within a 0.5m radius of the focal individual and in 33 (38%) observations, the focal spider interacted with at least one other *Habronattus*. We observed a total of 44 interactions between focal spiders and other *Habronattus*, 37 (84%) of which occurred between sexually mature adults; the nature of these interactions are summarized in Figure 5. Twenty-nine (78%) of these involved interactions between conspecifics and 8 (22%) involved interactions between heterospecifics. Twenty-two (50%) of all interactions occurred between sexually mature males and females (15 of which were between conspecifics and 7 between heterospecifics). In 100% of these 22 interactions, regardless of whether or not they were conspecifics or heterospecifics, males engaged in courtship. During courtship interactions, males were attacked in four cases; 3 of these occurred during conspecific courtship (between male and female *H. pyrrithrix*) and one during heterospecific courtship (between a male *H. clypeatus* and a female *H. pyrrithrix*). In one of the cases of conspecific aggression, the male was attacked several times by the female, but he continued to court and eventually copulated with her; copulation occurred in the leaf litter in full sunlight. In the heterospecific case the male was attacked several times and was eventually eaten by the female. One instance of aggression was observed between adult females, when an *H. clypeatus* attacked (but did not kill) a female *H. pyrrithrix*. No aggression was observed between males.

For *H. clypeatus, H. hallani*, and *H. pyrrithrix*, there were no significant differences between conspecific and heterospecific interaction rates (i.e., an individual was just as likely to interact with a heterospecific as they were with a conspecific, although *H. pyrrithrix* tended to have more conspecific than heterospecific interactions; *H. clypeatus*: S=-2.00, P>0.999; *H. hallani*: S=0.00, P>0.999; *H. pyrrithrix*: S=-19.5, P=0.057; Figure 6). For *H. hirsutus*, individuals had significantly more interactions with conspecifics than heterospecifics (S=-28.5, P=0.008; Figure 6). When we limited our analysis to interactions between sexually mature adults (excluding interaction with juveniles), there were again no significant differences between conspecific and heterospecific interaction rates in *H. clypeatus, H. hallani*, or *H. pyrrithrix,* although *H. pyrrithrix* tended to have more conspecific than heterospecific interactions (*H. clypeatus*: *S*=0.00, *P*=1.00; *H. hallani*: *S*=1.50, *P*=0.50; *H. pyrrithrix*: *S*=15.5, *P*=0.086). Again, in *H. hirsutus,* individuals had more interactions with conspecifics than heterospecifics (*S*=15, *P*=0.047).

To compare these interaction rates with other ecologically relevant events, in only 2 of 87 focal observations (0.02%) did we see the focal individual capture prey. In both cases, the focal spider was an adult female *H. hallani* attacking and eating a juvenile *H. hirsutus* (~3mm in size).

Discussion

In this study, we examined two hypotheses to explain how four sympatric *Habronattus* jumping spider species might avoid the high costs associated with heterospecific courtship in the field. Our results suggest that, while the four species utilize the habitat and light environment differently, there is still substantial overlap between all four species. This overlap leads to high interaction rates among species and high rates of heterospecific courtship, suggesting that aspects of communication likely play a role in mitigating the costs of these interactions.

In other species where reproductive interference is costly, habitat partitioning has been suggested as a mechanism that allows species to co-exist (e.g., Groening et al. 2007). In this study we show that females of four different species of *Habronattus* do indeed utilize the available microhabitats and light environments differently, which may reduce heterospecific interactions to some extent. Specifically, female *H. hirsutus* spend most of their time above the ground in the vegetation, while the other three species are predominantly ground-dwelling. Among the ground dwellers, all three species spent most of their time in cottonwood leaf litter, substantially overlapping in their habitat use. The four species also showed different light environment use, with *H. hallani* females spending the least time in the sun and *H. hirsutus* and *H. pyrrithrix* spending the most.

Because females generally spend most of their time at rest, while males spend most of their time actively searching for females (see Chapter 2), we went on to test if males searched for females in microhabitats where they would be most likely to find conspecifics, rather than heterospecifics. Our results indicate that there were significant positive correlations between female substrate use and the substrate use of conspecific males in some, but not all, substrate types suggesting that, in some cases, males may be searching for females on substrates where they are most likely to find conspecifics. However, there was no correlation between the light environment preferences of males and females across species suggesting that males are likely not biasing their mate search towards light environments where they are most likely to find conspecifics.

An alternative explanation for how *Habronattus* might mitigate the high costs of heterospecific courtship is that, rather than being segregated from one another in the environment, they simply rely on communication with every individual that they encounter. While we found some evidence of microhabitat partitioning, we also found very high rates of interaction among all four species, suggesting that communication is likely important in reducing the costs of misdirected courtship. To put these interaction rates in perspective, over the course of 87 focal observations (15 minutes each), we observed only two focal spiders capturing prey, but we observed 44 focal spiders interacting with other individuals. Of those interactions, 22 involved courtship. For each spider, this is approximately 2 interactions per hour (or 1 courtship interaction per hour), compared with only 0.092 prey items captured per hour. Interactions with other individuals are clearly common and ecologically relevant events for these spiders. Interestingly, for three out of the four species (*H. clypeatus*, *H. hallani,* and *H. pyrrithrix*), individuals were just as likely to interact with a heterospecific as they were with a conspecific. Not surprisingly, *H. hirsutus* was the only species where conspecific interactions were significantly more likely than heterospecific interactions; this is likely because *H. hirsutus* spends most of its time in the vegetation, away from the other three species. While misdirected heterospecific courtship was the focus of this study, the high rates of conspecific courtship were also notable, suggesting that females likely have the opportunity to be choosy and males likely have the opportunity to mate multiple times. Clearly, frequent communication both within and between species is an important aspect of *Habronattus* ecology in this community.

For male animals that provide no resources to their mate (e.g., food, parental care), selection may favor those that mate multiply and indiscriminately (Arnqvist & Rowe 2005). However, for male *Habronattus,* indiscriminate courtship comes with a risk that is not faced by males in many other taxa: female aggression and predation. In *Habronattus,* intraguild predation is most commonly a result of adult females feeding on either males or juveniles (see Chapter 2). In this study, every interaction involving a sexually mature adult male and female resulted in courtship by the male, regardless of whether they were of the same or different species. Our data show that female aggression can occur during both conspecific and heterospecific courtship in the field. In conspecific courtship, it is possible that this risk is outweighed by the possibility of successful copulation; the one focal male in our study that copulated was first attacked several times by the same female. However, in the case of heterospecific courtship, males are unlikely to gain any benefit from courting a heterospecific, but they pay the same cost; one male in our study was attacked several times by a heterospecific female who eventually captured and ate him. There is growing evidence in spiders that courtship displays incur both energetic and viability costs for males (Mappes et al. 1996; Kotiaho 2000; Hoefler 2008; Cady et al. 2011), as well as increased risk of predation (Hoefler et al. 2008). However, we argue that what makes spiders a particularly intriguing system to examine misdirected courtship is the risk of predation from females, a novel cost that, to our knowledge, has never been examined in this context (see review in Groening & Hochkirch 2008).

It may be that the colorful, species-specific ornaments and multimodal courtship displays of male *Habronattus* help to reduce the risks associated with misdirected courtship. If these ornaments and behaviors allow a male to identify himself from a distance, indiscriminate courtship may give him the opportunity to safely assess a female's receptivity or aggression. Work done with two other jumping spider species (*Cosmophasis umbratica* and *Phintella vittata*) has shown that blocking UV light affects mate choice decisions, suggesting that UV coloration plays an important role in sex and/or species recognition in these species (Lim et al. 2007; Lim et al. 2008; Li et al. 2008b). In *Habronattus* however, the role of color as a species recognition signal has received only limited support thus far. In *H. pyrrithrix*, the presence of a male's bright red face and green leg coloration are not required for successful copulation, although the presence of red facial coloration improves male courtship success in certain contexts (Chapter 4). If a male is courting in the sun, the presence of red facial coloration improves his success, but his coloration has no effect if he is courting in the shade (Chapter 4). Although not a required species recognition signal, elaborate male colors may help females assess a male's species identity under certain environmental conditions. More work is clearly needed on other *Habronattus* species to examine the roles that male ornaments play in recognition by females.

In the present study, females of the four species of *Habronattus* utilized the available substrate and light environment differently, and thus it is plausible that male species-specific colors have been selected not only to reduce the costs of misdirected courtship by signaling species identity, but also to maximize signal transmission when communicating with conspecific females in different light environments (e.g., Endler & Thery 1996). Male coloration is geographically variable in many species (see Griswold 1987) suggesting that color patterns may be locally adapted to specific attributes of their environment. While this hypothesis should be examined on a larger phylogenetic scale, results from the present study provide some intriguing patterns to be investigated further. First, female *H. hallani* spent the least time in the sun (see Figure 4); males of this species have iridescent markings (see Figure 2c) that might allow them to maximize signal transmission in the shade, where other colors are less effective (e.g., red, see Chapter 4). In contrast, female *H. pyrrithrix* spent the most time in the sun. As described above, the bright red face of *H. pyrrithrix* only improves male courtship success in the sun (but not in the shade), presumably due to the fact that sunlight is richer in red light while forest and woodland shade is relatively low in red light (Endler 1993). The fact that *H. hirsutus* also spends most of their time in the sun may not appear to fit this pattern, yet some males in this population do indeed exhibit red coloration on their faces (see Study Species section of Methods) and across their geographic range, red facial coloration in *H. hirsutus* is relatively common (Griswold 1987). Clearly, these qualitative relationships are preliminary and speculative, but warrant further study within a larger phylogenetically-controlled framework.

Learning may be an additional way for males to avoid the high costs of misdirected courtship. In some animals, males are able to learn to avoid heterospecific courtship, reducing the associated costs (e.g., Dukas 2004; Magurran & Ramnarine 2004). Jumping spiders exhibit extensive behavioral plasticity (e.g., Nelson & Jackson 2011a, b), a remarkable capacity for learning (reviewed in Jakob et al. 2011), and males are known to adjust their courtship repertoires depending on context (e.g., Jackson 1977; Jackson & Macnab 1991;

Jackson 1992; Jackson & Willey 1995; Cross et al. 2008). Because females often show aggression towards heterospecific males (LAT, pers. obs.), this presents an opportunity for males to modify their behavior through experience. In a situation where heterospecific females are abundant, males may learn through frequent attacks that caution is warranted. Alternatively, in an area with few heterospecifics and predominantly conspecifics, experience may cause males to adopt bolder, less discriminating courtship strategy. Using a mathematical model, Dukas et al. (2006) showed that learning is an improvement over indiscriminate courtship when female encounter rates are high and courtship durations are long. Both of these conditions apply to the *Habronattus* courtship examined here suggesting that this may be a promising group to examine the roles of learning in courtship, particularly in the context of species recognition and avoiding misdirected, heterospecific courtship.

Females may also incur costs associated with courtship; in some species of jumping spiders, evidence suggests that females actually face a higher predation risk than the males who are courting them (Su & Li 2006). In water striders, males of some species court and attempt matings with females indiscriminately (Arnqvist 1997). Females often struggle to deter or dislodge males that are attempting to copulate with them, but such struggling results in a 200% increase in energy expenditure (Watson et al. 1998). Female *Habronattus* also likely pay a similar cost for misdirected courtship. In this study, courting males who were rejected in the field often pursued females, even when they attacked males or tried to hop away. Given the high heterospecific interaction rates observed in our study, constantly hopping away from courting heterospecific males is likely to incur energetic costs for females. While females are often bigger than males and can readily attack them, we provide observational data in the field showing that initial attacks are not always successful and that males may dodge attacks while continuing to court. All of these observations suggest that misdirected courtship likely incurs costs for female *Habronattus,* as well as males.

The costs of misdirected courtship and heterospecific mating attempts are often density dependent and may affect interacting species in different ways depending on their relative abundance (e.g., Hochkirch et al. 2007). Interestingly, in the community of *Habronattus* examined in our study, *H. hallani* is the least abundant species of the four (LAT, unpublished data) and is also generally found in low abundance in other areas of its range (LAT, pers. obs.). As such, we might expect *H. hallani* females to incur higher relative costs due to misdirected courtship than the other species and thus females might benefit from additional mechanisms of signaling species identity to males. Interestingly, of the three species, *H. hallani* is the only species where females exhibit striking facial patterns (see Figure 2d). Future studies should examine the roles of female face markings within a larger phylogenetic framework to test the idea that they are more likely to evolve in situations where the costs of misdirected courtship are highest.

Given all of the costs associated with high rates of misdirected courtship, we might expect that for this system to evolve there must be substantial benefits to indiscriminate male courtship, at least under some circumstances. For example, this behavior may be particularly beneficial in habitats where there is little overlap among species and where most interactions occur between conspecifics or when closely related heterospecifics can hybridize to produce viable offspring. As landscapes are modified through increasing urbanization, we may see different combinations of species coming into contact at higher rates than they did historically.

Alternatively, it may be that this is an ideal strategy when conspecific interaction rates are particularly low and every possible courtship opportunity is extremely valuable. For example, in redback spiders, males experience exceptionally high mortality during mate searching; field data suggests that more than 80% of males die before finding a mate and that, on average, an individual male has less than one mating opportunity within his lifetime (Andrade 2003). These odds have likely driven the seemingly maladaptive behavior in which a male twists his abdomen into the female’s jaws where she feeds on it during copulation (Andrade 1996, 2003). While the *Habronattus* males in our study population had many opportunities to court females, there are likely situations where populations are less dense and courtship opportunities are less frequent. Given the similarity in appearance of the females of the four species studied here, males that are selective enough to avoid heterospecific females might risk avoiding the occasional conspecifics as well.

A recent review of the literature on misdirected courtship found a strong bias towards laboratory studies (n=27) compared with field experiments (n=8) and field observations (n=9) (Groening & Hochkirch 2008). Groening and

Hochkirch (2008) stress the limitations of laboratory experiments where limited space may inflate heterospecific interaction rates and they argue that more field studies are needed to understand the relevance and significant of such interactions in nature. Here we show that heterospecific courtship occurs at high rates among four species of sympatric *Habronattus* jumping spiders in the field and that these interactions can lead to female aggression and even predation. This high cost of misdirected courtship may help explain the evolution of colorful and complex communication of *Habronattus* jumping spiders.

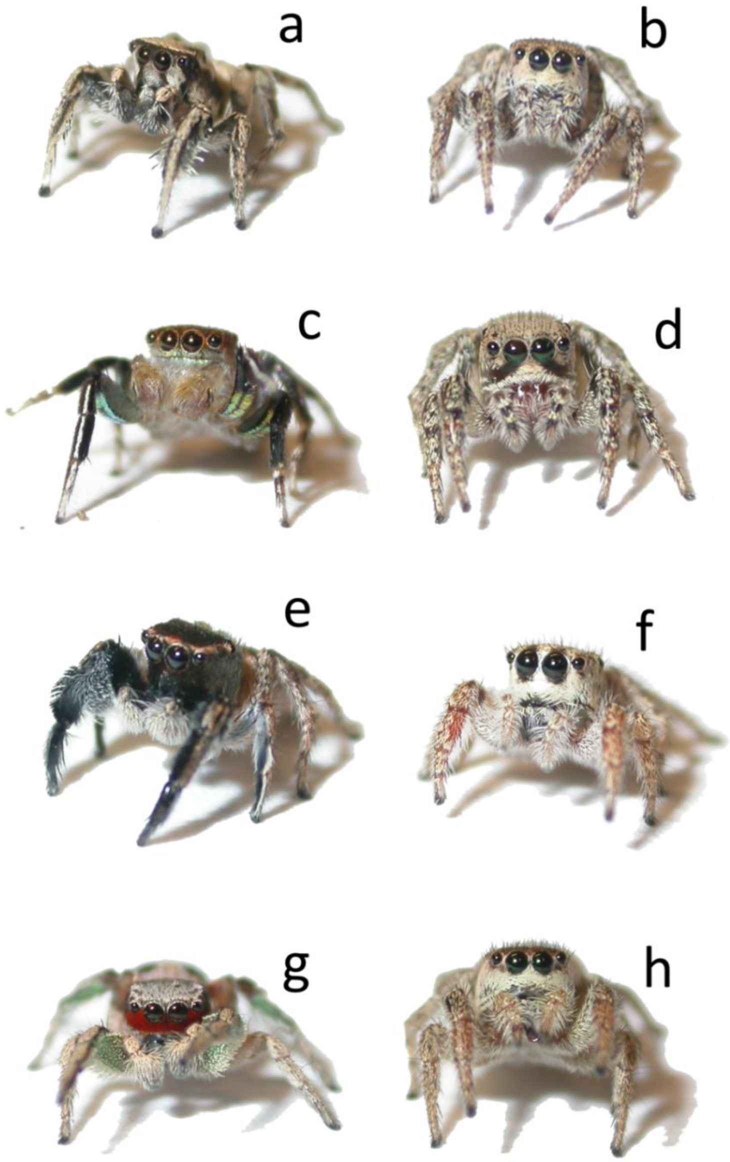

Figure 2. Adult sexual dimorphism in face and leg coloration in four sympatric species of *Habronattus*. *H. clypeatus* male (a) and female (b), *H. hallani* male (c) and female (d), *H. hirsutus* male (e) and female (f), and *H. pyrrithrix* male (g) and female (h). While females all look similar to one another, they can be identified based on subtle differences in dorsal and facial markings.

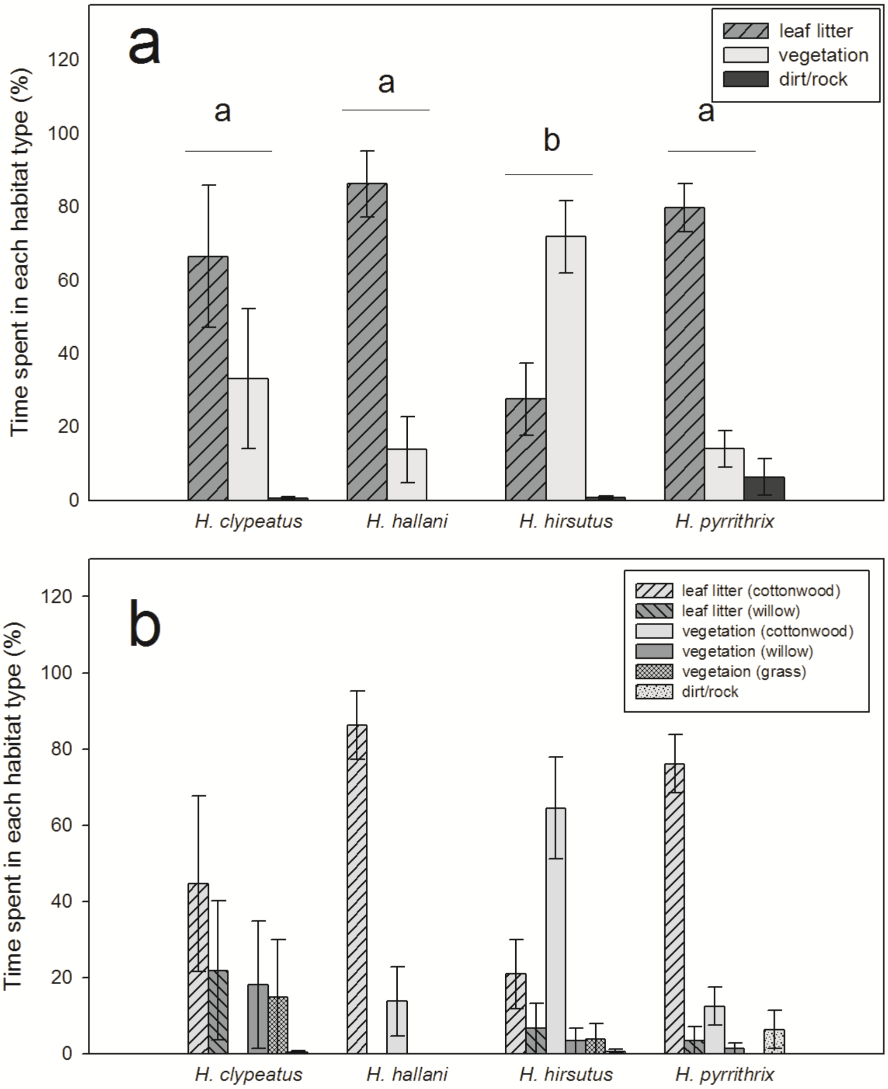

Figure 3. Comparison of the amount of time females of each species spent on different substrates during behavioral observations in the field (mean± SEM). Comparison of use of three broad categories of substrate (a), and comparison of finer categories of substrate use (b). In (a), different letters indicate significant differences between species in their use of the leaf litter and vegetation.

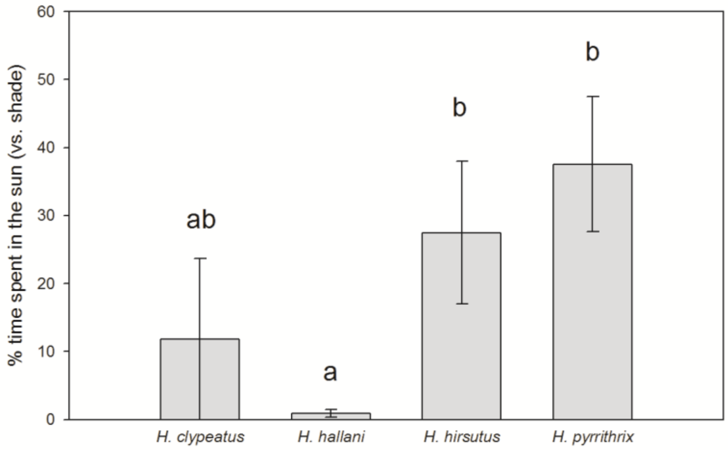

Figure 4. Comparison of the amount of time females of each species spent in the sun during behavioral observations in the field (mean± SEM). Different letters indicate significant differences between species.

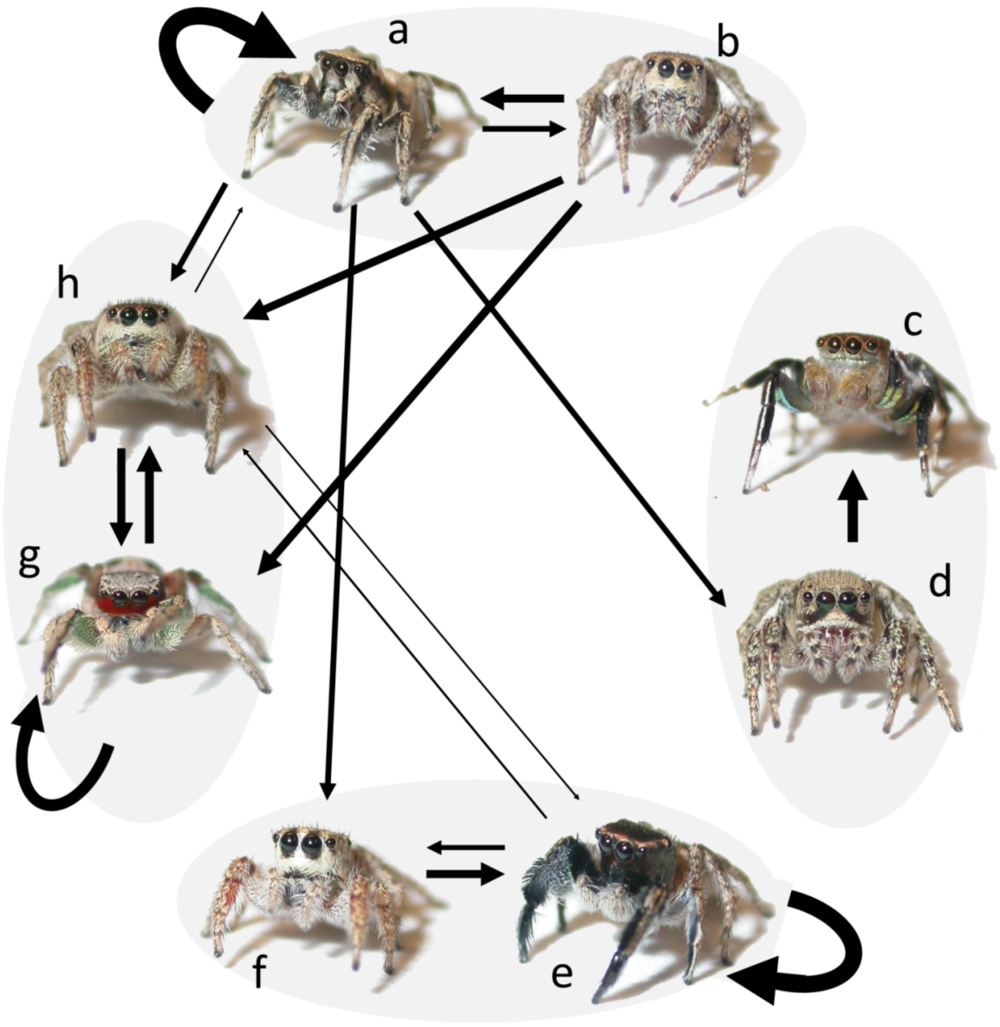

Figure 5. Summary of interactions between sexually mature adults in four species of sympatric *Habronattus*. The width of arrows represents the relative frequency of interactions per observation (with wider arrows indicating more frequent interactions). Curved arrows indicate interactions between individuals of the same species and sex. *H. clypeatus* male (a) and female (b), *H. hallani* male (c) and female (d), *H. hirsutus* male (e) and female (f), and *H. pyrrithrix* male (g) and female (h).

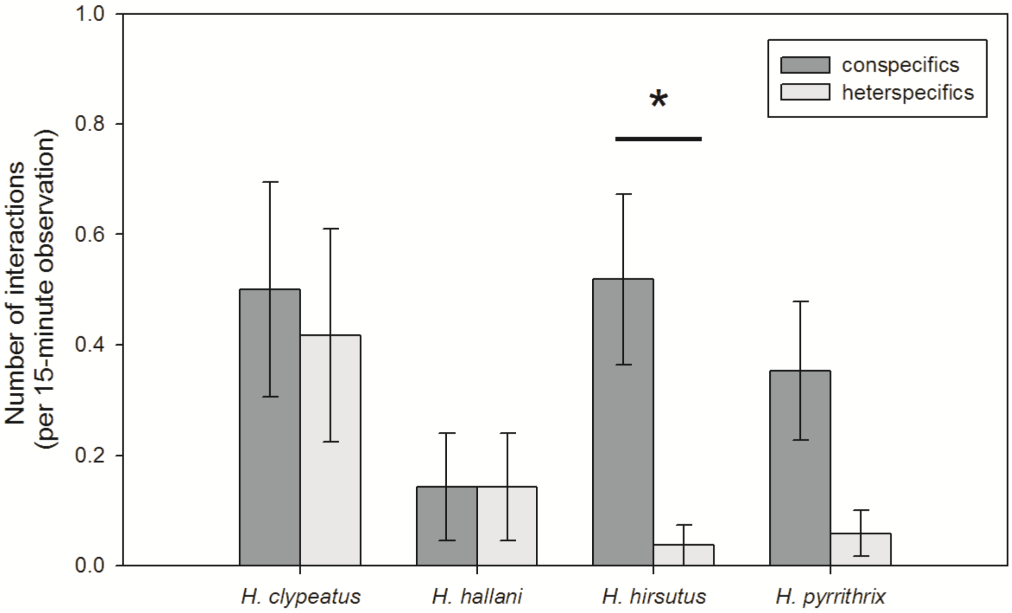

Figure 6. Comparison of the number of conspecific and heterospecific interactions during behavioral observations in the field (mean± SEM). Asterisks (*) indicate significant differences between conspecific and heterospecific interaction rates within a species.

Table 1. Spearman rank correlations between female habitat preference and the preference of conspecific males in four species of sympatric *Habronattus* (*H. clypeatus, H. hallani, H. hirsutus,* and *H. pyrrithrix*).

Microhabitat	**Rho (ρ)**	***P***
Light environment		
Sunlight (vs. shade)	0.400	0.600
Broad substrate category		
Leaf litter	1.000	<0.001
Vegetation	1.000	<0.001
Dirt/rock	0.770	0.225
Finer scale substrate categories		
Cottonwood* leaf litter	1.000	<0.001
Willow† leaf litter	0.632	0.368
Cottonwood* vegetation	0.316	0.684
Willow† vegetation	0.800	0.200
Grass	-0.544	0.456

* *Populus fremontii*
† *Chilopsis linearis*

Table 2. Spearman rank correlations between female habitat preference and the preference of conspecific males in three litter-dwelling species of *Habronattus* (*H. clypeatus*, *H. hallani,* and *H. pyrrithrix*). Note that the primarily vegetation-dwelling *H. hirsutus* is excluded from this analysis**.**

Microhabitat	**Rho (ρ)**	***P***
Light environment		
Sunlight (vs. shade)	0.500	0.667
Broad substrate category		
Leaf litter	1.000	<0.001
Vegetation	1.000	<0.001
Dirt/rock	0.867	0.333
Finer scale substrate categories		
Cottonwood* leaf litter	1.000	<0.001
Willow† leaf litter	1.000	<0.001
Cottonwood* vegetation	-0.867	0.333
Willow† vegetation	1.000	<0.001
Grass	-0.500	0.667

* *Populus fremontii*
† *Chilopsis linearis*

Chapter 2

SEX-DIFFERENCES IN DECEPTION: CAN ACTIVITY PATTERNS OR MICROHABITAT USE EXPLAIN SEXUALLY DIMORPHIC DORSAL COLOR PATTERNS IN *HABRONATTUS* JUMPING SPIDERS?

Abstract

In many animals, color pattern and behavior interact to deceive predators, resulting in morphological and behavioral camouflage or mimicry that ranges from nearly perfect to only subtle resemblance in color and behavior. Such strategies for avoiding predation often differ between males and females as a result of ecological selection pressures that differ between the sexes. While sex differences in mimicry are common in jumping spiders (Salticidae), to our knowledge, no one has examined the selective forces shaping these differences. Here we examine dorsal color pattern in a community of three sympatric species of male *Habronattus* jumping spiders (*H. clypeatus, H. hallani, H. pyrrithrix*); males of these species have bold and conspicuous patterns reminiscent of the coloration of wasps and bees, while females are dull and cryptic. We show that, compared with females, males of these conspicuously-patterned species exhibited increased leg-waving behavior that occurs outside of the context of courtship; such leg-waving behavior is common in jumping spiders that mimic hymenopterans because a mimic’s waving legs resemble antennae of ants, wasps, and bees. In a fourth species (*H. hirsutus*) found in the same habitat, males do not have conspicuous dorsal patterning, and these males did not exhibit increased leg-

waving behavior compared to females. These results are consistent with the idea that male color and behavior have co-evolved to deceive predators in three species of *Habronattus*. The second goal of our study was to test two hypotheses that could explain sex differences in coloration. We posited that higher activity and movement rates of males (who must wander to find females) and/or different use of the microhabitat (e.g., substrate, light environment) by the sexes could explain sexual dichromatism. Our results support the activity hypothesis; males and females generally did not differ in their use of the available microhabitat or light environment, but males of all three conspicuously colored species spent more time actively moving than females, who spent most of their time at rest. To our knowledge, this is the first study to suggest that conspicuous male dorsal coloration in *Habronattus* has a deceptive function.

Introduction

In many animals, color patterns and behavior interact to facilitate evasion or deception of potential predators; as such, selection by predators is thought to act on particular combinations of these traits (e.g., butterflies: Robbins 1981; snakes: Brodie 1989; grasshoppers: Forsman & Appelqvist 1998; hoverflies: Golding et al. 2005; moths: Rota & Wagner 2006). Metalmark moths and tephritid flies appear to mimic jumping spiders (family Salticidae); eyespots and leg-like markings on their wings paired with stereotyped postures and movements not only reduce predation, but also elicit displays from would-be salticid predators who mistake them for other salticids (Greene et al. 1987; Mather & Roitberg

1987; Rota & Wagner 2006). A variety of invertebrates are known to mimic ants; such mimics often use combinations of ant-like morphology as well as ant-like behaviors including erratic running and waving of their front legs in a way that mimics the waving of an ant's antennae (reviewed in: McIver & Stonedahl 1993; Cushing 1997). Hoverflies (Syrphidae) appear to mimic wasps and bees, and some species also appear to mimic the flight patterns of their models (Golding et al. 2005). Such examples range from precise, near-perfect Batesian mimicry (e.g., *Myrmarachne* jumping spiders that closely mimic ants (Nelson et al. 2005b)) to general or imperfect resemblance in color and behavior (e.g., *Syrphus* hoverflies that only subtly resemble wasps (Golding et al. 2005)).

Such defensive strategies often evolve differently between the sexes. While many of the best-studied examples of sexual dimorphism are traits shaped by sexual selection via mate choice or competition over mates (Andersson 1994), sexual dimorphism can also be shaped by ecological selection, where males and females experience different selection pressure as a result of sex-specific differences in diet, habitat use, activity levels, thermoregulatory requirements, or the different suites of predators to which they are exposed (Slatkin 1984). There are several examples of deceptive coloration (e.g., mimicry) evolving differently between the sexes (reviewed in Ruxton et al. 2004). For example, in butterfly mimicry complexes, it is often females that are mimics, while males are not; it has been suggested that slower-flying females need added protection from predators or that mimicry in males is less likely to evolve because a male's species-specific color patterns are crucial for mate recognition (reviewed in Ruxton et al. 2004).

Jumping spiders (family Salticidae) are an excellent group in which to examine questions about predator deception, as well as the selective pressures that shape sexual dimorphism in deceptive strategies. Across the family of more than 5000 described species (Platnick 2011), there are numerous species in which dorsal coloration differs dramatically between the sexes (e.g., Maddison 1995), yet in many cases the sexually dimorphic body regions are not overtly displayed in courtship (LAT, pers. obs.). In some of the most striking examples, one or both sexes appear to mimic brilliant and colorful hymenopterans, most notably wasps and bees (including the so-called ‘velvet ants’ in the family Mutillidae). For example, in *Phiale formosa*, males and females both appear to be mimics, but clearly mimic different species; males have a striking black and white color pattern while females are black and yellow (LAT, pers. obs.). In the genus *Phidippus*, velvet ant mimicry appears to be widespread and varies among species; in some species both sexes appear to be mimics, while in other species only adult males appear to be mimics (Edwards 1984, 2004). Apparent hymenopteran mimicry in salticids ranges from near-perfect resemblance to the model’s color pattern (e.g., *Phiale mimica* mimicking the velvet ant *Dasymutilla cressonii*, LAT pers. obs., see Figure 1c in Preface) to only subtle resemblance or imperfect mimicry (e.g., female *Phiale formosa* which appears to be a general wasp mimic, LAT pers. comm.). At both of these extremes, color pattern is usually paired with behavioral mimicry, in which the spiders frequently wave their first pair of legs, presumably to resemble the antennal movement characteristic of hymenopterans (LAT, pers. obs., see also Cushing 1997).

Despite this diversity of sexual dichromatism across the family, to our knowledge, no study in any jumping spider species has addressed the question of why males and females often differ so drastically in dorsal coloration that is not used in courtship display.

In this study, we attempt to understand the selective forces shaping sexually dimorphic dorsal coloration in a community of three species of jumping spiders in the genus *Habronattus*. Most of the behavioral work on *Habronattus* has focused on their complex ornamentation and exuberant courtship displays (e.g., Maddison & Stratton 1988; Richman & Cutler 1998; Elias et al. 2003; Hebets & Maddison 2005; Elias et al. 2006b; Elias et al. 2006a) and the role of sexual selection in driving diversification of these traits (Maddison & McMahon 2000; Masta & Maddison 2002). However, in many species of *Habronattus,* males also have conspicuous dorsal color patterns consisting of bold black and white stripes or chevrons (see Griswold 1987) that are visible from above and behind the spider, but which are not overtly displayed to females during courtship; in fact, these patterns are actually oriented away from the female during the entire courtship display (LAT pers., obs., Figure 8). In contrast, females of most species lack these conspicuous markings and are instead drab and cryptically colored (Griswold 1987, see Figure 7). The striking male dorsal patterns are reminiscent of the bold stripes on the dorsal surfaces of common hymenopterans that are frequently seen in the leaf litter in the same habitat as these spiders and can inflict a painful sting (e.g., velvet ants (Mutillidae), ground-nesting bees (e.g., *Lasioglossum* sp.); LAT, pers. obs.). Anecdotal observations of male behavior in the field suggested that these conspicuously patterned male *Habronattus* also frequently wave their front legs when moving through their habitat (outside of the context of courtship) in a way that appears to enhance their resemblance to hymenopterans (LAT, pers. obs., see online supplementary video: http://vimeo.com/31919620). Such leg-waving behavior (or false antennation) that occurs outside of the context of courtship is very common among other salticids that mimic hymenopterans (reviewed in Cushing 1997). These preliminary observations led us to the hypothesis that these conspicuous male color patterns combined with characteristic leg-waving behavior are functioning as deceptive signals, either to directly (but imperfectly) mimic hymenopterans (Sherratt 2002) or to exploit the perceptions of predators that have evolved to avoid such color patterns and behaviors (Ruxton & Schaefer 2011). Because these two hypotheses are difficult to disentangle (see discussion in Schaefer & Ruxton 2009; Ruxton & Schaefer 2011), we lump them together here as a single hypothesis which we refer to as the 'deception hypothesis'.

The present study has two main goals. First, we test initial predictions of the deception hypothesis described above that will allow us to qualitatively weigh support against other alternative explanations for conspicuous male dorsal coloration in *Habronattus* (e.g., that it functions as disruptive coloration (e.g., Cott 1940; Stevens & Merilaita 2009) or motion dazzle coloration (e.g., Stevens et al. 2008; Scott-Samuel et al. 2011). We focus our study on three sympatric species found within a single habitat in Phoenix, AZ, USA (*H. clypeatus, H. hallani,* and *H. pyrrithrix).* Because we are focusing on a small community of species, this is not intended to be a phylogenetic comparative study, but rather a behavioral study that is replicated across three sympatric species to increase the robustness and generality of our conclusions. The deception hypothesis posits that conspicuous male color and leg-waving behavior function together to deceive potential predators. As such, it predicts that these conspicuously colored males should also exhibit correspondingly higher leg-waving rates in a non-sexual context compared with drab and cryptic females. Interestingly, there is a fourth species of *Habronattus* in this habitat (*H. hirsutus*) in which males do not have conspicuous coloration (both males and females are solid in color, although males are often darker than females, Griswold 1987, LAT pers. obs.). This species is the most distantly related of the group (Maddison & Hedin 2003) and thus we use them as a comparison for the other three. Because male *H. hirsutus* lacks conspicuous markings, we predict to find comparatively less non-sexual leg-waving among males compared with females. To provide a context for understanding the selection pressures driving such color patterns, we report all predation events observed on all four species of *Habronattus*.

The second goal of this study was to address the question of why such conspicuous colors in *H. clypeatus*, *H. hallani*, and *H. pyrrithrix* are male-specific. Here we test two potential hypotheses. In the 'activity hypothesis', we posit that males and females are exposed to different selection pressures due to differences in their activity patterns (i.e., movement rates). Recent work with insect prey and fish predators has shown that movement and color pattern interact synergistically such that cryptic coloration is only beneficial to prey if they are motionless; if the prey become active, the benefits of crypsis go away (Ioannou & Krause 2009). This suggests that for an animal that is necessarily active, crypsis may not be effective and other coloration strategies may be more adaptive. In a phylogenetically-controlled study of butterflies, Merilaita and Tullberg (2005) found that aposematic and mimicry coloration were more likely to evolve in butterflies that are active during the day, whereas nocturnal species that rest during the day were more likely to be cryptic. This suggests that high rates of activity and movement may constrain the evolution of crypsis (Merilaita & Tullberg 2005). We know of only one other study that has examined movement patterns by tracking free-ranging jumping spiders; this study found that in *Phidippus clarus,* males spent more time moving than females and females spent more time feeding (Hoefler & Jakob 2006). In preliminary observations of *Habronattus* jumping spiders in our focal population, males appear to wander in search of mates and to initiate courtship whenever they find them. Such differences in behavior between the sexes may explain why females have evolved crypsis, while males have evolved deceptive coloration.

In the 'different microhabitat hypothesis', we posit that males and females face different selection pressures due to differences in aspects of their preferred microhabitats (e.g., the substrate and/or light environments in which they spend most of their time). Sex-specific differences in habitat use have been argued to be a driving force in the evolution of sex differences in coloration in other animals (e.g., grasshoppers: Calver & Bradley 1991; Ahnesjo & Forsman 2006; isopods: Merilaita & Jormalainen 1997). In the buprestid beetle *Chrysobothris humulis* males, but not females, mimic distasteful chrysomelid beetles; Hespenheide (1975) suggests that this may be a result of the fact that males spend their time in different habitats (i.e., legume twigs) where there are a greater number of their chrysomelid models and where they are exposed to higher predation compared with females, which exhibit metallic green coloration. It could be that male and female *Habronattus* have employed completely different coloration strategies due to differences in the background colors and light levels of their preferred microhabitat habitat types (e.g., leaf litter, vegetation, rocks, and dirt) or due to differences in the suites of potential predators or models in those different microhabitats.

Similarly, we posit that males and females may face different selection pressures because males are exposed to a wider variety of habitat types while searching for mates while females can wait for suitors to approach them and can therefore remain in a more homogeneous habitat. Empirical work with birds and artificial prey has shown that in heterogeneous habitats, survival is maximized if prey exhibit a compromise in crypsis using a color pattern that is intermediate in its match between the different microhabitats, rather than a close match to either one (Merilaita et al. 2001). Using an evolutionary simulation, Merilaita and Tullberg (2005) found that if animals had to move through multiple habitats of different background colors, then alternatives to crypsis (i.e., aposematic coloration) were more likely to evolve, suggesting that habitat heterogeneity may constrain the evolution of crypsis. In the jumping spider *Phidippus clarus*, females showed higher site fidelity than males (Hoefler & Jakob 2006) and thus it seems plausible that females might experience less habitat heterogeneity. If *Habronattus* females simply have to wait for males to find them, they may be able to remain in a single microhabitat type where they are well-suited for cryptic coloration. In contrast, if males need to seek out females, this may require them to travel greater distances through a larger number of habitat types, reducing the effectiveness of crypsis and making deceptive coloration a more beneficial strategy.

The 'activity hypothesis' and the 'different microhabitat hypothesis' are not mutually exclusive, yet each generates specific and testable predictions. Here we weigh support for each using direct behavioral observations of free-ranging individuals in the field. To our knowledge, only one study has examined salticid movement patterns using focal observations on free-ranging spiders (Hoefler & Jakob 2006) and no study has attempted to address hypotheses to explain sex-differences in salticid dorsal coloration that is not involved in courtship.

Methods

Study species

The genus *Habronattus* includes approximately 100 species, primarily in North America, with a diversity of elaborate visual ornaments and dramatic multimodal courtship displays (Griswold 1987; Maddison & Hedin 2003). In addition to the colorful ornaments that males display to females, males and females of many species are also strikingly sexually dimorphic in dorsal coloration (Griswold 1987). The four species examined in this study are all from different species groups (Maddison & Hedin 2003) and do not hybridize in the field or lab (LAT, pers. obs.).

Habronattus clypeatus (Banks), *H. hallani* (Richman), and *H. pyrrithrix* (Chamberlin) all exhibit similar patterns of sexual dimorphism in dorsal color pattern; males of all three species have striking and contrasting patterns of black and white stripes and/or chevrons, while females are brown and cryptic (Figure 7a-f). In *H. hirsutus* (Peckham and Peckham), males and females are both solid in color, although males are darker than females (Figure 7g,h). Geographic variation in coloration is common within the genus *Habronattus* (see Griswold 1987) and thus it should be noted that some subtleties of color pattern, as well as behavior, described here might be typical of this Phoenix, AZ population, and may vary across the species range. These four species are all relatively common in riparian areas and gardens around Phoenix.

Study site

All observations of spider behavior were made at the Rio Salado Habitat Restoration Area in Phoenix (33.42°N, 112.07°W). The mission of this restoration area is to reestablish native wetland and riparian habitats that were historically associated with the Salt River (Rio Salado), which used to flow year-round (City of City of Phoenix), and thus it is an ideal natural habitat to study the natural history and behavior of *Habronattus.* These spiders are commonly found on the ground wandering through the leaf litter, above ground in the vegetation of cottonwood trees (*Populus fremontii*) and desert willows (*Chilopsis linearis*), and occasionally in grass and on the dirt, rock, and gravel substrates (LAT, pers. obs.).

Data collection

Behavioral observations were conducted between 900 and 1500 hrs. from March to November in 2009 and 2010. We located spiders in the field by visually scanning the leaf litter and vegetation. When a spider was located, we conducted a two-part behavioral observation in which we followed the spider and recorded its behavior using voice recorders. Sample sizes vary among species due to differences in abundance (*H. clypeatus*: n= 12 (5 females, 7 males), *H. hallani*: n=14 (8 females, 6 males), *H. pyrrithrix*: n=34 (20 females, 14 males), *H. hirsutus*: n=27 (10 females, 17 males). During the first 15 minutes of the observation, we quantified the amount of time spent moving (i.e., walking, jumping) and stationary (i.e., not moving). While stationary, we quantified the amount of time spent in the sun versus the shade. In both the sun and shade, we further quantified the amount of time spent on different substrate types (cottonwood leaf litter, desert willow leaf litter, cottonwood vegetation, desert willow vegetation, grass, or rock/dirt. Finally, by marking the starting and ending location of the focal spider, we measured the total distance moved during the 15-minute observation period. While this ‘distance’ metric does not account for additional movement that did not occur in a straight line, that data is captured in the measurement of the amount of time the spider spent moving (see above).

If, after the 15-minute behavioral observation ended, the focal individual was *not* presently interacting with or oriented towards another individual, we conducted an additional 5-minute observation in which we quantified their leg-waving behavior. Outside of the context of courtship, individuals often raise and lower their front legs either simultaneously or in an alternating fashion (see online supplementary video: http://vimeo.com/31919620). For each focal spider, we randomly selected either the left or right leg (to avoid the difficulty of observing both moving legs at the same time) and counted the number of times that that leg was raised during the five minute period.

After all data were collected, we temporarily captured each individual in a clear plastic vial. For putative adult females, we confirmed maturity by examining their epigynum; mature females can be distinguished from immatures by the presence of a sclerotized epigynum (Foelix 1996). To ensure that we were not repeatedly observing the same individuals, we captured each spider after data were recorded and marked them with a small black dot of paint (~1mm in diameter) on the underside of their abdomen using non-toxic black liquid eyeliner (Urban Decay Cosmetics, Costa Mesa, CA, USA). If, after collecting data for a given individual, we discovered that the individual had already been marked, we excluded the data from our analyses.

To better understand the suite of potential predators that may be shaping male and female color patterns, we recorded all predation events on *Habronattus* that we observed in the field throughout the study (n=13), both within and outside of the context of our focal behavioral observations. Because we frequently saw several mud dauber wasps (family Sphecidae) in the area that we suspected were feeding on *Habronattus*, we also examined the contents of 23 abandoned mud dauber nests found on the underside of a cement bridge within the area where we carried out our spider behavioral observations. All of the nests that we examined with emergence holes were empty, and thus here we include data only on the nests that we found that were sealed and did not have emergence holes. Wasp nests were likely inhabited by *Sceliphron* sp. or *Chalybion* sp., both of which we have observed at this field site (LAT, pers. obs.). Both species specialize on spiders; *Sceliphron* build mud nests in which they provision their young, while *Chalybion* use the old nests of *Sceliphron* (see Landes et al. 1987; Elgar & Jebb 1999; Blackledge & Pickett 2000; Camillo 2002), making it difficult to determine which species is the owner of the provisions in a particular nest cell.

Statistical analyses

All predictions above involve comparing behaviors between the sexes of the three species that exhibit conspicuous male coloration (*H. clypeatus, H. hallani,* and *H. pyrrithrix).* For comparison with a species that does not exhibit conspicuous coloration, we also examined sex differences in behavior of the most distantly-related species, *H. hirsutus*. We used 2-tailed t-tests (if data met the relevant assumptions) or Wilcoxon rank sum tests (if assumptions of parametric tests were violated) for all comparisons.

To test initial predictions of the 'deception hypothesis', we compared leg-waving rates (number of leg-waves in five minutes) that occurred outside of the context of courtship between males and females. We then went on to further examine the hypotheses for why the sexes differ in their coloration strategies in *H. clypeatus, H. hallani,* and *H. pyrrithrix*. To test the predictions of the 'activity hypothesis', we compared the time spent moving (vs. time spent at rest) during behavioral observations between the sexes. To test the predictions of the 'different microhabitat hypothesis', we compared the time spent in each broad habitat category (leaf litter, vegetation, rock/dirt) between the sexes, and then we went on to compare time spent in finer scale microhabitat categories (cottonwood leaf litter, willow leaf litter, cottonwood vegetation, willow vegetation, grass, and rock/dirt). We then compared the time spent in different light environments (sun vs. shade) between the sexes. To understand if males and females differ in habitat heterogeneity, we compared the total distance traveled as well as the number of different microhabitat types individuals of each sex traveled through during the observation period. Finally, we calculated the proportion of time that each individual spent in their preferred habitat type and compared this between the sexes.

In this study, we were interested in broad-scale sex differences that could be replicated across sympatric species with conspicuous male color patterns (e.g., *H. clypeatus, H. hallani, H. pyrrithrix).* In testing our hypotheses, we place emphasis on patterns that held up across species rather than emphasizing the importance of any one significant result for any one species; for this reason, we did not employ Bonferroni corrections (see Cohen et al. 2008).

Results

Deception hypothesis

In the three species with conspicuous male dorsal patterns, males waved their legs more than females (*H. clypeatus*: $t_3=4.017$, $P=0.028$; *H. hallani*: $X^2=6.74$, $P=0.009$; *H.* pyrrithrix: $X^2=7.19$, $P=0.007$; Figure 9). In *H. hirsutus,* males and females did not differ in leg-waving rates ($X^2=0.514$, $P=0.474$; Figure 9).

Activity hypothesis

In *H. clypeatus, H. hallani,* and *H. pyrrithrix*, males spent more time moving than females, while females spent more time at rest (*H.* clypeatus: $t_{10}=2.763$, $P=0.020$; *H. hallani:* $X^2=4.33$, $P=0.037$; *H. pyrrithrix:* $X^2=16.194$, $P<0.0001$; Figure 10). For comparison, in *H. hirsutus*, males and females did not differ in movement rates ($t_{25}=1.34$, $P=0.194$; Figure 10).

Different microhabitat hypothesis

In *H. clypeatus, H. hallani,* and *H. pyrrithrix*, males and females did not differ in the time they spent resting within the three major habitat types (*H. clypeatus:* leaf litter: $X^2=0.347$, $P=0.556$; vegetation: $X^2=0.347$, $P=0.556$; rock/dirt: $X^2=3.05$, $P=0.081$; *H. hallani*: leaf litter: $X^2<0.001$, $P>0.999$; vegetation: $X^2<0.001$, $P>0.999$; *H. pyrrithrix:* leaf litter: $X^2=0.020$, $P=0.887$; vegetation: $X^2=0.324$, $P=0.569$; rock/dirt: $X^2=0.587$, $P=0.444$; Figure 11). When the different habitat types were divided into finer categories (cottonwood litter, willow litter, cottonwood vegetation, willow vegetation, grass, and dirt/rock), there was still no difference between the sexes on any habitat type for *H. clypeatus*, *H. hallani*, or *H. pyrrithrix* (see supplementary material in Appendix C). With the exception of *H. hallani* (where males spent more time in the sun than females; X^2=4.67, P=0.031), the sexes did not differ significantly in the amount of time spent in the sun vs. the shade (*H. clypeatus:* X^2=1.00, P=0.317; *H. pyrrithrix*: X^2=0.067, P=0.795, Figure 12)

For comparison, in *H. hirsutus*, there were no significant sex differences in the time spent resting on different substrate classes, although there was a tendency for females to spend more time on rocks and dirt than males (leaf litter: X^2=1.66, P=0.198; vegetation: X^2=1.66, P=0.198; rock/dirt: X^2=3.53, P=0.060). When the microhabitat types were divided into finer categories as above, there were still no significant sex differences (see supplementary material in Appendix C). There was a trend towards females spending more time in the sun than males, but this difference was not statistically significant (X^2=3.39, P=0.066; Figure 12).

In *H. hallani*, males traveled greater distances than females (X^2=6.75, P=0.009; Figure 13), and there was a non-significant trend in the same direction for *H. pyrrithrix* (X^2=3.30, P=0.069; Figure 13). However, in *H. clypeatus*, there was no sex difference in the total distance traveled over the course of behavioral observations (*H. clypeatus:* t_8=0.896, P=0.396; Figure 13). Despite some evidence that males travel further than females, there were no sex differences in the number of microhabitat types they moved through during behavioral observations (*H. clypeatus:* t_{10}=-0.97, *P*=0.356; *H. hallani:* X^2=0.108, *P*=0.742; *H. pyrrithrix:* X^2=0.991, *P*=0.320; Figure 14). Furthermore, males and females did not differ in the amount of time they spent in their preferred habitat type compared with others (*H. clypeatus:* t_{10}=-0.65, *P*=0.530; *H. hallani:* X^2<0.0001, *P*>0.999; *H. pyrrithrix:* X^2=0.613, *P*=0.434; Figure 15).

Similarly, in *H. hirsutus*, males and females did not differ in total movement distance (X^2=1.67, *P*=0.196; Figure 13), number of microhabitats utilized (X^2=2.38, *P*=0.123; Figure 14), or in the amount of time spent in their preferred habitat type compared with other habitats (X^2=1.94, *P*=0.164; Figure 15).

Predation on Habronattus

We directly observed thirteen predation events on *Habronattus* over the course of the study (see Table 3). 77% of these involved predation by conspecifics (n=6) or heterospecific *Habronattus* (n=4); the other three were by an ant, a wolf spider, and a different salticid species (Table 3). Of the *Habronattus* events, the predators were always the same size or larger than the prey. In 80% of cases, the predators were adult females, while the remaining 20% were juveniles (Table 3).

Examining the remains of old mud dauber wasp nests (n=23) indicated that salticids, and in particular *Habronattus,* make up a significant part of their diet (see Table 4). Of the 23 nests that we examined, 14 still had remaining spider provisions; the other 9 had dead wasps (adults, larvae, or pupae) that appeared to have eaten all of their provisions but for some reason not survived to emergence. Of the nests that still had spider provisions remaining, the total number of spiders per cell ranged from 4 to 51, with a total of 211 spiders, 31 of which were salticids. Three of the fourteen nests contained at least one salticid. Interestingly, for the nests that contained any salticids at all, 75% of the spiders that were in those nests were salticids. Of those salticids, 89% (n=26) were *H. hirsutus* while the rest were immature *Phidippus* sp. All *H. hirsutus* specimens found in nests were mature adults (n=12) or large juveniles (≥ 4mm, n=14) (Table 4).

Discussion

From direct observations of free-ranging spiders in the field, we show that bold and conspicuous sexually dimorphic dorsal coloration in males of three species of *Habronattus* (*H. clypeatus, H. hallani*, and *H. pyrrithrix*) is associated with increased male leg-waving behavior similar to the false antennation behavior exhibited by other spiders that mimic hymenopterans (e.g., McIver & Stonedahl 1993; Cushing 1997). Females of all three species are cryptic in coloration and do not engage in this putative false antennation behavior. Also consistent with the deception hypothesis is the finding that, in the one species in our study in which males do not have conspicuous markings (*H. hirsutus*), males correspondingly do not show the increased leg-waving behaviors seen in the other three species. When examining two hypotheses for why males and females from three species differ in their dorsal coloration strategies, we found support for the activity hypothesis, which posits that higher movement rates of males may have constrained the evolution of cryptic coloration, resulting in different coloration strategies between the sexes. Because sexes did not different in microhabitat use, our data failed to support the different habitat hypothesis for sex differences in coloration (discussed in more detail below).

Evidence for deceptive coloration and its alternatives in Habronattus *males*

While our observations and field data are suggestive of dorsal color and behavior working together to deceive predators, it is important to weigh support against alternative explanations that are commonly proposed for bold and conspicuous colors, such as disruptive coloration (e.g., Cott 1940; Stevens & Merilaita 2009) or motion dazzle markings (Stevens 2007; Stevens et al. 2008; Scott-Samuel et al. 2011). Disruptive coloration is a form of camouflage that employs markings that break up an animal's outline or create the appearance of false edges and boundaries, hindering the detection or recognition of an animal's true shape (e.g., Cott 1940; Stevens & Merilaita 2009). As such, disruptive pattern elements are expected to be biased towards borders and edges (Merilaita 1998); empirical work has shown that such markings are indeed more effective at deterring predation when they occur along body outlines (Cuthill et al. 2005; Schaefer & Stobbe 2006). In the conspicuously-colored *Habronattus* males in our study, the contrasting patterns are located in the center of the abdomen (see Figure 1). In contrast with disruptive coloration, which interrupts edges, in all three species, the abdomen is bordered with a conspicuous white margin, which clearly highlights the outline of the body, rather than disrupting it (see Figure 1a, c, e).

Furthermore, the frequent leg-waving of males attracts attention and clearly identifies the head of the spider, which appears to amplify the body shape rather than obscure it (LAT, pers. obs.), suggesting that this coloration is not functioning as disruptive coloration.

Another potential explanation for conspicuous markings that has received recent attention is the idea that they interact with movement creating a ‘motion dazzle’ effect, making it difficult for predators to estimate their speed and trajectory at high speeds (Stevens 2007; Stevens et al. 2008; Scott-Samuel et al. 2011). Empirical support comes from human subjects and computer animations; at high speeds, dazzle coloration affects the perceived speed of objects moving in a straight line (Scott-Samuel et al. 2011) and makes the capture of moving prey more difficult (Stevens et al. 2008). This, too, seems improbable for *Habronattus* males, whose movement patterns are relatively slow compared with the animations used in dazzle studies (15-20cm/s: Stevens et al. 2008; 357 cm/s: Scott-Samuel et al. 2011). Furthermore, movement patterns of *Habronattus* males are jerky, and involve frequent stopping and zig-zagging through their habitat as they search for females (LAT, pers. obs.), making this an unlikely system for dazzle coloration to be effective.

The present study provides the first step towards understanding if and how male dorsal patterns in *Habronattus* deceive predators. Further experiments with color-manipulated spiders and ecologically relevant suites of predators are clearly needed. Our study examines color patterns in their natural context with an emphasis on natural history; it has recently been argued that this emphasis is missing from many studies on protective coloration (Stevens 2007). Furthermore, our results identify two groups of organisms that likely to be important predators in this system (mud dauber wasps that specialize on spiders and other *Habronattus*), setting the stage for work that examines how predator perception and cognition influence the evolution of prey color patterns and behaviors (e.g., Chittka & Osorio 2007; Stevens 2007). Because mud dauber wasps hunt exclusively for spiders (e.g., Landes et al. 1987; Elgar & Jebb 1999; Blackledge & Pickett 2000; Camillo 2002), the deceptive strategy of male *Habronattus* may simply allow them to escape a mud dauber's search image.

Interestingly, in the single species in our study in which males do not exhibit conspicuous coloration (*H. hirsutus*), males correspondingly lack the increased leg-waving behavior relative to females. While this is consistent with the idea that male color pattern and leg-waving function together in *Habronattus*, a comparative study that examines a larger number of species both with and without conspicuous coloration is clearly needed. The genus *Habronattus* is both diverse and speciose (Griswold 1987), and the availability of a molecular phylogeny (Maddison & Hedin 2003) makes it an ideal group for testing hypotheses about the coevolution of dorsal color pattern and leg-waving behavior within a phylogenetic framework. Such phylogenetic studies may also help explain why males of different species, such as *H. hirsutus*, have not adopted conspicuous color patterns like those found in *H. clypeatus, H. hallani*, and *H. pyrrithrix* males. Data from this study indicate that *H. hirsutus* spends 80% of their time above ground in the vegetation compared with the other three species which all spend more than 74% of their time in the leaf litter on the ground (see Figure 11). Furthermore, *H. hirsutus* was the only species of *Habronattus* that we found in mud dauber nests (see Table 4). Such differences in natural history should be explored further as potential explanations for the selection pressures that have differently shaped male color patterns.

We tested two hypotheses to explain why male and female *H. clypeatus, H. hallani,* and *H. pyrrithrix* differ in dorsal color pattern. We failed to uncover support for the different habitat hypothesis. In none of these species did males and females differ in the time spent in different microhabitats, and with the exception of *H. hallani* none differed in the amount of time that the sexes spent in the sun vs. the shade. While there was some evidence that males traveled greater distances than females during observations, this did not lead to males traversing a larger number of habitat types during observations. Furthermore, there were no differences in the proportion of time spent in an individual's preferred habitat type relative to other habitat types. Instead, the activity hypothesis to explain male color pattern differences was supported in all three conspicuously colored species; males indeed spent more time actively moving than females. Interestingly, and as expected, this relationship did not hold up for *H. hirsutus.*

A novel twist on the activity hypothesis

In this study, we show that, in three species of *Habronattus,* females spend relatively small amounts of time moving compared to males, and thus, cryptic coloration may be an ideal strategy. In contrast, males must move, presumably in search of females, and thus they need to find an alternative strategy for protection. Our study lends support to the idea that males have solved this problem by pairing conspicuous patterns of bold stripes and chevrons with leg-waving behavior to deceive predators. The rationale behind the activity hypothesis is not new; both theoretical and empirical work support the idea that movement can limit the effectiveness and ultimately constrain the evolution of cryptic coloration, leading to alternative solutions for protection in other animals (e.g., Merilaita & Tullberg 2005; Ioannou & Krause 2009). Our evidence in support of the activity hypothesis comes from observations on males outside of the context of courtship, as leg-waving behavior was only quantified when males were not interacting with other spiders (see Methods). Yet observations of male courtship behavior suggest that the same color patterns that presumably protect males when wandering in search of females might also be strategically designed to function while males are actively courting. Leg-waving is a standard feature of jumping spider communication and courtship (Jackson 1982), and this waving and extension of the first pair of legs occurs throughout the entire courtship display in *Habronattus* (see Figure 8). Males must use extreme caution when displaying for females who are also generalist predators and often attack conspecifics (e.g., see Table 1, see also Jackson & Pollard 1997). Given this risk, it is not surprising that courting male *Habronattus* are extremely focused on courtship and often appear oblivious to external stimuli or threats when courting (LAT, pers. obs.). Here we propose the ‘focused courtship’ hypothesis as an extension of the activity hypothesis. Because males must already extend and wave their first pair of legs to communicate with females during courtship, adding conspicuous patterning to their backs creates the combination of conspicuous stripes and leg-waving that is characteristic of hymenopteran mimics. It has been suggested that in ant mimics, behavioral mimicry (i.e., false antennation) evolved before morphological mimicry (reviewed in Cushing 1997). In *Habronattus,* as well as other salticids, this transition to behavioral mimicry might be a relatively simple step as it involves taking a behavior that is already part of courtship (leg-waving) and extending it to the mate search context as well. In courting male *Habronattus* jumping spiders, actively and clearly identifying their location to females from a safe distance is a necessity; this goal likely conflicts with any attempt to avoid detection by potential predators (which include the very females whose attention they are trying to capture). As such, selection on *Habronattus* should drive protective strategies that continue work *after* detection and that do not require the male to risk taking his focus off of a potentially cannibalistic female; this may explain why mimicry is so widespread across the salticidae (e.g., Cushing 1997).

In conclusion, here we provide evidence that, in three sympatric species of *Habronattus,* male color and pattern work together, presumably, to deceive predators. We then provide support suggesting that this coloration likely differs between males and females as a result of differences in activity patterns (i.e., movement rates) between the sexes, and speculate that, perhaps, it is also a result of the unique need for males to remain focused on the potentially cannibalistic females that they court. Despite the extensive literature on the function and evolution of protective coloration (e.g., see reviews in Ruxton, 2004

#543}(Stevens 2007; Stevens & Merilaita 2009; Ruxton & Schaefer 2011), to our knowledge, no study has examined sex differences in non-display coloration in the Salticidae. This is surprising, given the degree to which non-display dorsal color patterns differ between the sexes across the family, often being more conspicuous in males (e.g., see Maddison 1995). Recent work on tropical caterpillars with large vertebrate-like eyespots and face-like markings suggest that mimicry complexes may play out in unexpected ways, depending on natural history (Janzen et al. 2010). Unlike most models of Batesian mimicry (e.g., see review in Ruxton et al. 2004), in these tropical caterpillars, avoidance by predators is a function of innate, rather than learned avoidance of eye-like and face-like patterns (Janzen et al. 2010). As such, there is no strong selection on mimics to perfectly resemble any specific species of model and due to the extremely high cost of ignoring the danger of recognizing eyes or faces on a real predator, harmless mimics may largely outnumber their models(Janzen et al. 2010). This system may be an example of where imperfect mimicry and perceptual exploitation are difficult to tease apart (e.g., Schaefer & Ruxton 2009; Ruxton & Schaefer 2011). The *Habronattus* system examined in the present study seems to resemble such a system; male leg-waving and striped patterns do not cause males to perfectly resemble a particular wasp or bee, but rather likely play on a potential predator's innate aversions to such cues (Kauppinen & Mappes 2003). Additionally, in our study populations, *Habronattus* males are abundant, perhaps even more so than their presumed models (LAT, pers. obs.), yet the high risk to predators of mistakenly attacking a hymenopteran may be enough to provide true benefits. In the case of avoiding attack from spider-hunting mud dauber wasps, looking like anything other than a spider might be enough to deter an attack. Such systems which challenge current thinking about Batesian mimicry are exciting and may provide new insights into the function and evolution of diversity in animal color patterns.

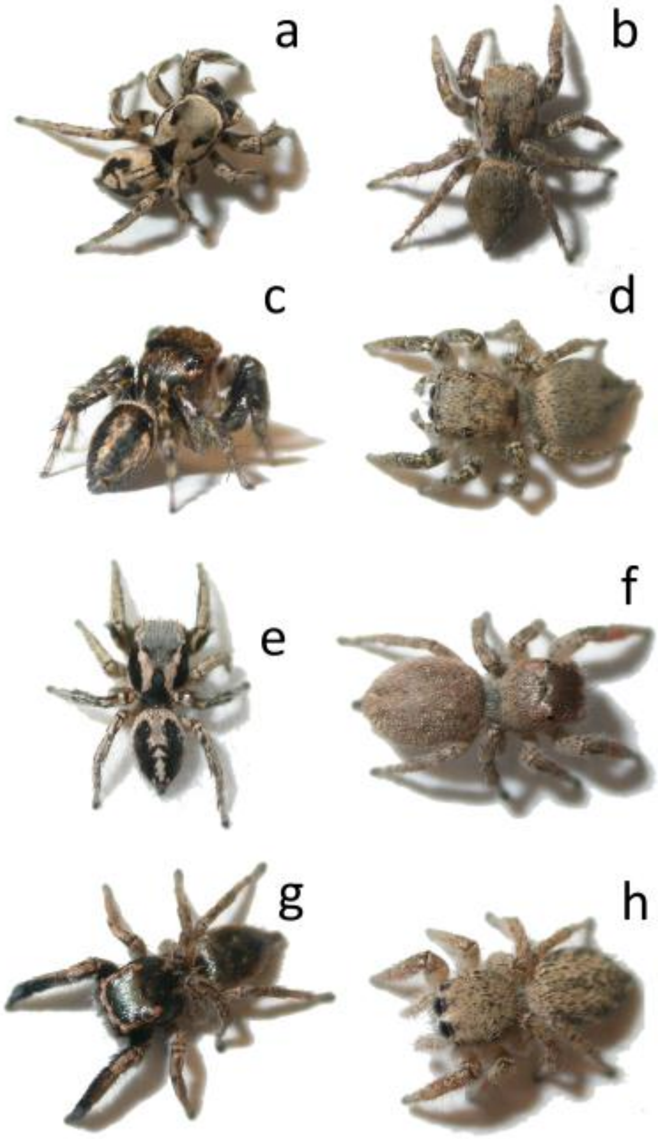

Figure 7. Sexual dimorphism in dorsal color pattern in four sympatric species of *Habronattus*. *H. clypeatus* male (a) and female (b), *H. hallani* male (c) and female (d), *H. pyrrithrix* male (e) and female (f), *H. hirsutus* male (g) and adult female (h). Note that males of *H. clypeatus* (a), *H. hallani* (c), and *H. pyrrithrix* (e) exhibit conspicuous dorsal markings, while males of *H. hirsutus* (g) do not. While females all look similar to one another, they can be identified based on subtle differences in dorsal and facial markings.

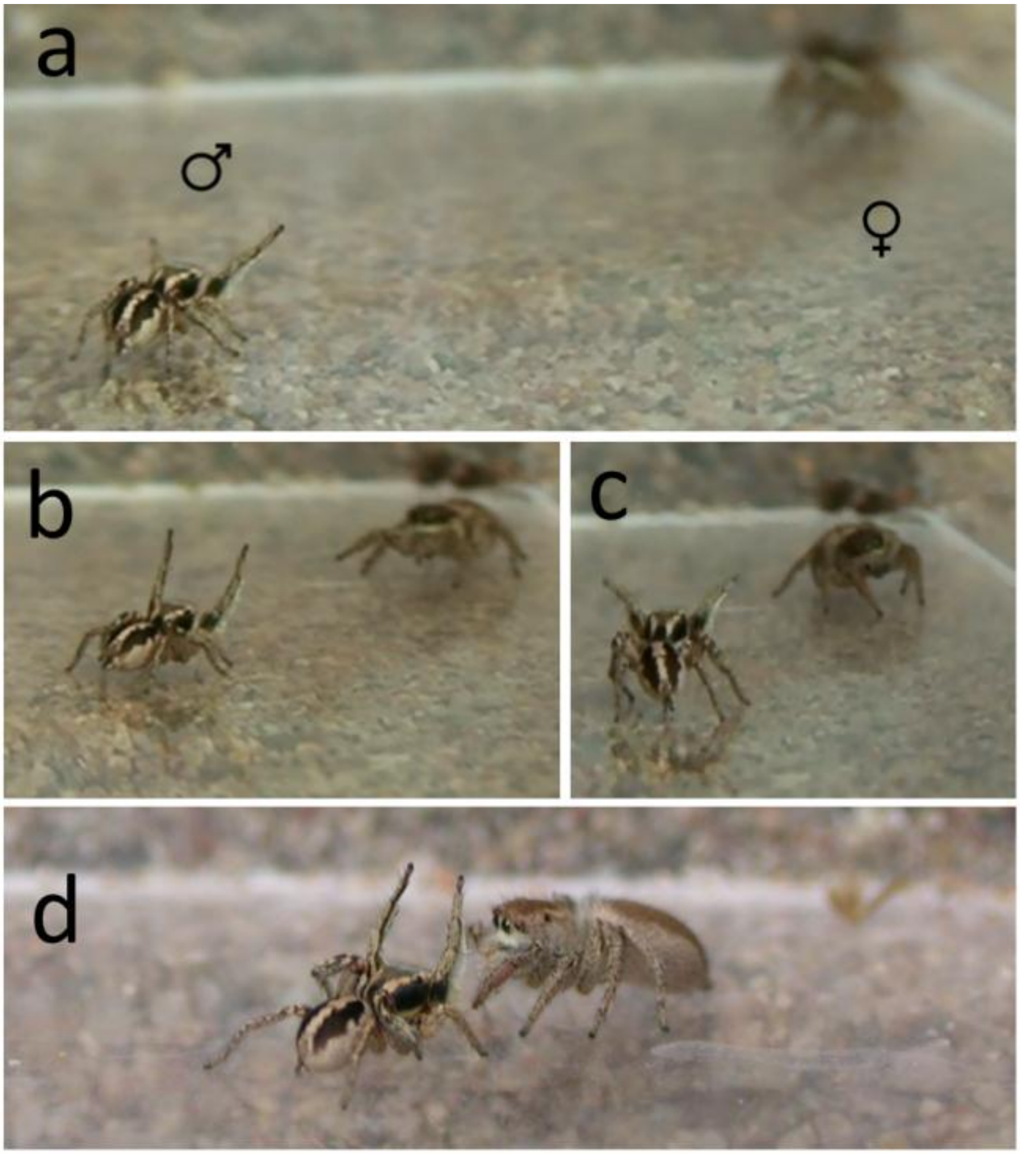

Figure 8. Courtship display in *Habronattus pyrrithrix*. In the first stage of display, the male approaches the female and displays his ornamented red face and the green undersides of his front legs (a, b, c). In stage 2 of display, he approaches and stops directly in front of the female and initiates an additional seismic component to his display (d). Note that the male’s conspicuous dorsal pattern is oriented away from the female throughout the entire display.

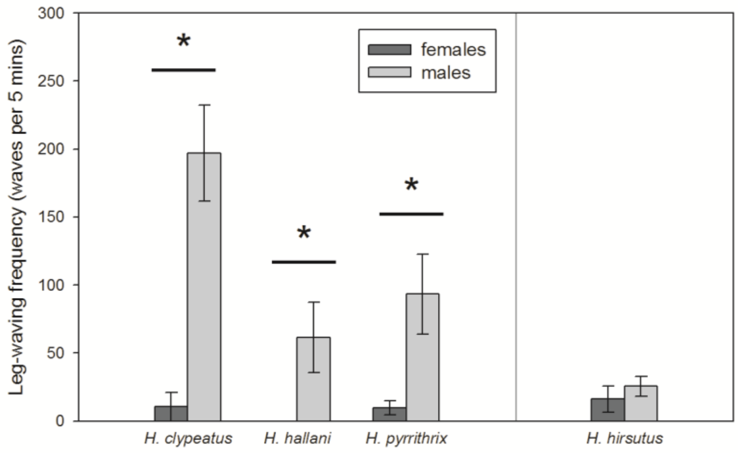

Figure 9. Comparison of leg-waving frequency between the sexes during behavioral observations in the field (mean± SEM). Asterisks (*) indicate significant sex differences within a species.

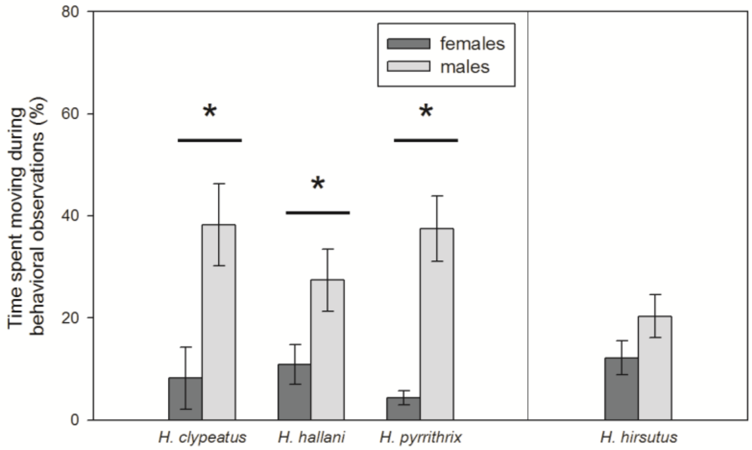

Figure 10. Comparison of time spent moving between the sexes during behavioral observations in the field (mean± SEM). Asterisks (*) indicate significant sex differences within a species.

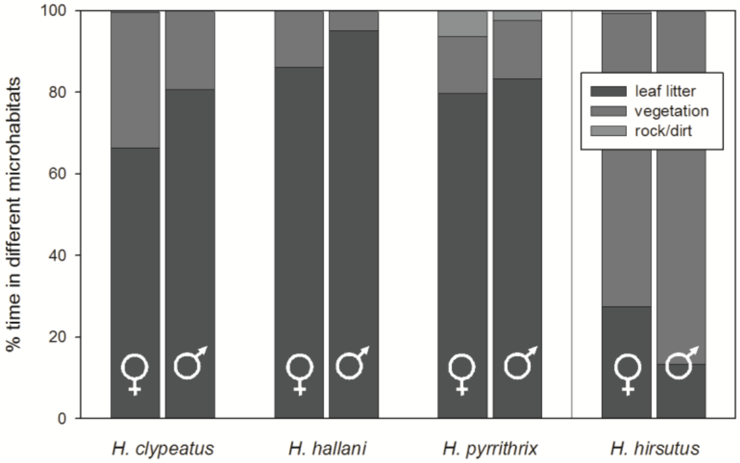

Figure 11. Comparison of mean proportions of time spent in different microhabitat types. Note that there are no significant sex differences for any species. For clarity, we include three broad habitat types in this analysis (leaf litter, vegetation, rock/dirt). However, in a second analysis, we broke up the microhabitats further by plant species (cottonwood leaf litter, desert willow leaf litter, cottonwood vegetation, desert willow vegetation, grass, and rock/dirt) and found that there were still no differences between the sexes (see Results).

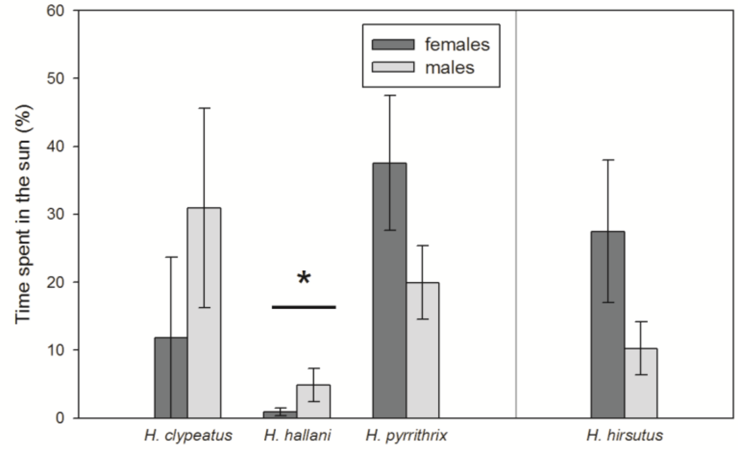

Figure 12. Comparison of % time spent in the sun between the sexes during behavioral observations in the field (mean± SEM). Asterisks (*) indicate significant sex differences within a species.

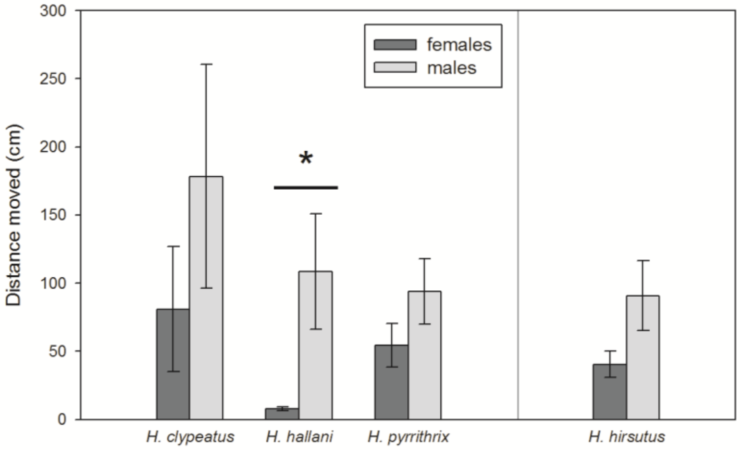

Figure 13. Comparison of the total distance traveled over the course of 15-minute behavioral observations (mean± SEM). Asterisks (*) indicate significant sex differences within a species.

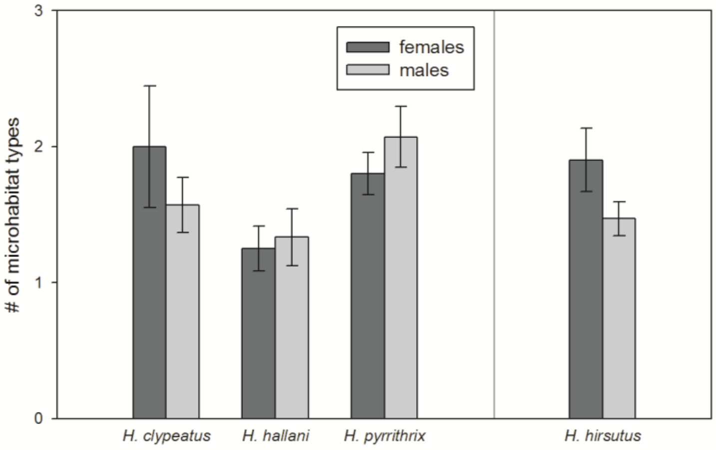

Figure 14. Comparison of the number of different habitat types individuals traveled through during the course of 15-minute behavioral observations (mean± SEM). Note that there are no significant sex differences for any species. Available habitat types included: cottonwood leaf litter, desert willow leaf litter, cottonwood vegetation, desert willow vegetation, grass, and rock/dirt.

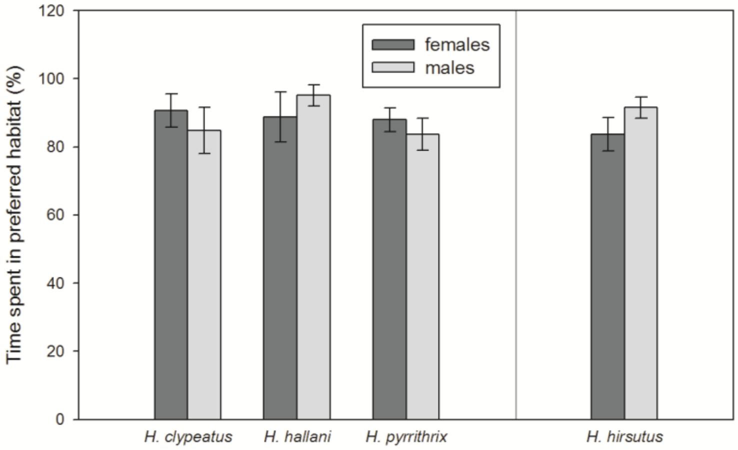

Figure 15. Comparison of the proportion of time individuals spent in their preferred habitat type (mean± SEM). Note that there are no significant sex differences for any species.

Figure 16. Contents of three cells of a mud-dauber wasp nest (Sphecidae) found in the vicinity of the Rio Salado Habitat Restoration Area, Phoenix, AZ, USA, indicating that at least for some individual wasps, *Habronattus* makes up a significant portion of their prey.

Table 3. List of predation events on *Habronattus* observed at the Rio Salado Habitat Restoration Area in Phoenix, AZ, USA.

Prey species	Prey sex	Size class (mm)	Predator	Predator sex	Size class (mm)
H. clypeatus	adult male	5	*H. clypeatus*	adult female	6
H. clypeatus	adult male	5	*H. pyrrithrix*	adult female	6
H. clypeatus	adult male	5	*H. pyrrithrix*	adult female	6
H. hirsutus	adult female	6	*H. hallani*	adult female	6
H. hirsutus	juvenile*	3	*H. hallani*	adult female	6
H. hirsutus	juvenile*	3	*H. hirsutus*	juvenile*	3
H. hirsutus	juvenile*	4	*H. hirsutus*	adult female	6
H. hirsutus	juvenile*	4	*H. hirsutus*	juvenile*	4
H. hirsutus	juvenile*	4	*H. hirsutus*	adult female	6
H. pyrrithrix	adult female	2	Formicidae (ant)	unknown	3
H. pyrrithrix	adult female	6	Lycosidae (wolf spider)	adult female	10
H. pyrrithrix	adult male	5	*H. pyrrithrix*	adult female	6
H. pyrrithrix	adult male	5	*Phidippus* sp. (Salticidae)	juvenile*	7

* In *H. hirsutus* and *Phidippus* sp., sex-specific color patterns do not appear until maturity; thus, the sex of juveniles is unknown

Table 4. *Habronattus* specimens found in mud dauber wasp nests (family Sphecidae) near the Rio Salado Habitat Restoration Area, Phoenix, AZ

Prey species	**Sex**	**Size class (mm)**	**Quantity**
H. hirsutus	adult female	6	10
H. hirsutus	adult male	6	2
H. hirsutus	subadult male†	5	2
H. hirsutus	juvenile*	4	12

† For all *Habronattus*, subadult males (just prior to sexual maturity), exhibit enlarged pedipalps, allowing us to identify them as males before their final molt into adult coloration

* In *H. hirsutus*, sex-specific color patterns do not appear until maturity; thus, the sex of most juveniles is unknown

Chapter 3

ONTOGENETIC COLOR CHANGE IN SEXUALLY DICHROMATIC *HABRONATTUS* PYRRITHRIX JUMPING SPIDERS

Abstract

Animals use colors for a variety of purposes, from acquiring mates to avoiding predators. In many animals, these color patterns are not static throughout life, but change drastically during development, maturity, and senescence. During development, selection pressures can shift as animals change in size, vulnerability to predation, habitat use, or reproductive status. Even after maturity, maintaining coloration can be costly, as the pigments and structures that produce colors may degrade over the course of the mating season and throughout an animal’s life. While recent work has focused on the signaling value of salticid colors, we know very little about how these colors change as spiders develop and age; such information can provide a context for understanding the functions of, and the constraints on, colorful signals. Focusing on the sexually dichromatic jumping spider, *Habronattus pyrrithrix*, our goals were to (1) examine the microscopic morphology of the elaborately colored body regions that males display to females during courtship (i.e., males’ red faces, green legs, and white pedipalps), (2) examine how the colors of these regions as well as dorsal color patterns change during development leading up to sexual maturity, and (3) examine how male condition-dependent red facial and green leg coloration changes as males age beyond sexual maturity. Although the bright white pedipalps and the green leg coloration of males appeared only at sexual maturity (after their final molt), males and females began to differentiate in red facial coloration and dorsal patterning as young juveniles (ca. 2.5 mm, or ca. 45% of their total mature adult body size), with males developing the red facial coloration and conspicuous black and white dorsal patterning typical of sexually mature adult males. Even after sexual maturity, color was not static; a male's green leg coloration (but not his red facial coloration) faded with age. Our results are discussed in the context of potential costs, constraints, and benefits of the production and maintenance of different types of color in salticids.

Introduction

Animal colors and patterns can serve a variety of functions. They are often displayed in courtship to aid in species recognition or to convey information about the quality of an individual as a mate (see reviews in Andersson 1994; Hill & McGraw 2006a). They are also frequently used to help animals avoid predators by aiding in aposematism or mimicry (see reviews in Cott 1940; Ruxton et al. 2004). In many animals, these color patterns are not static throughout life, but change drastically during development, maturity, and senescence, as well as seasonally (Booth 1990). When color patterns differ between the sexes, examination of ontogenetic color change is particularly interesting because the timing and extent of color differentiation between the sexes can give us a more holistic understanding of the costs and benefits of different color patterns and their functions and constraints across contexts throughout an animal's life.

In many animals, individuals undergo dramatic and seemingly strategic color changes as they mature; such color changes likely reflect different selection pressures as individuals change in size, vulnerability to predation, habitat use, or reproductive status (Booth 1990). In animals where bright male colors have evolved via sexual selection, sex-specific color patterns often appear suddenly upon sexual maturity, presumably because they are costly and unnecessary for juveniles, who do not engage in courtship or male-male agonistic interactions (Andersson 1994). When sexually selected colors appear before sexual maturity, they are particularly interesting because they may hint at previously overlooked functional roles (e.g., Kilner 2006; Kapun et al. 2011). When the sexes differ in color pattern due to different ecological selection pressures (e.g., Slatkin 1984), the timing of color pattern divergence can help us understand shifting selection pressures. For example in *Eremias lugubris* lizards, adults and older juveniles are tan and cryptic, whereas young juveniles have highly conspicuous markings, mimicking noxious oogpister beetles (Huey & Pianka 1977); in this system, subtle and changing functional roles of color would be missed by limiting study to adult stages.

In addition to the apparently strategic color changes that occur with development, mature organisms can also change color more subtly as they age, particularly if pigments or structures that produce colors break down over time (Booth 1990). There is growing evidence that animal colors, including those that involve pigments or structures contained within dead tissue (e.g., feathers, scales), can fade with age as a product of abrasion, soiling, or photobleaching (Ornborg et al. 2002; McGraw & Hill 2004; Delhey et al. 2006; Kemp 2006). If maintenance of coloration is costly, such age-based fading can have important consequences for signaling with the ability to maintain bright colors (i.e., the ability to resist tissue/pigment damage) acting as an indicator of quality (e.g., Delhey et al. 2006). Alternatively, color fading may provide direct information about an individual's age (Manning 1985). Such information could help individuals identify older, more viable mates (reviewed in Kokko & Lindstrom 1996). Alternatively, if older individuals are more likely to carry disease or parasite infection (e.g., Tarling & Cuzin-Roudy 2008), or if they are more likely to accumulate deleterious mutations in their germ-line (Beck & Promislow 2007), age-based color variation might enable individuals to identify and select younger mates. A deeper understanding of how, and ultimately, why colors change with age will enable us to generate informed predictions about their potential signal content.

Jumping spiders are an understudied, yet excellent group in which to examine ontogenetic color change from development through senescence. Adult males are often more colorful than females, and in many cases they display these colors to females during courtship or to other males during competitive interactions (e.g., Peckham & Peckham 1889, 1890; Lim & Li 2004; Girard et al. 2011). In addition, sexual dichromatism in dorsal color that is not displayed during courtship may reflect different predator-avoidance strategies of males and females (see Chapter 2). To date, only three jumping spider species have had any aspects of their colors quantified using modern color measurement techniques (i.e., spectrophotometry) (*Cosmophasis umbratica* (Lim & Li 2006a), *Phintella* *vittata* (Li et al. 2008a), and *Habronattus pyrrithrix* (Taylor et al. 2011, Appendix B)), and in only one study were juvenile colors measured (Lim & Li 2006a). To our knowledge, no study has documented age-based changes in salticid colors as they develop from spiderlings through sexual maturity. Because species descriptions and dichotomous keys typically include details on only adults, with anatomy of mature genitalia required for proper identification (e.g., Ubick et al. 2005), the salticid literature is lacking even in qualitative descriptions of juvenile color patterns.

The jumping spider genus *Habronattus* is one of the most highly ornamented groups with approximately 100 species; males are typically elaborately and conspicuously colored, whereas females are drab and cryptic (Griswold 1987; Maddison & Hedin 2003). Furthermore, patterns of juvenile coloration also vary across the genus (LAT, pers. obs.). For example, in *H. hirsutus*, juveniles of both sexes are indistinguishable from one another and resemble cryptic adult females until sexual maturity (LAT, pers. obs.). In *H. hallani*, juveniles of both sexes are indistinguishable from one another but have striking dorsal color patterns unlike either adult males or females (LAT, pers. obs.). In *H. pyrrithrix*, juvenile males and females exhibit color patterns similar to those of sexually mature adults; males have red faces and striped dorsal patterns, whereas females are drab and cryptic throughout their life (LAT, pers. obs). This diversity in ontogenetic color change suggests that the costs, benefits, and functions of juvenile colors might be just as interesting and diverse as those of adults. Additionally, there is evidence that, after reaching maturity, adult male ornamental colors in *H. pyrrithrix* continue to undergo additional age-related changes, which could have important implications for sexual signaling (Taylor et al. 2011, Appendix B).

Focusing on *Habronattus pyrrithrix*, our goals were to (1) examine the microscopic morphology of the elaborately colored body regions that males display to females during courtship (i.e., males' red faces, green legs, and white pedipalps), (2) examine how the colors of these regions as well as dorsal color patterns change during development leading up to sexual maturity, and (3) examine how male condition-dependent red facial and green leg coloration changes as males age beyond sexual maturity. The red facial coloration and white pedipalp coloration of *H. pyrrithrix* are contained within modified setae, or scales (e.g., Hill 1979), while the green leg coloration is present on the surface of the cuticle of the leg (e.g., Parker & Hegedus 2003; Ingram et al. 2011), which is further adorned with white scales (LAT, pers. obs.). Recent work on *H. pyrrithrix* suggests that adult male facial and leg colors that are displayed to females during courtship are condition-dependent (Taylor et al. 2011, Appendix B), and that the presence of red coloration improves courtship success in certain contexts (see Chapter 5), yet we know nothing about the role of red facial coloration in juvenile males. Conspicuous dorsal coloration in sexually mature adult males (compared with drab and cryptic coloration of females) appears to be associated with the higher movement rates of males while searching for females (Chapter 2), yet we know nothing about the potential factors that might shape color differences in sexually inactive juveniles. Even after maturity, male ornamental colors do not appear to be static (Taylor et al. 2011, Appendix B). Throughout the mating season, the scales that produce the colors may undergo natural wear and degradation, which may result in predictable, post-maturity, age-related deterioration of color (e.g., Kemp 2006; Kemp & Macedonia 2006); this may allow such colors to be used by females to assess a male's age during courtship (e.g., Manning 1985).

To our knowledge, this will be the first study to quantify ontogenetic color changes throughout development in any of the more than 5000 species (Platnick 2011) of jumping spider. Standard portable spectrophotometers used in animal coloration studies (reviewed in Andersson & Prager 2006) typically have a minimum reading area of 1 mm (e.g., Lim & Li 2006a; Moreno et al. 2006; Galvan & Moller 2009); thus, precise quantification of color can only be done on relatively large body regions (>1 mm). Thus, using standard equipment makes the study of minute patches of color on small species of spiders challenging and makes the detailed study of color on particular body regions of juvenile salticids (e.g., faces, legs, pedipalps) impossible. Here we use a custom-designed microspectrophotometer (See Methods, see also Taylor et al. 2011, Appendix B) allowing us to carefully measure minute patches of color on juveniles and compare colors with those same precise areas on adult spiders.

Methods

Study species

Habronattus pyrrithrix Chamberlin 1924 is found throughout southern California and Arizona, USA south to Sinaloa, Mexico (Griswold 1987). In Phoenix, Arizona, they are quite common and found at high densities in riparian areas, grassy backyards, and agricultural fields (LAT, pers. obs.). Geographic variation in coloration is common within the genus *Habronattus* (see Griswold 1987) and thus some subtleties of color pattern described in the present study may be typical of this Phoenix, AZ population, and may vary across the species range. Additional details on the biology and courtship display behavior of *H. pyrrithrix* are provided in Chapters 4 and 5.

Scale morphology of adult male ornaments

Using sexually mature adult specimens, we imaged the color patches on the males' red face, green front legs, and white pedipalps that they display to females using a Leica-Cambridge Stereoscan 360 field emission scanning electron microscope (SEM) (Leica Microsystems, Wetzlar, Germany) at an acceleration voltage of 2kV. Prior to imaging, we allowed frozen specimens to air-dry overnight and then mounted the carapace, legs, and pedipalps onto standard SEM stubs using conductive graphite paint.

Color study 1: Ontogenetic color changes in juveniles

To examine how male and female coloration changes during juvenile development in the field, we collected spiders (n=135) from a range of developmental stages (i.e., size classes) between May and October 2008 from a single, dense population within an agricultural area in Queen Creek, Arizona, USA (Maricopa County, 33.224744 N, 111.592825 W). This population was chosen because, in contrast with other sites where multiple species are abundant and interact (see Chapter 1), the only species of *Habronattus* that we have ever seen at this site in five years is *H. pyrrithrix*. This allowed us to be confident that all spiderlings and juveniles included in the present study were *H. pyrrithrix*. Specifically, we collected spiderlings (before they are able to be sexed, ca. 1.5-2.0 mm in length, n=15), small juveniles, (ca. 2.5 mm, n=15 males, n=15 females), large juveniles (ca. 3 mm, n=15 males, n=15 females), subadults (ca. 4-6 mm, n=15 males, n=15 females) and sexually mature adults (ca. 5-7 mm, n=15 males, n=15 females). Immediately after collection, we froze spiders (-80° C) for later color analysis.

Color study 2: Post-maturity age-related changes in condition-dependent male ornaments

To examine how adult male color changes with age post-maturity, we collected 12 gravid adult females in July and August 2008 from the same population described above and brought them back to the lab and allowed them to lay eggs. Spiderlings were housed together until they were large enough to be sexed (ca. 2.5mm in length), at which point the first three males from each female's egg sac were removed, housed separately in clear plastic containers (6x6x13cm), and fed a constant diet of small crickets (*Acheta domesticus*). Spiders (n=36; three from each of 12 egg sacs) were checked daily to determine if they had molted; within each clutch, as males reached their final molt to maturity, they were randomly assigned to one of three different age groups (0, 60, and 120 days post-maturity). These age ranges were chosen because they likely represent the difference in ages of males in the field during the most active part of the mating season at this site (approximately May-August; LAT, pers. obs.). When males reached the appropriate randomly assigned age (0, 60, or 120 days post-maturity), we euthanized them and placed them in the freezer (-80° C) for color analysis.

Color measurement and analysis

Body colors were quantified following methods described in Taylor et al. (2011, Appendix B). Briefly, we used a reflectance spectrophotometer (USB2000, Ocean Optics, Dunedin, FL, USA) coupled to a modified Leica DMLB2 fluorescence light microscope with a 40x quartz objective lens (Leica Microsystems, Wetzlar, Germany) and illuminated with a full-spectrum Leica 75 W xenon arc lamp (Leica Microsystems, Wetzlar, Germany). This setup allowed us to quantify the minute color patches of all size classes of these spiders that are too small to measure accurately with standard spectrophotometry equipment. Unfortunately, the optics of the microscope cut out a portion of the UV spectrum, so this instrument only provides spectral data from 375-700nm. In some jumping spider species, UV reflectance appears to be important in communication (Lim et al. 2007; Li et al. 2008b; Lim et al. 2008), and thus we must use caution when excluding UV wavelengths from our analyses. However, we confirmed in a previous study that, though reflectance does extend into the UV for the green legs and white pedipalps, there are no UV peaks in either region, so the benefit of using an instrument that allows precise and repeatable measures on minute color patches of these tiny spiders far outweighs the disadvantage of excluding UV (Taylor et al. 2011, Appendix B).

For Study 1, where we were interested in color changes of the faces, front legs, and pedipalps of males and females that occurred during juvenile development through maturity, we took the average of two reflectance measures from each of these three body regions. From these spectral data, we calculated the single color variable that captured the most sex- and age-related variation. Specifically, because face color among the different sex/age classes varied from white to red, the metric that captured most of this variation was ‘red chroma’ (i.e., the proportion of total reflectance in the red region of the spectrum, between 600 and 700 nm). Similarly, because the front legs varied from white to green, the metric that captured most of this variation was ‘green chroma’ (the proportion of total reflectance between 450 and 550 nm). Finally, because the pedipalps varied in coloration from gray to bright white, brightness (total reflectance over the entire spectrum) was the metric that captured most of this variation. For a detailed discussion of the rationale behind selecting relevant color variables, including those used here, see Montgomerie (2006). In addition, we qualitatively characterized the dorsal color pattern of individuals as either (1) tan and cryptic in coloration, similar to the dorsal coloration of adult females, or (2) consisting of black and white stripes and chevrons, characteristic of adult males (see Chapter

2); all individuals examined fit clearly into one of these two categories. Because these categorizations were based on pattern rather than reflectance properties of the colors, we did not quantify dorsal coloration spectrophotometrically.

For Study 2, where we were interested in more subtle, age-based fading of display colors in adult males, we limited our analysis to the coloration of the red face and green legs, because previous studies showed that these two color patches were condition-dependent in the field, presenting the possibility that such condition-dependence could be explained in part by the fading of colors as males age (Taylor et al. 2011, Appendix B). We took the average of two reflectance measures from each individual's face and front legs and used these spectral data to calculate three color variables that were found to be condition dependent (and thus, potentially driven by age) in a previous study: (1) the hue of the red face (the wavelength corresponding to the inflection point of the red curve), (2) the red chroma of the face (the proportion of total reflectance between 600 and 700 nm), and (3) the brightness (mean reflectance) of the green front legs, following the methods described in Taylor et al (2011, Appendix B). We also determined the relative size of the male's red facial patch, which was also previously found to be condition dependent (see Methods in Taylor et al. 2011, Appendix B). Three males died over the course of the study for unknown reasons and were thus excluded from our analyses.

Statistical analysis

For Study 1, we used analyses of variance (ANOVA) to examine effects of developmental stage (i.e., size class), sex, and their interaction on face color (red chroma), front leg color (green chroma), and pedipalp color (mean brightness). Data did not meet normality and equal-variance assumptions and thus were rank transformed (Conover & Iman 1981). For Study 2, we used ANOVA to examine the effects of age on the hue, red chroma, and size of a male's red face and on the brightness of his green legs. Because we used three males from each clutch (one assigned to each age category), we included the clutch (i.e., mother's identity) as a random factor in the model. Following ANOVA, we compared the colors among age classes using Tukey-Kramer pairwise comparisons with an alpha level of 0.05. All data from Study 2 met the assumptions of parametric statistics. All statistical analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC, USA).

Results

Scanning electron microscopy revealed varied scale structure on the three different body regions of males (Figure 17). Examination of the red scales on the face revealed ridged protrusions covering the surface of each scale (Figure 17a,b). The green legs were ornamented with long spatulate scales, the flattened ends of which were covered with fine ridges (Figure 17c,d). The white scales on the pedipalps were similar in size and shape to the red facial scales, but were relatively smooth in comparison (Figure 17e,f).

In Study 1, we found a significant age x sex interaction on all three color metrics examined (Table 5) indicating that colors developed differently between the sexes. Although spiderlings of both sexes had sparse red scales around their anterior median eyes (Figure 18a), development of red coloration on the face was apparent in small juvenile males and it increased into adulthood, whereas small juvenile females developed white facial scales (Figure 19a, Figure 18). Similarly, the conspicuous dorsal color pattern of males was also fully developed in small juveniles (ca. 2.5mm), whereas all spiderlings and juvenile females had a cryptic, tan dorsal color pattern similar to adult females (Figure 20). In contrast, the green coloration of the legs and the bright white pedipalp coloration typical of adult males showed a sudden onset at sexual maturity (Figure 19b,c).

In Study 2, the green leg coloration of adult males was brighter (lighter) with increasing age ($F_{2,21}=4.17$, $P=0.029$; Figure 21d), but we found no effect of age on any aspect of red facial coloration (hue: $F_{2,21}=0.37$, $P=0.694$; red chroma: $F_{2,21}=0.53$, $P=0.595$; size of red facial patch: $F_{2,21}=1.97$, $P=0.165$; Figure 21a,b,c).

Discussion

Here we document unique scale morphology associated with the three colored body regions in male *Habronattus pyrrithrix* that are prominently displayed to females during courtship. Then, using field-collected spiders, we show how the colors of these three regions (i.e., red face, green front legs, and bright white pedipalps) develop as individuals grow from spiderlings through sexual maturity. Finally, given that two of these body regions (i.e., red faces and green front legs) are condition-dependent (Taylor et al. 2011, Appendix B), we examined the possibility of age-related fading of these traits in adult males and show that green leg coloration, but not red facial coloration, fades (i.e., becomes lighter) with age.

Ontogenetic color change in juveniles

In examining color development, we found that both the bright white pedipalps and the green leg coloration of males appeared only at sexual maturity. This is typical of many ornaments used in mating or aggressive competitions over mates; because such colors typically incur costs, it is not surprising that these ornaments are not expressed in juvenile stages (Andersson 1994). In contrast, males and females began to differentiate in red facial coloration and dorsal patterning as young juveniles (ca. 2.5 mm). During these stages, young males began to develop red facial scales and conspicuous black and white dorsal patterning typical of sexually mature adult males. The red coloration of adult males is prominently displayed in courtship and has been shown to improve courtship success in certain contexts (Chapter 4), yet it is unclear whether this coloration might have any functional role for juvenile males who do not engage in courtship. Red coloration has been shown to have important effects on receivers in a variety of taxa (reviewed in Pryke 2009); it could be that juvenile males use their red face for signaling in non-sexual contexts, either with conspecifics, potential predators, or prey.

Regarding conspicuous dorsal patterning in adult males, this appears to be linked to higher movement rates associated with mate-searching, compared with cryptic females who spend more time at rest; the pairing of conspicuous body patterns with false antennation (i.e., leg waving behavior) presumably helps adult males avoid predators as they move through the leaf litter searching for and courting females (Chapter 2). Again, it is unclear what benefits, if any, this dorsal coloration might provide to young juvenile males. It is possible that even as juveniles, males and females might face different ecological selection pressures (e.g., different dispersal or movement rates) that may drive such sex-differences in juvenile dorsal patterning (Booth 1990); in future work, such ideas should be examined in more detail. Finally, it is possible that juvenile sexual dichromatism does not have a functional role (e.g., Johnston 1967); it may simply indicate relaxed selection pressure for crypsis, compared with other species in which males are cryptically colored until maturity. It is interesting, however, that this species is an exception to the general pattern of salticid color development, where juveniles of both sexes typically resemble females in color pattern until reaching maturity (LAT, pers. obs.). To date, studies of any aspect of the biology of juvenile jumping spiders are rare (e.g., Bartos 2008; Nelson et al. 2005a), yet they have revealed interesting aspects of life history that would have been missed by simply focusing on adults, as most studies do. *H. pyrrithrix* is a particularly good system to examine sex differences in juveniles because, unlike most salticid species, color patterns allow small juveniles to be accurately sexed before reaching maturity.

Post-maturity age-based color fading

In addition to age-related changes that occur during development prior to sexual maturity, our study also uncovered post-maturity, age-based color change. Previous studies have suggested that structural coloration in jumping spiders may be linked to male age (Lim & Li 2007; Taylor et al. 2011, Appendix B), yet both of these studies used comparisons of two groups of spiders, one that had been collected from the field and measured immediately and a second that was field-collected and measured after a certain period of time in the lab. While differences in the two groups may likely be due to age, we cannot rule out confounding effect of diets and captivity; in both cases, the first group experienced a field-based diet/environment for its entire life while the second group was collected from the field and then switched to a lab-based diet/environment prior to color measurement. Here we remove these confounding effects of diet and captivity to show that, even when spiders are raised entirely in the lab, the green leg coloration of adult males fades (i.e., increases in mean brightness) with age. This is also consistent with correlational findings from a previous study (Taylor et al. 2011, Appendix B); this same aspect of male leg color (brightness) is condition-dependent in the field, suggesting that younger males in better condition have darker legs, while older males in poorer condition have lighter legs.

Interestingly, this pattern of age-based fading did not hold for the males' red facial coloration, which is also condition-dependent (Taylor et al. 2011, Appendix B, see also Chapter 6). Previous studies have linked red facial coloration to a male's juvenile diet (Taylor et al. 2011, Appendix B). Collectively, these studies support the idea that the two different colors (red faces and green legs) have the potential to signal different aspects of male quality (reviewed in Hebets & Papaj 2005). A male's red facial coloration potentially signals a male's nutritional status and foraging ability (but not his age), while green leg coloration may signal age while containing no information about his diet or foraging ability. An interesting next step will be to examine how the mechanisms of coloration (e.g., specific pigments, structures, etc.) for these jumping spiders might facilitate or constrain the information content of a specific color (e.g., McGraw et al. 2002). Work with butterflies suggests that structural colors are more likely to fade with age than pigmentary colors (Kemp 2006). A better understanding of the detailed mechanisms of color production in *H. pyrrithrix,* including the specific pigments and structure types, will allow us to test the generality of these ideas.

Our examination of the morphology of the males' green legs offer preliminary insight into the mechanisms of age-based fading observed in our study. The green leg coloration is produced in the cuticle, while additional white light is reflected off of the long, fragile spatulate scales (LAT, pers. obs., see Figure 17c,d). Fading of leg color could thus be a result of the breakdown of structures in the green cuticle, or alternatively, could be a result of damage to white spatulate scales, causing them to reflect more light. Males use these front legs in prey capture (LAT, pers. obs.), and thus damage to their scales over time may be difficult to avoid. Closer examination of the morphological changes that occur with age may help to elucidate the mechanisms behind age-based fading in *H. pyrrithrix* leg color.

Here we show that, in addition to sexually dichromatic male display colors that show a sudden onset at maturity (e.g., brilliant green legs, bright white pedipalps), males also have bright sexually dimorphic colors that begin to develop when males are still small juveniles (e.g., red faces and conspicuous black and white dorsal patterning). Furthermore, these colors are not all static at maturity; in particular, the green front legs of males are subject to age-based fading. To our knowledge, this is the first study to quantify age-based changes in juvenile coloration of any species of jumping spider, an important first step towards understanding the costs, benefits, and potential functions of juvenile coloration. Work on salticid coloration has only begun recently, and has provided some interesting and promising systems to examine general questions about color communication and evolution (Lim et al. 2007; Li et al. 2008a; Lim et al. 2008; Taylor et al. 2011, Appendix B). Examination of ontogenetic changes in spider coloration, particularly in groups such as *Habronattus*, may help us elucidate some of the more subtle costs and benefits of color expression and change throughout an animal's life.

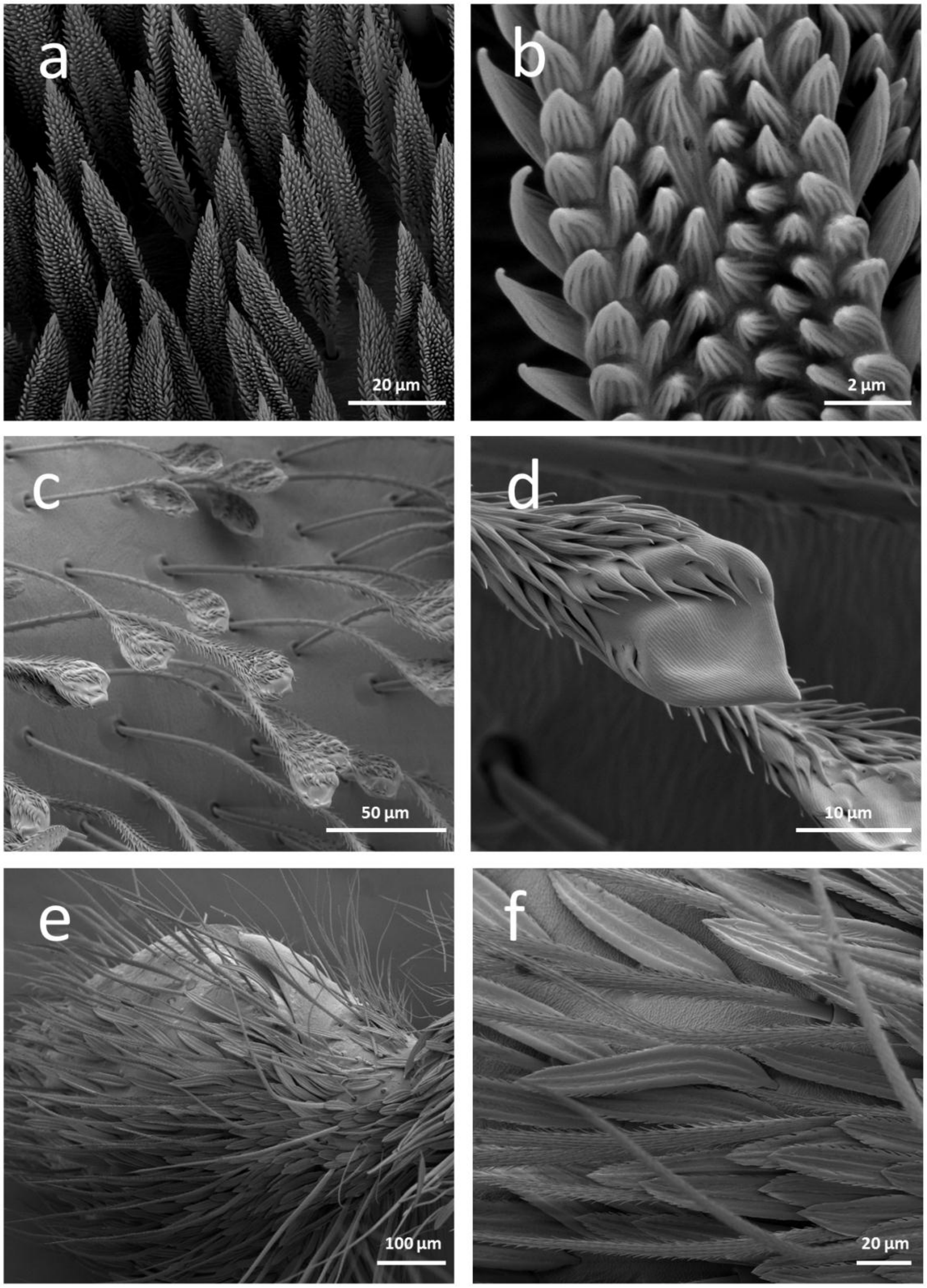

Figure 17. Morphology of the colored body regions of adult male *Habronattus pyrrithrix.* Red scales on the face showing ridged protrusions (a, b), white spatulate scales ornamenting the green front legs (c, d), and flat white pedipalps scales (e, f).

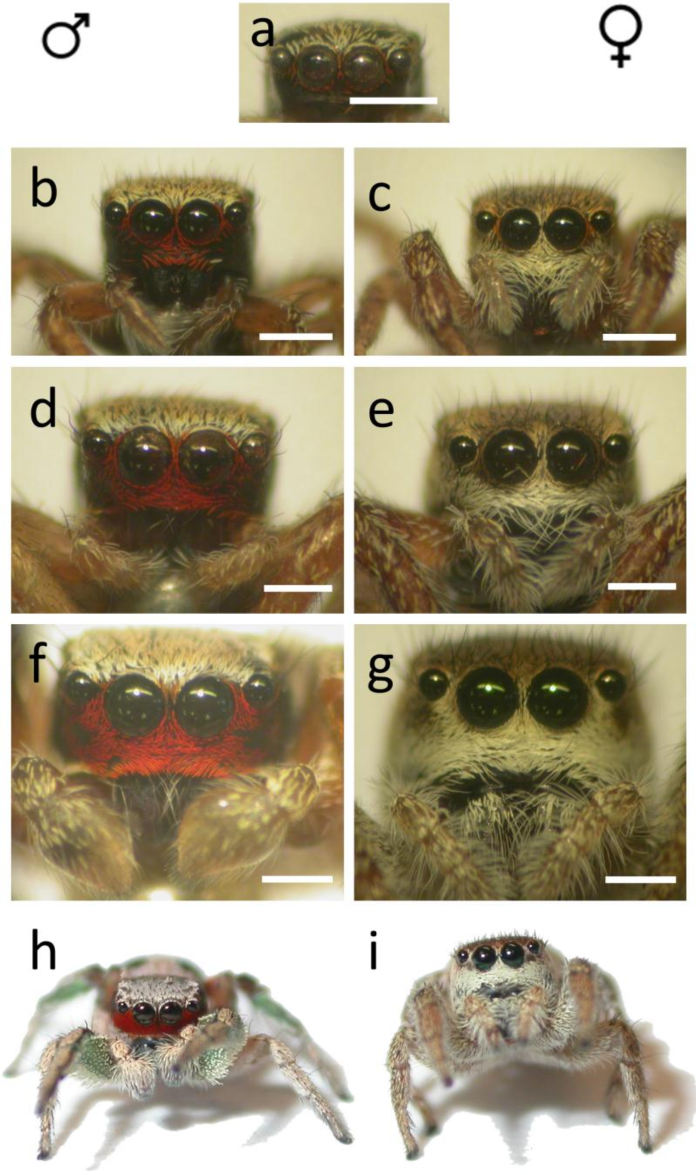

Figure 18. Ontogenetic changes in coloration in males and females as spiders develop from spiderlings through sexual maturity. (a) spiderling stage (where sexes are indistinguishable), (b) small juvenile male, (c) small juvenile female, (d) large juvenile male, (e) large juvenile female, (f) subadult male, (g) subadult female, (h) sexually mature adult male, (i) sexually mature adult female. Scale bars represent 0.5 mm.

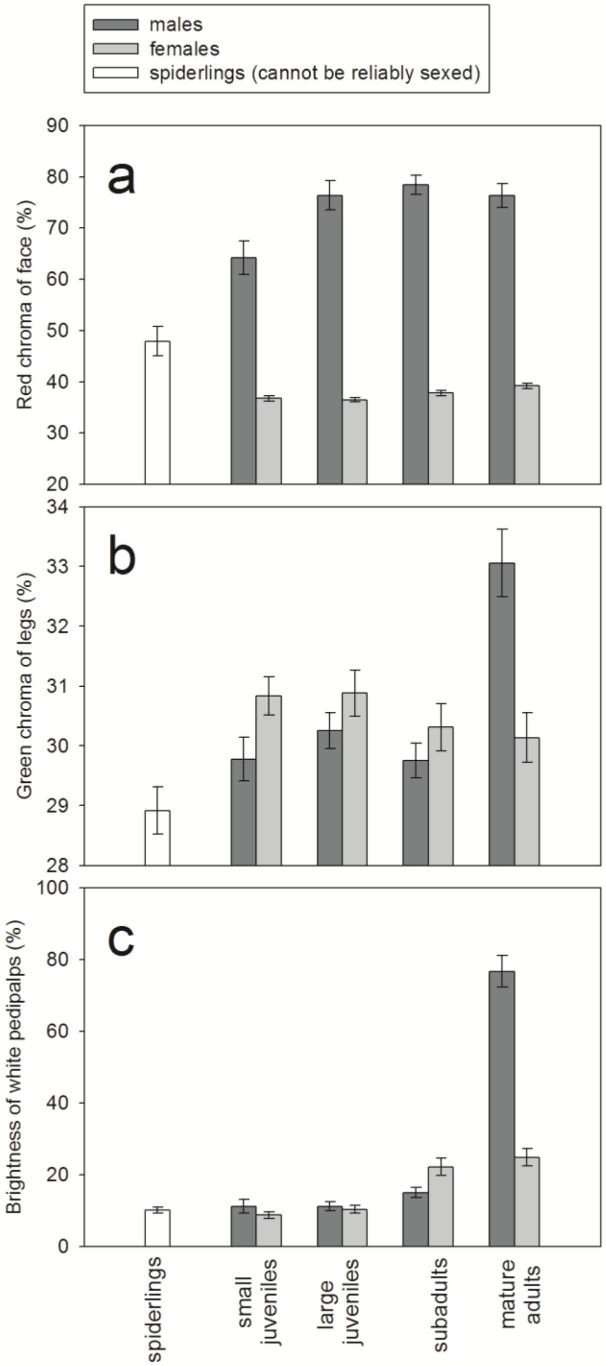

Figure 19. Ontogenetic changes in coloration in males and females as spiders develop from spiderlings through sexual maturity (mean ± SEM). (a) facial coloration, (b) front leg coloration, and (c) pedipalp coloration.

Figure 20. Sexual dichromatism in dorsal coloration in juvenile and adult male and female *H. pyrrithrix*. (a) juvenile male, (b) juvenile female, (c) adult male, (d) adult female.

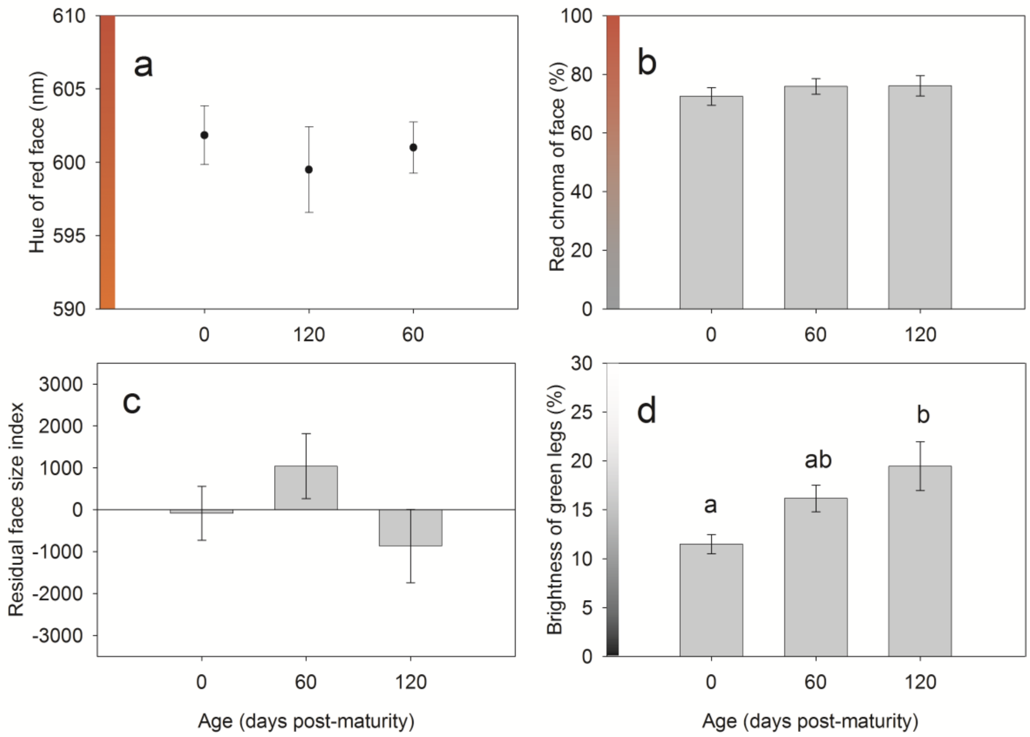

Figure 21. Effect of adult age (post-maturity) on condition-dependent male display colors (mean ± SEM). Aspects of red facial coloration (a, b, c) did not change with age, yet the brightness (lightness) of male green leg coloration increased as males aged (d). Different letters indicate significant differences at *P*<0.05.

Table 5. Results of ANOVA examining the effect of sex, age (i.e., size class), and their interaction on color metrics associated with the face, legs, and pedipalps during development in *H. pyrrithrix* jumping spiders.

Red chroma of face	Df	*F*	*P*
sex	1,140	304.960	<0.001
age	4,140	6.300	<0.001
sex x age	4,140	20.270	<0.001
Green chroma of legs	Df	*F*	*P*
sex	1,140	0.280	0.596
age	4,140	9.370	<0.001
sex x age	4,140	5.100	<0.001
Brightness of pedipalps	Df	*F*	*P*
sex	1,140	1.430	0.234
age	4,140	41.540	<0.001
sex x age	4,140	3.330	0.012

Chapter 4

MALE ORNAMENTAL COLORATION IMPROVES COURTSHIP SUCCESS IN A JUMPING SPIDER, BUT ONLY IN THE SUN

Abstract

In many animals, males display colorful ornaments to females during elaborate courtship dances, and the effectiveness of these color signals depends on the ambient lighting environment. While a variety of hypotheses exist to explain both the presence of and variation in such traits, many of these propose that they function as signals and that their *presence* is either required for or improves successful sex/species recognition and mating. In *Habronattus pyrrithrix* jumping spiders (family Salticidae), males are adorned with conspicuous, condition-dependent red facial coloration and green leg coloration, which they actively display to drab gray and brown females during complex courtship. These spiders also live in heterogeneous lighting environments (e.g. leaf litter, grass), so we designed two experiments to test if brilliant colors affect mating success under varying light conditions. In Experiment 1, we paired individual males with virgin females under full-spectrum lighting in the laboratory and found that blocking either the red facial coloration or green leg coloration, or both, had no effect on any measure of mating success (likelihood of copulation, latency to copulation, copulation duration) or female behavior (aggression or cannibalism) when compared to sham-treated control males. In Experiment 2, we gave virgin females the choice between two simultaneously courting males, one with his facial coloration blocked and the other that received a sham treatment, and ran trials outdoors in both the sun and shade. We found that blocking red facial coloration reduced a male's ability to approach a female, but only when he was courting in the sun. These results suggest that ornamental coloration is not required for successful mating in *H. pyrrithrix*, but that red coloration improves mating success in certain environmental contexts. We discuss implications of these findings for the evolution of elaborate, multimodal courtship displays by animals that interact in complex, variable environments.

Introduction

Selection for effective communication between potential mates has resulted in elaborate and complex ornaments and behaviors in a variety of taxa. Many hypotheses have been proposed and tested to explain the functions of traits that are involved in courtship (reviewed in Andersson 1994). Some of these hypotheses make predictions about how receivers will respond to the *presence* (or absence) of certain display traits. For example, hypotheses positing that such traits function in species recognition (e.g., lizards: Losos 1985; fish: Couldridge & Alexander 2002), sex/mate recognition (e.g., damselflies: Sherratt & Forbes 2001; spiders: Elias et al. 2005; Lim et al. 2007), or attracting receiver attention (e.g., spiders: Clark & Morjan 2001; lizards: Ord & Stamps 2008) all predict that the *presence* of the signal will either ensure or improve the chances of successful mating. Other functional hypotheses make predictions about how receivers respond to natural, more subtle *variation* in trait expression. Such hypotheses posit that such signal variation encodes aspects of individual identity (e.g., birds: Dale et al. 2001; wasps: Tibbetts 2002), age (e.g., crickets: Simmons & Zuk 1992; butterflies: Kemp 2006), health (e.g., birds: McGraw & Ardia 2003; fish: Pike et al. 2007), foraging ability (e.g., Karino et al. 2005), or nutritional condition (e.g., birds: McGraw et al. 2002; Velando et al. 2006). These two broad categories of hypotheses are not mutually exclusive – for example, it is plausible that the presence/absence of a trait might provide important information for species recognition from a distance, while subtle variation in that trait might provide additional information about individual quality as courtship progresses.

While no single experiment can disentangle all of these hypotheses simultaneously, a good starting point is to remove the trait of interest completely and assess behavioral effects on the putative receiver. This approach allows us to test the explicit predictions of hypotheses about species recognition, sex recognition, and attention-altering described above. In addition, such experiments can help us to establish the social or sexual context in which the putative signal likely functions. For example, early experiments that involved blacking out the red epaulets of red-winged blackbirds (*Agelaius phoeniceus*) established their role as an important signal for settling territorial disputes (Peek 1972; Smith 1972). These initial studies provided the foundation for the numerous studies that have followed to probe more deeply into the complex roles that natural variation in these color patches plays in different situations (review and meta-analysis in Yasukawa et al. 2010).

In many animals, courtship occurs in complex, variable, and unpredictable environments, yet the successful transmission of courtship signals depends much on the environmental conditions in which they are sent. For example, the transmission of seismic signals depends on the vibratory properties of the substrate on which they are produced (e.g., spiders: Elias et al. 2004; Hebets et al. 2008; Gordon & Uetz 2011), and the transmission of vocalizations depends on the acoustic properties of the surrounding habitat (e.g., birds: Brown & Handford 2000; frogs: Castellano et al. 2003). The distances between signaler and receiver can also affect the efficacy of different types signals (e.g., Clark & Biesiadecki 2002). Given this effect of the environment on signal transmission, we might expect that the role and importance of certain traits within a display repertoire will change depending on environmental conditions (e.g., Gordon & Uetz 2011; Wilgers & Hebets 2011).

Brilliant colors, because of their production and maintenance costs (e.g., McGraw 2006; Kemp & Rutowski 2007) and their widespread use in animal courtship displays, are good examples of signals that can play important roles in mating (reviewed in Andersson 1994; Hill 2006). Because of the importance of available light in the transmission and perception of color signals (Endler 1991, 1992; Endler & Thery 1996), they are also good examples of traits whose efficacy might vary considerably depending on subtle differences in the ambient environment (e.g., fish: Wong et al. 2007; Heuschele et al. 2009; butterflies: Obara et al. 2008). With the use of modern spectrophotometry, colors (as well as the light environment in which they are transmitted) can be easily quantified and manipulated to tease apart their effects on receivers in both the field and lab (Endler 1990; Andersson & Prager 2006).

Jumping spiders (family Salticidae) are excellent models for understanding the functions of colorful displays. Their charismatic and colorful courtship has intrigued biologists for years (e.g., Peckham & Peckham 1889, 1890), yet surprisingly little empirical work has been aimed at understanding how male color may influence mating success. Many of the more than 5000 species of jumping spiders (Platnick 2011) are sexually dichromatic and engage in dramatic and colorful courtship displays for potential female mates (e.g., Maddison 1995; Oxford & Gillespie 1998), and certain aspects of these displays, such as male dancing, have been shown to increase female receptivity (e.g., Jackson 1981). Jumping spiders have excellent vision (Land 1969; Devoe 1975; Yamashita & Tateda 1976; Williams & McIntyre 1980; Blest et al. 1981; Peaslee & Wilson 1989; Harland & Jackson 2000), and behavioral experiments indicate that they can discriminate between different colors (blue, green, yellow, and red: Nakamura & Yamashita 2000; red vs. blue: Jakob et al. 2007; red vs. yellow: VanderSal & Hebets 2007). Work done with *Cosmophasis umbratica* and *Phintella vittata* (jumping spiders from Asia) has shown that blocking UV light affects mate choice decisions, suggesting that UV coloration is playing an important role in courtship signaling in these species (Lim et al. 2007; Lim et al. 2008; Li et al. 2008b). To our knowledge, only one study, conducted more than 60 years ago, has used direct manipulations of male salticid color patterns (Crane 1949). Crane (1949) painted various male body regions on five tropical salticid species; the study was descriptive in nature and results indicated that if male color patterns were altered, females would often still mate with them (Crane 1949). To our knowledge, the present study will be the first to systematically manipulate male color patterns using current color measurement technology and compare mating success with control males using modern statistical approaches.

Males in the genus *Habronattus* are among the most highly ornamented of jumping spiders, with a striking diversity of colorful and sexually dimorphic display traits (Richman 1973; Richman 1982; Griswold 1987; Maddison 1995; Richman & Cutler 1998; Maddison & Hedin 2003). Throughout the genus, male displays consist of various combinations of brilliant colors (e.g., Taylor et al. 2011, Appendix B), motion (e.g., Elias et al. 2006b), and seismic components (e.g., Maddison & Stratton 1988; Elias et al. 2003). Evidence from geographically isolated sky island populations of *Habronattus* suggests that sexual selection is responsible for driving this striking male diversification (Maddison & McMahon 2000; Masta & Maddison 2002), making this an ideal group to examine the functions of color in mating.

Here, we designed two experiments to test if brilliant colors of male jumping spiders play a role in mating and whether or not their effectiveness depends upon ambient lighting conditions. First, in Experiment 1, we wanted to determine if the presence of condition-dependent display colors are either *required* for species recognition or improve mating success in *Habronattus pyrrithrix*, a sexually dimorphic jumping spider in which males display bright red faces and green legs to dull, drab females during courtship (Figure 22). We paired individual males with virgin females in the laboratory in a 2x2 factorial design to examine the effect of blocking either the red facial coloration or green leg coloration, or both, on multiple metrics of mating success and female behavior when compared to sham-treated control males.

After finding that neither of these colors were required for or improved successful mating under laboratory conditions (see Results below), we went on to conduct Experiment 2 to examine the sexual significance of red facial coloration more closely under natural lighting conditions, which might give females greater opportunity to fully assess male colors and therefore be more choosy. Specifically, we presented virgin females with two simultaneously courting males, one of which had his red facial coloration concealed and the other which was given a sham treatment. We ran all trials outdoors under natural light, and each trial was repeated in both the direct sunlight and the shade. In field observations of two natural populations of *H. pyrrithrix* in Phoenix, AZ (Maricopa County), USA, densities and interaction rates are high and we have frequently observed multiple males simultaneously courting females in both the sun and shade (LAT, pers. obs.). Thus, this design allowed us to extend our study across a range of biologically relevant courtship scenarios.

Methods

Study species

Habronattus pyrrithrix Chamberlin 1924 is a sexually dichromatic jumping spider in which males display colorful ornaments to females during elaborate courtship displays. In particular, males display condition-dependent red facial patches and green front legs to inconspicuously colored gray and brown females (Taylor et al. 2011, Appendix B, Figure 22). The red coloration is contained within body scales on the face (e.g., Hill 1979), while the green coloration is present on the surface of the leg cuticle, which is then further adorned with white scales (LAT, pers. obs.). The distribution of *H. pyrrithrix* extends from southern California and Arizona south to Sinaloa, Mexico (Griswold 1987). In Phoenix, Arizona, they are common and often found in high densities in leaf litter in natural riparian areas (e.g., cottonwood (*Populus fremontii*), desert willow (*Chilopsis linearis*)) as well as grassy backyards and agricultural areas (LAT, pers. obs.). These complex leaf litter and grassy microhabitats create patchy areas of sun and shade in which spiders interact (LAT, pers. obs.).

Like other species of *Habronattus*, males engage in complex courtship displays for females (e.g., Richman 1973; Richman 1982; Griswold 1987; Maddison 1995; Richman & Cutler 1998; Maddison & Hedin 2003), which consist of both visual and seismic components (LAT, pers. obs.). The red faces and green legs of males are oriented towards females during courtship and are generally concealed when the male is viewed from above or from the side (LAT, pers. obs.). Male courtship typically begins when a male locates a female from several centimeters away and begins to wave his front legs and expose his red face, gradually approaching in a zigzag fashion (stage 1 courtship, Figure 22a). If the female remains stationary and does not chase the male or retreat, the male will proceed to stage 2 of courtship, in which he approaches to within a few millimeters of the female’s face and immediately stops and extends his first pair of legs straight up, nearly perpendicular to the plane of his body (Figure 22b) and initiates the seismic component of his display (LAT, pers. obs.; D.O. Elias, pers. comm.). Both of these stages can last from several minutes to hours, depending on the reaction of the female (LAT, pers. obs.). Stage 3 of courtship consists of the male gently tapping the female’s carapace with his front legs; if she does not jump away, he typically mounts and copulates with her (LAT, pers. obs.). Because females are often larger than males and are voracious generalist predators, courting males are frequently cannibalized prior to copulation in both the field and the lab (LAT, pers. obs.). Males occasionally display by waving their legs in the direction of other males, but these displays are rare and typically last for only a few seconds compared to the displays performed for females that can last for hours (LAT, pers. obs.).

Experiment 1: Color manipulation and mating success

The goal of Experiment 1 was to determine if the presence of male facial and leg coloration is required for successful mating, and how these colors influence various metrics of mating success. We conducted a color manipulation experiment in which we blocked male colors (described in more detail below) and assessed the effects on a male’s ability to successfully copulate with a female and also to avoid cannibalism. We focused specifically on the red face and green legs of males, because they have been found to be condition-dependent and are clearly displayed to females during courtship (Taylor et al. 2011, Appendix B).

While standard mate-choice studies in many animals, including some jumping spiders, typically involve pairing a single female with multiple males that are physically isolated and therefore unable to interact with one another (e.g., Cross et al. 2007; Lim et al. 2008; Li et al. 2008b), this type of design does not work with *Habronattus*. To our knowledge, there is no known behavior other than copulation (e.g., see Masta & Maddison 2002) or a female's willingness to allow a male to approach and make contact with her (LAT, pers. obs.) that clearly indicates receptivity in any *Habronattus* species. For this reason, mating success studies with *Habronattus* typically involve paring a single female with a single male and measuring 'mating success' directly in terms of whether or not copulation occurs (e.g., Masta & Maddison 2002; Hebets & Maddison 2005; Elias et al. 2005; Elias et al. 2006a) . Thus, this was our approach for Experiment 1.

We reared all spiders in the lab for Experiment 1, which allowed us to create a design that reduced variation associated with genetics as much as possible (described in more detail below). Additionally, rearing all spiders in the lab allowed us to minimize variation associated with factors such as diet, mating history, and other experience, which is not possible with field-collected individuals. In July and August 2008, we collected 18 gravid females from Queen Creek, Arizona (Maricopa County), USA and brought them back to the lab and allowed them to lay eggs. Throughout the study, we housed all spiders individually in clear plastic boxes (10.16 x 10.16 x 12.86 cm) at approximately

28°C. Light was provided from full-spectrum lights (30W compact full-spectrum light bulbs, Mercola, Hoffman Estates, IL, USA) on a 14:10 light-dark cycle, and each cage was fitted with a mesh top to allow adequate light to reach the inside of the cage. Irradiance data from our artificial lighting is provided as supplementary material in Appendix D. Each cage was also provided with an artificial green plant (approx. 10 cm long, Ashland fern collection, Michael's Stores, Irving, TX, USA) affixed to the side of the cage to provide enrichment (e.g., Carducci & Jakob 2000). Upon hatching and emerging from the egg sac (as soon as spiders were large enough to determine their sex, ca. 2.5mm) we removed either four male or four female spiders from each clutch (one clutch per female) and raised them to maturity in individual cages (as described above) on a constant diet of small crickets (*Acheta domesticus*) amounting to approximately their own body weight three times per week. We chose this diet because pilot studies suggested that this diet results in spiders with body condition indices comparable to those collected from the field (LAT, unpublished data). Opaque barriers separated the spider cages during rearing so that they could not see and interact with one another.

Upon maturity, each group of four brothers was randomly assigned to an unrelated group of four sisters, to which they were paired in the mating success experiment. Because the mothers of these spiders may have mated either singly or multiply in the field, individuals in each clutch may be either full or half siblings. Within each group of four brothers, we assigned individuals randomly to one of four treatments in a 2x2 factorial design: (1) red facial color manipulated (see below for more details on color manipulations), (2) green leg color manipulated, (3) both colors manipulated, or (4) neither color manipulated (sham control). Individual males were then randomly assigned to a female (unrelated) from their paired clutch, resulting in 36 male-female pairs.

We began trials when females were between 10 and 40 days post-maturity. All trials were started between 0800 and 1100 hrs. Immediately before a trial began, we weighed spiders to the nearest 0.0001 g with an electronic balance (Mettler-Toledo, Columbus, OH, USA) and photographed each spider next to a size standard using a Nikon Coolpix 4500 digital camera (Nikon Inc., Melville, NY, USA). From these photographs, we measured carapace width (just behind the posterior lateral eyes) using Photoshop software (Adobe Systems Inc., San Jose, CA, USA). Because these spiders' carapace width is fixed at maturity, while the abdomen stretches with feeding, we used the residuals of a regression of mass on carapace width as an estimate of body condition that is uncorrelated with body size, and is a common method of estimating body condition in spiders (e.g., Jakob et al. 1996; Taylor et al. 2011, Appendix B).

For each female, we introduced the assigned male partner into the plastic cage in which the female had been raised and videotaped all interactions for the first hour under the laboratory conditions described above. After the one-hour videotaped period, we continued to house the spiders together for an additional 48 hours. After the 48-hour period ended, we removed the male from the female's cage. Females were then fed in excess (approximately three times their body weight in food was provided daily) for two weeks to allow them the opportunity to lay eggs. To confirm that eggs were fertile, all were allowed to hatch and we recorded whether or not spiderlings emerged.

From the videotapes, we used the freeware program Cowlog (Hänninen & Pastell 2009) to record each male's courtship effort (amount of time spent actively courting) and latency to begin courting. We also measured female aggression (number of attacks by females) and whether or not cannibalism or copulation occurred. In instances where copulation did occur in the first hour (11 out of 36), we recorded copulation latency and duration. For the subsequent 48 hours, we checked the pair of spiders every 24 hours and recorded additional instances of cannibalism.

Experiment 2: Simultaneous choice in sunlight and shade

Because our results from Experiment 1 suggested that color was not a requirement for successful mating and did not affect any aspect of mating success in the laboratory (see Results), the goal of Experiment 2 was to determine if color would affect a male's mating success in contexts that more closely resembled natural conditions and might be more conducive to color signaling. Specifically, each female was paired with two males simultaneously and trials were run outdoors and repeated in both the sun and the shade. Because this experiment involved pairing each female with two males simultaneously, we could not manipulate both facial and leg coloration using the same 2x2 factorial design that we used in Experiment 1 (as this would necessitate pairing a single female with four simultaneously courting males from different treatments). For this reason, in

Experiment 2 we focused on manipulation of the red facial coloration only. We chose to focus on the red coloration rather than the green because red coloration (but not green) is dependent on juvenile diet (Taylor et al. 2011, Appendix B), and thus we presently have greater evidence that it might contain useful information relevant to mate choice. Furthermore, various degrees of sexually dichromatic red male facial coloration are found commonly and sporadically throughout *Habronattus*, some of which exhibit striking and unexplained geographic variation (e.g., *H. hirsutus, H. americanus* (see Griswold 1987)). Thus, experimental studies of red color might provide insights into the function of this particular trait that is relevant to many other species in the genus.

As discussed above, the lack of obvious female receptivity displays in *Habronattus* prevented us from being able to run a typical simultaneous choice test in which females are allowed to view two physically isolated males and choose between them. To interpret female 'choice' in *H. pyrrithrix*, males must be allowed to approach and make contact with females (LAT, pers. obs.) and the only way to do this was to place all three spiders (one female, two males) into an experimental chamber and allow them to interact freely. Consequently, our experimental design, and in fact, any possible design that could be used with this species, would not prevent males from seeing and interacting with one another. Thus, our data from this experiment do not allow us to completely disentangle male-male competition from female choice *per se*. However, when two males are courting the same female, males typically direct their displays at the female simultaneously, rather than at the competing male, and thus we expect that any major effect of color manipulation that we see in our experiments will be a result of female behavior rather than male-male competition (LAT, pers. obs). Despite these expectations, we also quantified male aggression (described in more detail below) as a means of examining possible effects of color on the other male in the trial (see Results).

In contrast with Experiment 1, in which we were able to carefully control the relatedness, rearing environment, and experience of all subjects, our approach in Experiment 2 was to perform the experiments under conditions that more closely resembled those in nature. For this reason, we used field-collected rather than lab-reared spiders from the same Queen Creek population described above. Twenty-four male and 12 female spiders were collected in July and August 2009. Because female mating history affects mate choice in some jumping spiders (e.g., Jackson 1981), females were collected as penultimates (i.e., the developmental stage just prior to sexual maturity) and kept in the laboratory on a diet of *Acheta domesticus* as described above until reaching maturity. This ensured that all females in the experiment were virgins, and is common practice in jumping spider mate choice experiments (e.g., Clark & Biesiadecki 2002; Elias et al. 2005; Elias et al. 2006a; Lim et al. 2008). Males will actively court females regardless of their mating history (LAT, pers. obs.) and thus we collected males as adults and housed them in the laboratory until experimentation (days in captivity =33±5.24 (mean±SE)). In this experiment, spiders were housed indoors (using the same cages described above) on a natural light/dark cycle at approximately 30°C. Natural light was provided from five large windows. Opaque dividers separated the spiders so that they could not see and interact with each other prior to experiments.

We grouped males by collection date, paired them by body size (carapace width), and then randomly assigned each pair of males to a female. Within each male pair, individuals were randomly assigned to one of two treatment groups. Males either had their red facial coloration blocked or underwent a sham treatment in which their red facial coloration was left intact (see below for color manipulation details, including sham treatment).

When females were between 10 and 28 days post-maturity, we moved their cages outdoors to allow them to acclimate overnight before beginning their outdoor trial the next morning. All trials were run between 0700 and 1100 hrs., when the mean temperature was from 31-42°C. Our field observations indicate that these spiders are typically active and courting throughout the day during this time of year, despite the intense heat (LAT, unpublished data). Outdoor cages were surrounded by cottonwood (*Populus fremontii*) leaf litter to simulate the natural background color of the habitat of these spiders in the field. The location of the trials was in an area that received direct sunlight that could be manipulated with shade cloth (color: hunter, Springs Creative Products Group LLC, Rock Hill, SC, USA) hanging above. Immediately before the trial began, we took photographs of each spider to measure the carapace width following the methods described above. We did not have access to an electronic balance at this site, so we estimated mass from photographs by measuring the total area of the carapace and abdomen. Pilot data showed that body area measured this way is highly correlated with body mass in *H. pyrrithrix* (linear regression, $F_{1,43}$=62.03, R^2=0.591, P<0.0001, LAT unpublished data). This body mass estimate was then used to calculate residual body condition index as described above.

At the start of the trial, the two males were placed in random order into the female's cage in immediate succession and all interactions were videotaped for the duration of the trial. Each trial consisted of two hour-long periods, one in the sun and the other in the shade. These periods occurred in succession but in random order. The 'sunny' period of each trial was conducted with approximately 75% of the cage receiving direct sunlight, while 25% was shaded from above with shade cloth (to provide a retreat from the intense Arizona sunlight). The 'shady' period of the trial was conducted in the same location, but with the shade cloth adjusted so that the cage was entirely in the shade. Irradiance data from our 'sun' and 'shade' treatments are provided as supplementary material in Appendix D.

From the videotapes, we again used Cowlog (e.g., Hänninen & Pastell 2009) to quantify the amount of time that each male spent courting and his latency to begin courting. Because we were concerned about keeping the spiders in plastic cages for more than two hours outdoors in the summer heat of Arizona (average daily high temperature during experiment = 41° C), and because having two simultaneously courting males appeared to distract the female and extend copulation latency (LAT, pers. obs.), we anticipated (and found) low levels of copulation success during the outdoor trials. Our data from Experiment 1 indicated that mean distance between a male and a female over the course of the trial was an excellent predictor of successful copulation (i.e., males that got closer, and remained closer, to females were more likely to copulate, resulting in fertile eggs; $\chi^2=19.08$, $p<0.0001$); thus we used this metric as a proxy for courtship success in Experiment 2. Distances between each male and the female were measured at five-minute intervals and used to calculate a mean for each male over the entire trial. Finally, we recorded the number of attacks by females on each male, the number of male attacks directed towards one another, and all instances of cannibalism.

Color manipulation methods

We manipulated the red faces of males by covering the entire red area with black liquid eyeliner (Color: ‘Perversion’, Urban Decay Cosmetics, Costa Mesa, CA) (Figure 23a,b), which closely matches the reflectance properties of the underlying black cuticle (Figure 24). On control males, we applied the same amount of eyeliner to an equivalent area on the top of their carapace just behind their anterior median eyes, an area that is not clearly visible to females. For males receiving the leg color manipulation, we covered their green legs with light tan makeup powder (bareMinerals foundation, color: ‘Light’, Bare Escentuals, San Francisco, CA, USA) (Figure 23c), which closely matches the reflectance properties of their other, non-ornamented legs (Figure 24). For control males, we applied the same amount of powder to the same area on the second pair of legs (which are not ornamented and are not displayed to females during courtship). To manipulate their colors, we anesthetized all males with carbon dioxide for approximately five minutes, on the day before their trial began. After waking up from anesthesia, males were offered a cricket to confirm that they had recovered fully and were capable of capturing prey. We compared levels of courtship activity (% time spent courting) and latency to begin courting between treatment groups to confirm that the color manipulation did not affect courtship activity or motivation.

Statistical analyses

In Experiment 1, to confirm that our color manipulations did not have adverse effects on male behavior, we used analyses of variance (ANOVA) to determine if blocking either the red facial or green leg coloration influenced male courtship activity (amount of time spent courting over the course of the trial) or latency for males to begin courting. We used binary logistic regression to determine if facial or leg color manipulation, male body size, or male body condition predicted whether or not a male would mate successfully (leading to fertile eggs). In cases where copulation occurred in the first hour, we used analyses of covariance (ANCOVA), with male body size and condition as covariates, to determine if color manipulations affected male copulation latency or duration. Finally, we used ANCOVA (again with male body size and condition as covariates) to determine if color manipulation had any effect on the number of aggressive attacks that males received from females and binary logistic regression to determine if facial or leg coloration, male body size, or condition predicted whether or not a male would be cannibalized by females.

In Experiment 2, to again confirm that our color manipulations had no adverse effects on male behavior, we used ANOVA to determine if the average courtship intensity (i.e., time spent courting) and latency to begin courting differed between males with their facial color manipulated and control (sham-treated) males. Because each trial was repeated in both the sun and shade, we used repeated-measures ANCOVA (with male body size and condition as covariates) to determine if color manipulation affected the ability of males to approach females in the two different light environments. Because two males were paired simultaneously with each female, we included female ID as a random factor in the model. In cases where females cannibalized males (n=2 trials, both during the first phase of the trial), we removed the trial from analyses. Because we found a significant interaction between light environment and color treatment ($F_{1,7}$=7.30, P=0.031, see Table 6), we went on to conduct two separate ANCOVAs (one for each light environment) to more closely examine the effects of male color manipulation, body size, and body condition on a male's ability to approach the female in each. Finally, we used repeated-measures ANCOVA (again, with male body size and condition as covariates and female ID as a random factor) to determine if color manipulation affected the level of aggression (# of attacks) from the female or the partner male.

With the exception of the female and male aggression data (# of attacks), to which we applied a square-root transformation to improve normality, all data met the assumptions of parametric statistics. All statistical analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC, USA).

Results

Experiment 1: Color manipulation and mating success

Neither face nor leg color manipulation had any effect on male courtship activity ($F_{2,33}$=0.08, P=0.926) or latency to begin courting ($F_{2,33}$=0.21, P=0.812).

Copulation that resulted in fertile eggs (i.e., hatched spiderlings) occurred in 19 of 36 trials (~53%). Blocking either the red facial coloration or green leg coloration had no effect on this measure of male mating success (face: χ^2=0.073, P=0.787; legs: χ^2=2.63, P=0.105, Figure 25a); however, mating success was affected by male size and condition, with larger males in better condition being more likely to copulate (size: χ^2=5.85, P=0.016, condition: χ^2=5.58, P=0.018, Figure 26). Among the 11 males that copulated in the first phase of the experiment, there was no relationship between color manipulation and a male's latency to copulate ($F_{4,6}$=0.77, P=0.580) or copulation duration ($F_{4,6}$=1.04, P=0.459).

Color manipulation had no effect on the number of aggressive attacks by females ($F_{4,31}$=0.70, P=0.597). Females cannibalized males in 12 out of 36 trials (~33%), but the probability of cannibalism was not affected by our color manipulation (χ^2=3.42, P=0.489, Figure 25b).

Experiment 2: Simultaneous choice tests in the sun and shade

Male color manipulation had no effect on courtship activity (i.e., time spent courting, $F_{1,38}$=0.49, P=0.486) or latency ($F_{1,38}$=1.58, P=0.217).

There was a significant interaction between male color manipulation and light environment on the approach distance of males to females (Table 6). When trials were run in the sunlight, sham-treated males, with their red facial coloration intact, were able to get closer to females than males with their red facial coloration blocked, while neither male size nor condition had any effect on the distance that males were able to get to females (Figure 27a,b, Table 7). In contrast, when trials were run in the shade, there was no effect of color manipulation on the distance that males were able to get to females (Figure 27c, Table 7). Rather, male size (but not condition) was important, with larger males able to get closer to females than smaller males (Figure 27d, Table 7).

Neither color manipulation nor light environment affected levels of aggression that males received from females (light: $F_{1,7}$=0.38, P=0.556; color manipulation: $F_{1,7}$=2.47, P=0.160) or from their partner male (light: $F_{1,7}$=0.05, P=0.832; color manipulation: $F_{1,7}$=1.66, P=0.238). Full ANCOVA tables are provided as supplementary material in Appendix D.

Discussion

Despite being condition-dependent in field and laboratory experiments and thus likely candidates for honest mating signals, we found that red facial and green leg coloration of male *Habronattus pyrrithrix* was not required for successful mating. In Experiment 1, blocking either or both of these colors on males in a 2x2 factorial design laboratory experiment had no effect on any metric of mating success. In Experiment 2, we examined the effect of red facial coloration more closely under ecologically relevant conditions, and found that males with their red facial coloration intact were better able to approach females than males with their red facial coloration blocked, but only when trials were run in the sunlight. This finding suggests that color may play different roles in courtship depending on subtle environmental conditions.

Experiment 1

The results of Experiment 1 allow us to rule out the hypothesis that male red and/or green coloration is a required species or sex recognition signal in *H. pyrrithrix,* as these colors were neither necessary nor sufficient for successful mating. Just over half of the males in the experiment mated successfully (leading to the production of viable spiderlings), yet this was unaffected by blocking either the red or green coloration (or both). Furthermore, we found no evidence that these colors affected any metric of mating success (time to copulation or copulation duration) or female behavior (aggression or cannibalism). These results were surprising, especially given the effort with which males display these colors to females (LAT, pers. obs.). Overall, our data from Experiment 1 suggest that, even if the colors are used as important signals in some contexts, they are clearly not consistently important in all contexts in which spiders might engage in courtship.

In the field, *H. pyrrithrix* males are found courting in a variety of habitats, from open areas in full sunlight, to patchy areas of mottled sunlight beneath tree cover to completely shaded areas beneath the leaf litter (LAT, unpublished data). In Experiment 1, we used full-spectrum light bulbs in an attempt to create an environment with irradiance profiles as close to natural light as possible, while maintaining controlled laboratory conditions (see Methods). However, a comparison of irradiance data from this artificial lighting with data from natural Arizona sunlight suggests that these two light environments differ in both the overall intensity of light as well as the shape of the irradiance spectra (see supplementary material in Appendix D). Males that mated successfully in Experiment 1 were larger and in better condition than those that were unsuccessful, suggesting that under artificial lighting conditions, females may base their mating decisions on size and condition over male facial and leg coloration.

Experiment 2

Our results from Experiment 2 suggest that red facial coloration may play different roles in courtship depending on the natural light environment in which males are displaying. Because ambient light environment affects the transmission of color signals (e.g., Endler 1992, 1993), it is not surprising that the role of a trait such as red coloration might vary in different lighting environments, and particularly in sunny compared with shady areas. Typically, both forest shade (where available light is mostly reflected from vegetation) and woodland shade (where most light comes from the sky, but is outside the path of direct sunlight) are rich in greenish and bluish light, respectively, and relatively low in red light (Endler 1993). In contrast, sunlit areas (including both large and small forest gaps as well as open sky) contain higher proportions of red light (Endler 1993). Similarly, irradiance data from our experimental setup were consistent with Endler's (1993) characterization of different light environments; specifically, full sunlight had relatively more red light (600-700nm) than did our "shade" treatment (see supplementary material in Appendix D). Given the lower proportion of red light that occurs in the shade compared with the sun, it might be expected that the color red would be a more effective signal in sun, which is what we found. Indeed, our estimates of radiance (or ambient light that is reflected from a particular surface in a given light environment (e.g., see Macedonia 2001; Macedonia et al. 2003)) indicate that more red light would radiate from the red faces of males when viewed in sunlight compared with either shade or artificial light (see Supplementary Material in Appendix D).

H. pyrrithrix is abundant in the natural outdoor area in which this study was conducted and thus the variation in lighting environment examined in this study represents relevant ecological conditions. Recent work has demonstrated that spectral sensitivities, particularly in the longer wavelengths, vary widely among salticids (I-Min Tso, personal communication) making it difficult to interpret our results in the context of an appropriate salticid visual system. An interesting next step would be to explore the specific spectral sensitivities of *Habronattus* so that we can better understand how the red facial patch would appear to females under different light environments and backgrounds (e.g., Endler 1991).

Implications for multimodal communication in Habronattus

In some animals, males are able to adjust the timing, location, lighting, or visual background of their display to maximize its conspicuousness for potential mates (e.g., guppies: Endler 1991; forest birds: Endler & Thery 1996; manakins that create a display court: Uy & Endler 2004; bustards that display in the sun: Olea et al. 2010). In *H. pyrrithrix*, males are unlikely to have this opportunity in the field; they appear to wander in search of females and to court them whenever and wherever they encounter them (LAT, pers. obs.). Courtship has been observed in a variety of substrate types (e.g. grass, leaf litter, rock) and in a variety of lighting conditions, ranging from full sunlight to full shade (LAT, pers. obs.). Female *H. pyrrithrix* are voracious generalist predators and have been observed cannibalizing males in both the field and lab (LAT, pers. obs.) and thus a male’s need to begin courtship immediately when he locates a female might reduce his ability to be choosy about his exact location or his position relative to the available light.

Jumping spiders in the genus *Habronattus* are among the most highly ornamented of all jumping spiders, with highly complex courtship displays incorporating color, motion, and seismic components (see Maddison & Stratton 1988; Elias et al. 2003; Elias et al. 2005; Elias et al. 2006b; Taylor et al. 2011, Appendix B). A major theme in behavioral ecology that has received much recent attention is the question of why animals use multiple signals when one signal might suffice and reduce costs (reviewed in Candolin 2003; Hebets & Papaj 2005). A potential hypothesis is that, for animals that court in complex or unpredictable habitats, environmental variability creates situations in which no single signal component of a display is sufficiently efficacious under all possible conditions and thus, by signaling with multimodal displays, different signaling components (often in different modalities) can act as backups in different environments (Johnstone 1996; reviewed in Candolin 2003; Hebets & Papaj 2005). Because male *H. pyrrithrix* live in a variety of habitats and have limited control over where and when they display, selection might favor the use of backup signals.

Our study suggests that the light environment in which courtship occurs affects the way that females respond to visual displays during courtship. While our study focused on the colors involved in the visual display, *H. pyrrithrix* males also produce seismic signals as part of their display (LAT, pers. obs.; D.O. Elias, pers. comm.). Seismic signals in other arthropods, including spiders, are often linked to body size, suggesting that such signals can convey important information to females (e.g., De Luca & Morris 1998; Gibson & Uetz 2008; Rundus et al. 2011). Interestingly, when in the sunlight, red coloration allowed males to get closer to females, while body size had no effect (see Figure 27a,b). In contrast, in the shade, bigger males were able to get closer to females, regardless of their color treatment (see Figure 27c,d). If the general link between body size and seismic signaling holds for *H. pyrrithrix*, it is plausible that females rely more on seismic cues in the shade and rely more on color cues in the sunlight, which would lend support to the hypothesis that these colors and seismic cues serve as ‘backups’ for each other to increase transmission in variable environments (Johnstone 1996). An alternative but similar explanation is that male body size itself is simply a ‘backup’ visual cue that females pay attention to when the light environment is less conducive to color signaling (Johnstone 1996). Recent studies with wolf spiders whose courtship also involves both visual and seismic components support the ‘backup’ hypothesis and provide evidence that courting males modify their display depending on the transmission properties of the environment (e.g., Gordon & Uetz 2011; Wilgers & Hebets 2011). While it was not the focus of the present study, post-hoc tests suggest that male *H. pyrrithrix* in this study did not modify aspects of their displays (i.e., latency to begin visual displays, duration of visual display) in the two different light environments (LAT, unpublished data). Similarly, in a related species of *Habronattus* (*H. dossenus*) that also has a complex display, males did not modify their displays on different substrates despite a clear difference in the attenuation of their seismic signals on these different substrates (Elias et al. 2004). Additional mate-choice studies that manipulate the transmission properties of both the visual environment (e.g., light levels) and the seismic environment (e.g., different substrates) simultaneously will help elucidate the functions and interaction of different display components.

In the field, *H. pyrrithrix* often occur at high densities in sympatry with other abundant species of *Habronattus* (e.g., *H. hirsutus, H. hallani,* and *H.*

clypeatus; LAT, pers. obs.) While females of these species are all relatively cryptic in coloration (i.e., gray and brown), the males exhibit striking and conspicuous species-specific display coloration (Griswold 1987). Interspecific interaction rates in the field are high (LAT, unpublished data), suggesting that there may be a benefit to females for reliably assessing the species of a courting male from a distance. Moreover, *H. pyrrithrix* males frequently court heterospecific females both in the field and lab (LAT, unpublished data). Thus it might be beneficial to a male to signal his species identity early in courtship (and from a safe distance, see Figure 22a), to prevent wasting significant amounts of time and unnecessarily placing himself dangerously close to a potentially cannibalistic heterospecific female. Such observations of their natural history suggest that male coloration might be a crucial species recognition signal, yet our results surprisingly demonstrate that a male's red facial and green leg colors are not required to achieve copulation. However, our results do suggest that red facial coloration can improve courtship success under certain lighting conditions and thus are consistent with the idea that male color may improve species recognition by females (e.g., Lim et al. 2007; Lim et al. 2008).

The color red is a common component of male display both within the genus *Habronattus* (Griswold 1987) and across the Salticidae more generally (e.g., face of *Lyssomanes viridis* (Jackson & Macnab 1991), face of *Evarcha culicivora* (Wesołowska & Jackson 2003), face and legs of *Saitis barbipes* (Hill 2009)). Our results indicate that, in certain light environments (e.g., sunlight), red facial coloration in *H. pyrrithrix* enables males to get closer to females during courtship; approaching females is necessary to achieve successful copulation (LAT, pers. obs.). The color red has been shown to have interesting and unique effects on receivers in a variety of species; many animals show innate avoidance of red foods over other colors in prey choice (e.g., Mastrota & Mench 1995; Gamberale-Stille & Tullberg 2001; Skelhorn 2011) and red has been argued to be a general signal of intimidation across the animal kingdom (see Pryke 2009). During jumping spider courtship, males must approach voracious and potentially cannibalistic females, a process during which they must strike a balance between stimuli that attract or provoke females and stimuli that inhibit predation (see discussion in Jackson & Pollard 1997; Nelson & Jackson 2007). The unique psychological effects attributed to the color red might make it particularly well-suited to helping courting male spiders achieve this balance, and this might explain its prevalence in male jumping spider displays.

Implications for animal behavior studies

Taken together, the results of these experiments suggest that caution should be taken when interpreting the results of behavioral experiments conducted under artificial lighting conditions, or even under a small subset of possible natural lighting conditions. Due to logistical constraints, many behavioral experiments must be done indoors and/or under controlled or simplified conditions; however such conditions can adversely affect both the physiology and behavior of many animals (reviewed in Calisi & Bentley 2009). Even when experiments are done outdoors, they are often done under only one lighting regime, yet in our study the inclusion of both light environments is what uncovered the interesting relationships between color, body size, and courtship success in different lighting regimes.

This study suggests a functional role for the presence of red coloration in the courtship signaling of *Habronattus pyrrithrix* and suggests that this role may vary depending on the environmental context. Recent work on multimodal communication has been taxonomically biased towards vertebrates (Coleman 2009), yet insights into the complex courtship behavior of spiders, in which males must balance the risks of courting females that are also voracious predators, might reveal interesting and unexpected patterns.

Figure 22. Male courtship in *H. pyrrithrix.* (a) stage 1: male (right) is approaching and displaying his red face and green legs to a potential female mate (left), (b) stage 2: the male (right) has successfully approached and stopped directly in front of the female (left) and is now performing a display consisting of both visual and seismic components.

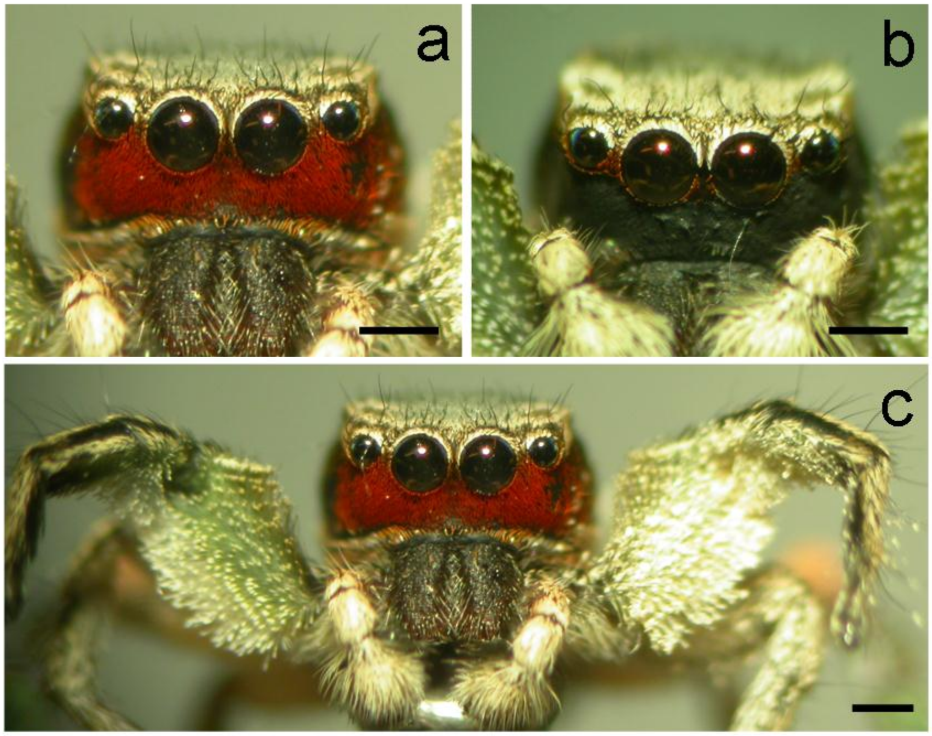

Figure 23. Manipulation of coloration in *H. pyrrithrix.* (a) pre-manipulated natural appearance (i.e., red facial coloration intact), (b) red facial coloration concealed with black liquid eyeliner, (c) male with one leg that has green coloration intact (left) and the other (right) concealed with foundation powder. Scale bars represent 0.5 mm.

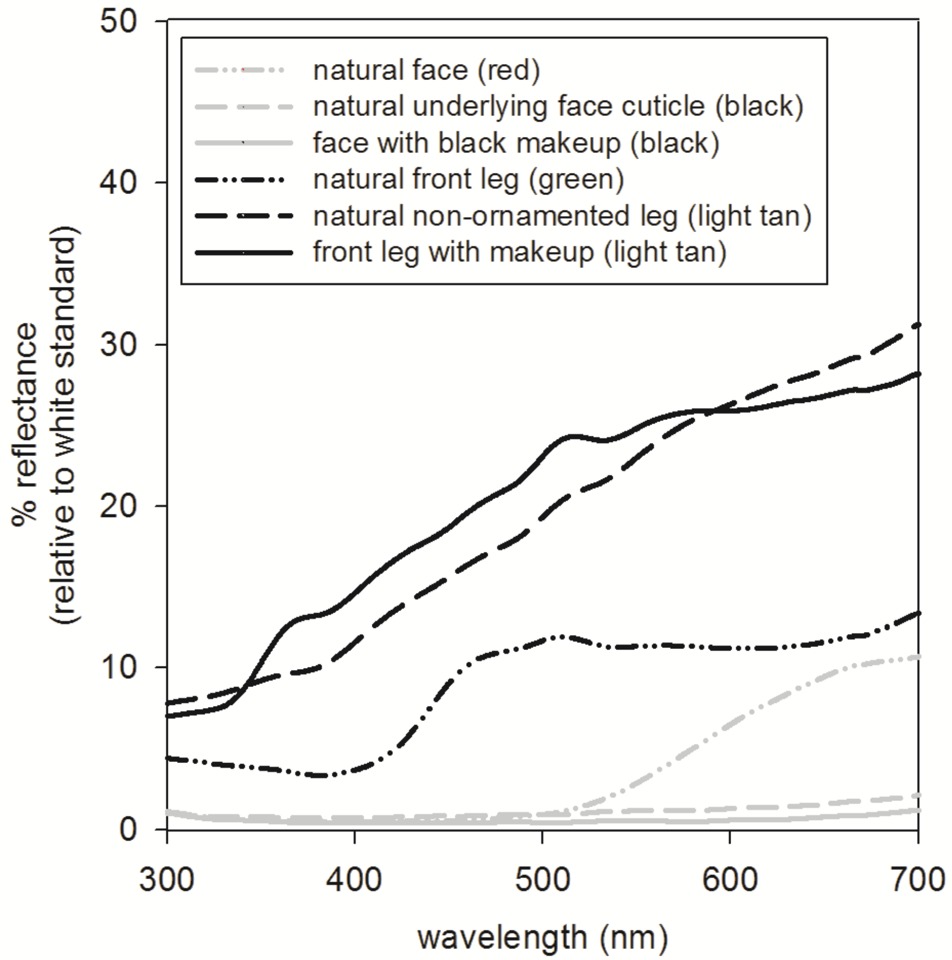

Figure 24. Representative reflectance spectra for the natural and color-manipulated regions of male *H. pyrrithrix* that were the foci of this study.

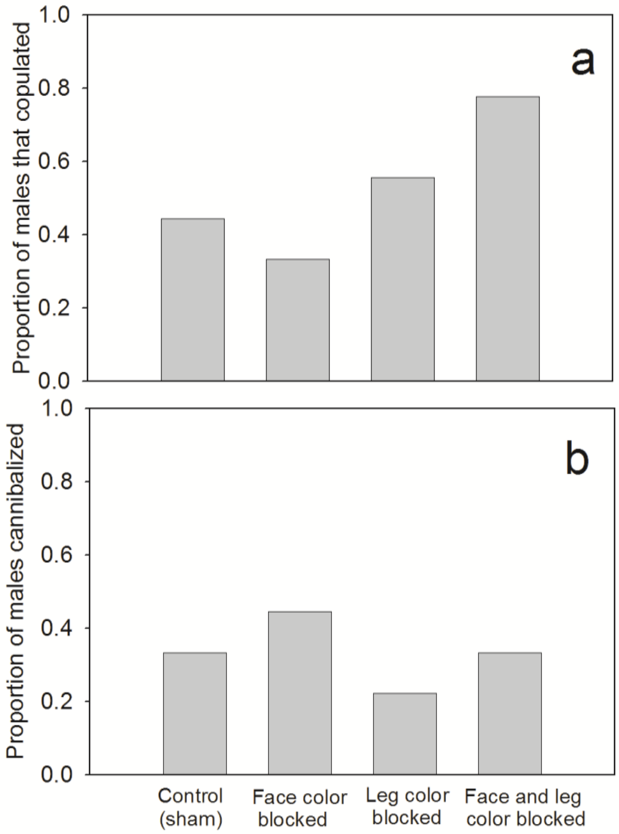

Figure 25. Effect of male color manipulation on copulation success (a) and sexual cannibalism (b) under laboratory conditions in Experiment 1.

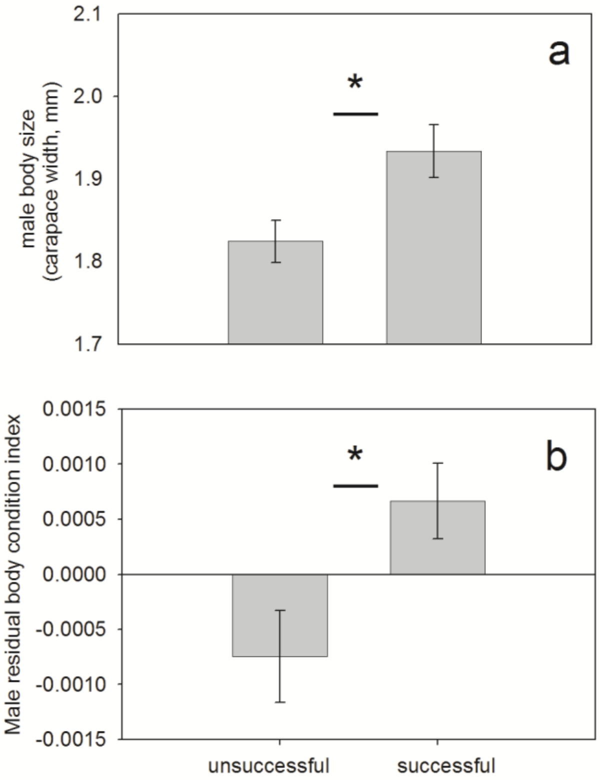

Figure 26. Comparison of male body size (a) and body condition (b) between successful and unsuccessful males in Experiment 1 (mean ± SEM). Successful males achieved copulation, which resulted in fertile eggs. Asterisks (*) indicate significant differences between groups.

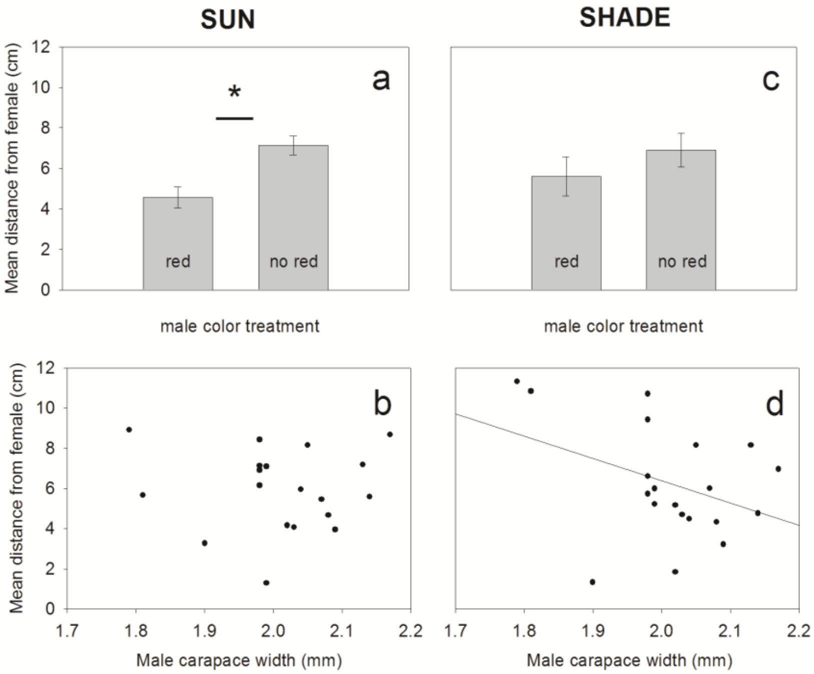

Figure 27. Effect of color manipulation and body size on the ability of males to approach females when courting in the sun (a,b) and shade (c,d) in Experiment 2. The presence of an asterisk (*) indicates a significant difference between treatment groups, and the presence of a regression line indicates a significant relationship between male size and distance from the female.

Table 6. Results of repeated measures ANCOVA for Experiment 2 examining the effect of color manipulation, body size, body condition, and light environment on the mean distance that males were able to get to females over the course of the trial.

within subjects effects	df	*F*	*P*
light	1,7	2.030	0.197
light*treatment	1,7	7.300	0.031
light*size	1,7	2.150	0.186
light*body condition	1,7	0.070	0.801
light*female ID	9,7	5.070	0.022
between subjects effects	df	*F*	*P*
treatment	1,7	4.640	0.068
size	1,7	3.780	0.093
body condition	1,7	0.570	0.476
female ID	9,7	3.460	0.058

Table 7. Results of ANCOVA for Experiment 2 examining the effect of color manipulation, body size, and body condition on the mean distance that males were able to get to females over the course of the trial. Because there was a significant interaction between light environment and color manipulation (Table 6), analyses were run separately for the sun and the shade.

SUN	df	*F*	*P*
treatment	1,7	8.11	0.025
body size	1,7	1.77	0.225
body condition	1,7	0.63	0.453
female ID	9,7	1.54	0.291
SHADE	df	*F*	*P*
treatment	1,7	0.90	0.374
body size	1,7	5.70	0.048
body condition	1,7	0.38	0.557
female ID	9,7	5.94	0.014

Chapter 5

NATURAL VARIATION IN CONDITION-DEPENDENT DISPLAY COLORATION DOES NOT PREDICT MALE COURTSHIP SUCCESS IN A JUMPING SPIDER

Abstract

In many animals, males display elaborate, costly, and condition-dependent colorful ornaments to choosy females. Indicator models of sexual selection predict that females should choose mates based on natural variation in such traits. In *Habronattus pyrrithrix* jumping spiders (family Salticidae), males have conspicuous, condition-dependent red facial coloration and green leg coloration that they actively display to drab gray and brown females during a complex courtship display. In a correlational study using field-collected spiders, we paired individual males with virgin females under full-spectrum lighting in the laboratory and found that condition-dependent natural variation in male coloration did not predict mating success (likelihood of copulation) or levels of female aggression. Rather, mating success was best predicted by male body condition. We then went on to conduct an outdoor experiment under natural sunlight where we gave both virgin and mated females the choice between two simultaneously courting males, one with his facial coloration experimentally reduced and the other that received a sham treatment. Again, we found no relationship between male coloration and courtship success. Our previous studies have shown that the presence (vs. absence) of male red facial coloration improves male courtship success, but here we found no evidence that natural variation in this trait has any effect on success. We discuss these findings in the context of complex, multimodal signaling in *Habronattus*. Finally, we argue that, while many studies report negative results for condition-dependent quality-signaling alongside positive ones, few discuss the implications of the negative results. We argue that, as empirical data continue to accumulate, we should move towards asking why some condition-dependent traits are consistently important to females while other equally condition-dependent traits are either ignored by females, or only matter in certain contexts.

Introduction

In many animals, males engage in costly behaviors or display elaborate ornaments to females during courtship interactions (reviewed in Andersson 1994). Indicator models of sexual selection posit that these elaborate traits, due to the costs associated with their production and maintenance, can honestly signal the individual quality of the bearer (Andersson 1982). Empirical work supporting this idea has grown over the years and there are now countless examples showing that natural variation in costly courtship signals that females find attractive is correlated with different aspects of male quality, such as nutritional condition (e.g., calling in field crickets: Scheuber et al. 2003), health status (e.g., scent marks in house mice: Zala et al. 2004), ectoparasite load (e.g., bowerbird bowers: Doucet & Montgomerie 2003), physical strength (e.g., dancing ability in humans: Hugill et al. 2009), foraging ability (e.g., nuptial gifts in scorpionflies: Missoweit et al. 2007), parental ability (e.g., courtship rate in damselfish: Knapp & Kovach 1991), and cognitive ability (e.g., song complexity in house finches: Boogert et al. 2008).

Because of the costs associated with producing and maintaining colorful ornaments, studies of animal coloration have been at the forefront of work on honest signaling (see reviews in Andersson 1994; Hill & McGraw 2006b, a). For example, many animals use carotenoid pigments to produce elaborate red, orange, and yellow colors that are used by females in mate choice; variation in these colors can signal various types of information relevant to females (e.g., Hill & Montgomerie 1994; Candolin 2000; McGraw & Hill 2000; Mateos-Gonzalez et al. 2011). In addition to pigmentary colors, there is also growing evidence that females prefer elaborate structural colors that are linked to attributes of male quality (e.g., McGraw et al. 2002; Doucet & Montgomerie 2003; Siefferman & Hill 2003; Kemp & Rutowski 2007).

Jumping spiders (Salticidae) are an excellent group to examine if variation in condition-dependent coloration affects mating success. Jumping spiders have excellent vision (e.g., Land 1969; Williams & McIntyre 1980; Blest et al. 1981; Harland & Jackson 2000) including the ability to distinguish between different colors in various contexts (e.g., Nakamura & Yamashita 2000; Jakob et al. 2007; VanderSal & Hebets 2007). In many of the more than 5000 species of jumping spiders (Platnick 2011), males display brightly colored ornaments to dull and inconspicuous females during complex courtship (e.g., Maddison 1995; Oxford & Gillespie 1998) and evidence suggests that some of these traits have been driven by sexual selection (Masta & Maddison 2002). Surprisingly little work has been aimed at understanding how condition-dependent variation in male color might affect male mating success. Recent studies have revealed that several aspects of coloration that are displayed to females during courtship contain reliable information about male diet and age (Lim & Li 2007; Taylor et al. 2011, Appendix B, Chapter 4). Work done with two UV-reflecting species (*Cosmophasis umbratica* and *Phintella vittata*) has shown that blocking UV light affects mate choice decisions, suggesting that UV coloration plays an important role in courtship signaling (Lim et al. 2007; Lim et al. 2008; Li et al. 2008b). In *Habronattus pyrrithrix*, directly blocking male red facial coloration using makeup reduces courtship success of males displaying in bright sunlight (Chapter 4). Thus, we have evidence in *H. pyrrithri*x that male colors are condition-dependent and evidence that the *presence* of male colors improves courtship and mating success in some contexts, but we do not yet know whether females specifically pay attention to natural, condition-dependent variation in male coloration as indicator models predict.

The goal of the present study was to test the hypothesis that natural variation in male display coloration in *Habronattus pyrrithrix* mediates mate choice. Per the indicator model, we predict that condition-dependent attributes of male coloration (i.e., larger, redder facial patches and darker green legs) (Taylor et al. 2011, Appendix B) should be preferred by females. We first test this idea correlationally by pairing field-collected males and virgin females in a mating-success study in the laboratory to determine if these aspects of coloration predict the likelihood that a male will successfully copulate. Because male red facial coloration is sensitive to juvenile diet, with males reared on high-quality diets maturing with larger, redder facial patches (Taylor et al. 2011, Appendix B), this color patch seems like the most likely candidate to signal a male's nutritional status or foraging ability; thus, in a second study, we focused solely on this red coloration. Outdoors, under natural sunlight, we gave both virgin and mated females the choice between two simultaneously courting males, one with his facial coloration experimentally reduced and the other that received a sham treatment (while his natural red facial coloration remained intact), and we measured courtship success of both males. Because female spiders of different mating status (i.e., virgin vs. mated) are expected to exhibit different levels of choosiness (e.g., Jackson 1981), using only virgin females is common practice in jumping spider mate choice experiments (e.g., Clark & Biesiadecki 2002; Elias et al. 2005; Elias et al. 2006a; Lim et al. 2008, see Chapter 4). However, male *H. pyrrithrix* interact with both virgin and mated females in the field (LAT, pers. obs.), and thus inclusion of both classes of females increases the likelihood that our experiment will capture ecologically relevant levels of choosiness. To our knowledge, this is the first study to directly manipulate jumping spider color patterns within natural levels of variation to test the hypothesis that condition-dependent colors function as quality-indicating sexual signals.

Methods

Study species

Habronattus pyrrithrix Chamberlin 1924 is found throughout southern California and Arizona, USA south to Sinaloa, Mexico (Griswold 1987). In Phoenix, Arizona they are quite common and found at high densities in riparian areas, grassy backyards, and agricultural areas (LAT, pers. obs.). Similar to other species of *Habronattus,* males engage in complex courtship display repertoires consisting of both visual and seismic components (LAT, pers. obs.). Male *H. pyrrithrix* orient their red faces and green legs towards females during display, yet these colors are generally not visible from above (LAT, pers. obs.). Courtship typically begins when a male orients towards a female from several centimeters away and waves his front legs while approaching in a zigzag fashion (stage 1 of courtship, Figure 28a). If the female does not retreat or attack the male, he will continue to approach until he is within a few millimeters of the female's face where he abruptly stops, extends his first pair of legs straight up, and begins the seismic component of the display (stage 2 of courtship, Figure 28b; LAT, pers. obs.; D.O. Elias, pers. comm.). Both of these stages are highly variable in length and appear to depend both on the female's response (e.g., remaining stationary, attacking the male, or hopping away) as well as the male's persistence (LAT, pers. obs.). In stage 3 of courtship, the male extends his front legs and gently taps the female's carapace; if she does not retreat or attack, he typically proceeds to mount and copulate with her (LAT, pers. obs., Figure 28d). Agonistic display between males is uncommon in *Habronattus* (Richman 1982; Cutler 1988); in *H. pyrrithrix* males will occasionally wave their legs in the direction of other males, but these displays are rare and short in duration, lasting only a few seconds, compared with displays for females that can continue for hours (LAT, pers. obs.).

Correlational mate choice study

The goal of our correlational mating success study was to determine if naturally-occurring variation in male display coloration explains variation in male mating success. Many mate choice studies in jumping spiders involve exposing a single female to multiple potential male mates that are physically isolated from one another and from the female and then assessing female choice based on differential receptivity behaviors exhibited by the female (e.g., Cross et al. 2007; Lim et al. 2008; Li et al. 2008b). However, to our knowledge, there is no known behavior other than copulation or a female's willingness to let a male approach and make contact with her that reliably signals receptivity in *Habronattus* (LAT, pers. obs., see also Masta & Maddison 2002). For this reason, most mate choice studies with *Habronattus* involve presenting a single male to a single female and assessing mating success by directly observing whether or not copulation occurs (e.g., Masta & Maddison 2002; Hebets & Maddison 2005; Elias et al. 2005; Elias et al. 2006a); thus we used this approach for the correlational mating-success study.

In June 2007, we collected 21 mature adult males and 21 penultimate females (in their final instar, just prior to sexual maturity) from Queen Creek, Arizona, USA (Maricopa County, 33.224744 N, 111.592825 W). We housed spiders individually in clear plastic cages (10.16 x 10.16 x 12.86cm). Light was provided from full-spectrum light bulbs on a 14:10 light-dark cycle (Mercola, Hoffman Estates, IL, USA) and temperature was maintained at approximately 28^{0}C. We fitted each cage with a mesh top to allow full-spectrum ambient light to reach the inside, and we glued an artificial green plant (Ashland fern collection, Michael's Stores, Irving, TX, USA) to the side of the cage for enrichment (Carducci & Jakob 2000). Irradiance data for our artificial lighting is provided as supplementary material in Appendix E. Spiders were fed crickets (*Acheta domesticus*) that were approximately their own body weight three times per week, as this feeding regime resulted in spiders with body condition indices were comparable to those observed in the field (LAT, unpub. data).

When females molted to sexual maturity in the lab, we randomly paired each with a male. Trials began between 0800 and 1100 hrs. when females were between 13 and 29 days post-maturity. Just before each trial began, we weighed spiders to the nearest 0.0001 g with a digital scale and photographed them next to a size standard. From digital photographs, we measured each spider's carapace width using Photoshop software (Adobe Systems Inc., San Jose, CA, USA). Because the carapace width of *H. pyrrithrix* is fixed at maturity, while the abdomen stretches with feeding, we used the residuals of a regression of body mass on carapace width as an estimate of body condition that is uncorrelated with body size and is a common metric of estimating condition in spiders (e.g., Jakob et al. 1996; Taylor et al. 2011, Appendix B).

At the start of each trial, the randomly paired male was introduced into the female's cage and we videotaped all interactions for the first hour. After the hour-long videotaped trial, males and females were housed together for an additional 48 hours to give them additional opportunities to court and mate. We then removed the male and fed the female in excess (approximately three times her own body weight in crickets daily) for two weeks to allow her to lay eggs if she had mated. We confirmed the fertility of all eggs by monitoring them and allowing them to hatch. From the videos, we recorded the levels of female aggression directed towards males (i.e., number of attacks). We quantified mating success by recording whether or not females laid fertile eggs (leading to hatching spiderlings).

At the conclusion of each mating trial, spiders were euthanized and placed in the freezer until color analysis. Colors were quantified following methods described in Taylor et al. (2011, Appendix B). Briefly, we used a reflectance spectrophotometer (USB2000, Ocean Optics, Dunedin, FL, USA) coupled to a modified Leica DMLB2 fluorescence light microscope fitted with a 40x quartz objective lens (Leica Microsystems, Wetzlar, Germany) and illuminated with a full-spectrum Leica 75 W xenon arc lamp (Leica Microsystems, Wetzlar, Germany). Reflectance measures were taken in a dark room and were measured relative to a Spectralon diffuse reflectance white standard (Labsphere Inc., North Sutton, NH, USA). This setup allowed us to quantify the minute color patches on the male’s red faces and green front legs (ca. 0.4 and 0.5 mm in width, respectively) that are too small to measure accurately with standard spectrophotometry equipment. Unfortunately, the optics of the microscope cuts out a portion of the UV spectrum and so this instrument only provides spectral data from 375-700nm. In some jumping spider species, UV reflectance appears to be important in communication (Lim et al. 2007; Li et al. 2008b; Lim et al. 2008), and thus we must use caution when excluding UV reflectance from our analysis. However, in a previous study, we confirmed that, while reflectance does extend into the UV for the green legs, there are no UV peaks and so the benefit of using an instrument that allows precise and repeatable measures on minute color patches that dominantly reflect long-wave light far outweighs the disadvantage of excluding UV (Taylor et al. 2011, Appendix B).

We took the average of two reflectance measures from each male's red facial patch and from each of the male's green front legs. From these spectral data, we calculated (1) the hue of the red face (the wavelength corresponding to the inflection point of the red curve), (2) the red chroma of the face (the proportion of total reflectance between 600 and 700nm), and (3) the brightness (mean reflectance) of the green front legs, as these were the color metrics found to be condition-dependent in a previous study (Taylor et al. 2011, Appendix B). Additionally, because male faces are relatively large surfaces that are only partially ornamented with red scales, we also measured the size of this red patch. We photographed the face of each male through a Leica MZ 125 stereo microscope at 50x magnification using a Spot Insight 11.2 digital camera (Diagnostic Instruments, Sterling Heights, MI, USA; image resolution 1,600 x 1,200 pixels) and Image-Pro Express software (Media Cybernetics, Silver Spring, MD, USA). All photos were taken using the same light and camera settings. We used Photoshop software (Adobe Systems Inc., San Jose, CA, USA) to calculate the total area of red scale coverage. Because larger males had larger patch sizes, we calculated a 'relative patch size index' using the residuals of a regression of patch area on carapace width (Taylor et al. 2011, Appendix B).

Color manipulation experiment

Because the results of our correlational study in the laboratory suggested that natural variation in male coloration does not play a major role in determining mating success (see Results), we went on to explore this idea further using a color manipulation experiment under circumstances in which we might expect color signaling to be most prominent. Specifically, we presented both virgin and mated females with two simultaneously courting males (one of which had his facial coloration experimentally reduced) and we ran all trials outdoors under natural sunlight. Because a male's red facial coloration has been found to be consistently condition dependent in field-collected individuals (see results of correlational study, see also Taylor et al. 2011, Appendix B), to reflect the quality of an individual's juvenile diet (Taylor et al. 2011, Appendix B), and, at least in some contexts, to act as a signal in courtship interactions (see Chapter 4), we currently have the most evidence that red color, as opposed to other ornaments, contains reliable information relevant to mate choice. Thus we focused on this color ornament for the present experiment.

As described above, in order to interpret female choice in a simultaneous mate choice experiment with *H. pyrrithrix*, males must be allowed to approach and make contact with females, and thus cannot be physically isolated from them.

Thus, the best methodology for simultaneous choice tests in this species is to place two males and one female into an experimental chamber where all three can interact freely (see Chapter 4). Consequently, our experimental design does not prevent males from seeing and interacting with one another and as such, we cannot completely disentangle male-male competition from female choice *per se.* However, when two males are courting the same female, they typically direct their displays at the female simultaneously, rather than towards one another (LAT, pers. obs.); thus we expect that any major effect of color manipulation that we see in our experiments will be a result of responses by the female, rather than from interactions between males (LAT, pers. obs).

In March 2011, we collected 42 mature adult male spiders and 21 female spiders from the same population described above. Of the females, 10 were mature upon collection and appeared to be gravid, while 11 were in their final (penultimate) instar just prior to sexual maturity, ensuring that they were virgins. We housed these spiders in the lab (as described above) for one month before the trials began, which allowed putatively gravid females to lay eggs (to confirm that they had indeed already mated) and allowed penultimate females to reach maturity. Because our previous studies suggested that both male size and body condition consistently predicted mating success (see Results; see also Chapter 4) we wanted to eliminate variation associated with size and condition as much as possible to attempt to identify effects of male color *per se*. The month-long period of housing males in the laboratory on a constant diet (see above) allowed us to reduce variation associated with body condition as much as possible. To further reduce variation associated with body size, we paired males by body size so that each pair differed in carapace width by only 0.0087±0.023mm (mean±SE).

Within each male pair, one male was randomly assigned to the color-manipulation group while the other male was assigned to the sham-treated control group. Males in the color-manipulated group had their red facial coloration experimentally reduced so that it closely matched the mean reflectance curve of the 10 least colorful males from this same population in a previous study (Taylor et al. 2011, Appendix B, see Figure 29a,c). To reduce male coloration, we applied a mixture of water and black liquid eyeliner (Color: ‘Perversion’, Urban Decay Cosmetics, Costa Mesa, CA) (Figure 29c). This color-manipulation method reduced the red chroma of the male’s face (see Figure 29a). We also applied additional eyeliner along the edges of the facial patch to reduce the size of the red area to approximate the mean of the 10 smallest patch sizes from a previous study (Taylor et al. 2011, Appendix B). On control males, we applied the same amount of eyeliner and water to an equivalent area on the top of their carapace just behind their anterior median eyes, while their red facial coloration was left intact (Figure 29b). On the day before mating trials, we anesthetized males with carbon dioxide for approximately five minutes while we performed color manipulations. After waking up from anesthesia, males were offered a cricket to confirm that they had recovered fully and were capable of capturing prey. To confirm that our color manipulation did not affect courtship activity or motivation, we recorded the latency for males to begin courting and the overall courtship effort of each male during trials.

Before a trial began, we moved the female's cage outdoors to allow her to acclimate for one hour. All trials were run in full sunlight between 0900 and 1500 hrs., when the mean temperature was 26-32° C. These outdoor trials were run in a riparian habitat on the campus of Arizona State University where *H. pyrrithrix* is found in high abundance (LAT, pers. obs.), thus providing natural light and natural vegetation to serve as background colors. Irradiance data collected in this outdoor area is provided as supplementary material in Appendix E. At the start of the first phase of the trial, the two males were weighed (to determine current body condition, as described above) and placed in the female's cage in immediate succession, in random order. All interactions were videotaped for 40 minutes.

From the videos, we used the freeware program Cowlog (Hänninen & Pastell 2009) to quantify the latency for each male to begin courting and the overall time spent courting. Results from previous studies indicated that the distance that males were able to get to females was an excellent predictor of mating success (i.e., males that were able to get closer to females were more likely to proceed through all stages of courtship and eventually copulate, leading to fertile eggs, see Chapter 4). Because having two simultaneously courting males seemed to distract the female and extend the latency to copulation (LAT, pers., obs.), we found low levels of copulation, comparable to previous studies (see Chapter 4). Thus we used the mean distance that males were able to get to females over the course of the trial as an estimate of courtship success. We also recorded the number of attacks by females on each male and all instances of copulation.

Statistical analyses

Correlational mating success study

First, to corroborate previous findings on the condition-dependence of male colors, we used linear regression to examine the relationships between a male's body condition at collection and color variables that were found to be condition dependent in previous experiments (specifically, the hue, red chroma, and size of the red facial patch and brightness of the green legs, Taylor et al. 2011, Appendix B). We then used logistic regression to examine if these color variables predicted successful copulation, and we used linear regression to determine if they predicted levels of female aggression. We also used logistic regression and linear regression, respectively, to determine if either male size or male body condition predicted copulation success and levels of female aggression.

Color manipulation experiment

First, to confirm that our experimental manipulations did not have adverse effects on male behavior, we used analysis of variance (ANOVA) to determine if the treatment groups (color manipulated vs. sham control) differed in the latency for males to begin courting or in their total courtship effort (amount of time spent courting over the course of the trial). We used logistic regression to confirm that the approach-distance of males to females predicted a male's likelihood to reach stage 2 of courtship, which is required for achieving successful copulation (see Figure 28).

To test the focal hypothesis of our study, we used mixed-model nested ANOVAs to determine if male color treatment, female mating status (virgin or mated), or their interaction affected courtship success (the distance that males were able to get to females) or levels of female aggression. Because each pair of males was tested simultaneously with a single female, female ID was included as a random factor, nested within female mating status. Because both male size and body condition have been found in previous experiments to affect courtship and mating success in *H. pyrrithrix* (see Results for correlational study; see also Chapter 4), we also ran mixed-model nested analysis of covariance (ANCOVA) as above, but with the addition of male size and condition as covariates.

In light of the finding that males were more successful when courting virgin rather than mated females (see Results), we went on to examine whether this pattern might be related to male courtship effort. We used mixed-model nested ANOVAs to determine if female status predicted a male’s latency to begin courting or his total courtship effort. Again, because each pair of males was tested simultaneously with a single female, female ID was included as a random factor, nested within female mating status.

In light of the surprising finding that females were more aggressive towards *larger* males in *better* condition (see Results), we went on to examine the idea that female aggression is an inherent risk associated with proceeding through courtship. Specifically, we used ANOVA to determine if males that were successful at proceeding to stage 2 of courtship received more aggression from females during the courtship process than males who did not progress successfully to stage 2. Because pairs of males were paired with a single female, we included female ID as a random factor in the model.

All data met assumptions of parametric statistics with the exception of the female aggression data (for both the correlational study and the color manipulation experiment); these data were thus rank transformed prior to analyses (Iman & Conover 1979).

Results

Correlational mating success study

As in our previous study, males in better condition had larger, redder facial patches (hue: R^2=0.196, $F_{1,19}$=4.63, P=0.045; red chroma: R^2=0.253, $F_{1,19}$=6.43, P=0.020; relative patch size: R^2=0.343, $F_{1,19}$=9.93, P=0.0053). However, in contrast to previous studies, the brightness of the male's legs was not related to his condition (R^2=0.050, $F_{1,19}$=1.01, P=0.328)

Eleven out of 21 males (52.4%) copulated during trials, leading to fertile eggs. Aspects of condition-dependent male red facial coloration did not significantly predict successful copulation (hue: χ^2=0.731, P=0.393; red chroma: χ^2=0.639, P=0.434; relative patch size: χ^2=0.021, P=0.886; Figure 30) or affect rates of female aggression (hue: R^2=0.012, $F_{1,19}$=0.23, P=0.635; red chroma: R^2=0.016, $F_{1,19}$=0.30, P=0.589; relative patch size: R^2=0.036, $F_{1,19}$=0.71,

P=0.409). Similarly, aspects of male green leg coloration also did not predict successful copulation (χ^2=2.741, P=0.098) or affect levels of female aggression (leg brightness: R^2=0.021, $F_{1,19}$=0.41, P=0.529).

Males in better condition were significantly more likely to copulate (χ^2=4.15, P=0.041; Figure 30), but male size had no effect on this measure of success (χ^2=0.078, P=0.779). Neither male condition nor size affected female aggression (condition: R^2=0.016, $F_{1,19}$=0.31, P=0.582; size: R^2=0.052, $F_{1,19}$=1.03, P=0.322)

Color manipulation experiment

Our experimental color manipulations had no adverse effects on male behavior, as there were no differences between the treatment groups in the latency for males to begin courtship ($F_{1,40}$=1.99, P=0.166) or the total time that males spent courting ($F_{1,40}$=0.07, P=0.799). As in previous studies, the distance that males were able to get to females was a strong predictor of a male's ability to proceed to stage 2 of courtship (χ^2=12.55, P=0.0004).

Males were more successful in courting virgin compared with mated females, but a male's color treatment had no effect on his success (Figure 31, Table 8). Neither female mating status nor male color treatment affected levels of female aggression (Table 8). When male size and condition were included as covariates, these factors had no effect on male courtship success (Table 9). Surprisingly, though, females were more aggressive towards males in better condition, and also tended to be more aggressive towards larger males (Table 9).

Males began courting virgin females more quickly than mated females ($F_{1,21}$=30.96, P<0.0001) and devoted more total time to courting virgins compared with mated females ($F_{1,21}$=4.57, P=0.044). Males that were successful in proceeding to stage 2 of courtship (n=16 out of 42) tended to receive higher levels of aggression from females than did males that did not proceed to stage 2 ($F_{1,20}$=4.12, P=0.056).

Discussion

In both a correlational study and a color manipulation experiment with *Habronattus pyrrithrix*, male color variables that are consistently condition-dependent across studies had no effect on female mate choice. First, using field-collected spiders, we paired individual males with virgin females under full-spectrum lighting in the laboratory and found that natural variation in male red facial and green leg coloration did not predict mating success (likelihood of copulation) or levels of female aggression. Rather, mating success was best predicted by male body condition. We then went on to conduct an outdoor experiment under natural sunlight, where we gave both virgin and mated females the choice between two simultaneously courting males, one with his facial coloration experimentally reduced and the other that received a sham treatment. Again, we found no relationship between male coloration and courtship success. Previous studies have shown that the presence (vs. absence) of male red facial coloration improves male courtship success (Chapter 4), but here we found no evidence that natural variation in this trait has any effect on success.

Patterns of condition dependence

Consistent with previous studies on the condition dependence of coloration in *H. pyrrithrix* (Taylor et al. 2011, Appendix B), field-collected males in better condition had larger and redder patches of color on their faces. Previous studies have also shown that the brightness of a male's green front legs correlates with condition in the field, yet that pattern did not hold up in the present correlational study. Unlike red face coloration, which is dependent on juvenile diet (Taylor et al. 2011, Appendix B), the brightness of a male's green legs is dependent on male age (Chapter 3). Because the present correlational study was conducted earlier in the season (early June) compared with the previous study that established the condition dependence of leg coloration in July and August, it may be that the population earlier in the season included mostly recently matured (i.e., young adult) males. Later in the season, as more males mature, we would expect a larger variation in age among mature males. Our color data are consistent with this idea; the mean leg brightness observed in this study (mean =21.0% reflectance) was on the darker end of the range found by Taylor et al. (range of male leg brightness = 11.79-48.89% reflectance, Taylor et al. 2011, Appendix B), suggesting that these males were relatively young. Unlike the green leg coloration, once a male's red facial coloration is produced at maturity, it does not change with age (Chapter 3). Juvenile diet seems to be responsible for variation in red facial coloration (Taylor et al. 2011, Appendix B), which may explain why this pattern has been more consistent among field studies. In some systems, the condition dependence of particular display traits varies with environmental heterogeneity; such traits may only provide useful information under relatively poor conditions when not all individuals can afford to invest in them (Vergara et al. 2012). The fact that the red coloration examined here has been found to be consistently condition dependent in this population suggests that it might be a particularly good candidate for a reliable signal of quality in this species (e.g., Andersson 1982).

Predictors of mating success

Contrary to our hypothesis, our correlational study revealed that natural variation in male red facial and green leg coloration did not predict mating success. When we went on to manipulate red facial coloration in our simultaneous choice test, again, male coloration had no effect on courtship success; males whose red facial coloration was experimentally reduced were just as successful at approaching females as sham-treated control males. As in previous studies with *H. pyrrithrix* (Chapter 4), males in our correlational study that were in better condition were more likely to copulate; this pattern of condition improving success holds up for other groups of ground-dwelling spiders (e.g., Gibson & Uetz 2008). In the color manipulation experiment, we deliberately reduced variation in male body size (by ensuring that paired males were very similar in size) and condition (by keeping males on a constant diet in captivity for one month), yet we still saw no effect of color on courtship success.

There are several possible explanations for our findings that male ornamental coloration was less important for male mating success than other morphological traits. First, males in the genus *Habronattus* engage in extremely elaborate and complex courtship displays consisting of various combinations of brilliant colors (e.g., Taylor et al. 2011, Appendix B), motion (e.g., Elias et al. 2006b), and seismic components (e.g., Maddison & Stratton 1988; Elias et al. 2003). One hypothesis to explain the evolution of complex signals is that each signal component contains different information, or that they contain similar information but act to back each other up when other signal components are more difficult to transmit (reviewed in Hebets & Papaj 2005). If this is the case, it may be that the suite of information presented is very complex, and thus the effect of any one component of the display (such as facial color) on a female's overall mate choice may be subtle. Examining these traits in isolation might make subtle effects difficult to detect.

A second explanation is that variation among females in their preferences for different display traits obscures overall, general preference patterns for any one trait, such as red facial color. Studies of the complex displays of bowerbirds suggest that there are age-specific differences in the male traits that females use in mate assessment (Coleman et al. 2004). Similarly, in buntings, there is extreme temporal variation in female mate choice, with females preferring different traits from year to year, presumably depending on their specific needs in a changing environment (Chaine & Lyon 2008). In both cases, it has been argued that such variation in female preferences may have driven male signal complexity

(Coleman et al. 2004; Chaine & Lyon 2008). If similar female preference variation is responsible for the complex displays in *Habronattus*, significant preferences for any one trait such as red facial coloration, again, may be difficult to detect in a single year or study. Because previous work has shown that female choosiness and female preferences change with mating status in other jumping spiders (Jackson 1981; Cross et al. 2007), in our experiment, we included both virgin and mated females. While we found no evidence of an interaction between female mating status and her preferences for male red facial color, there are other factors that might create variation in female preferences that should be explored in this system. In wolf spiders, female preferences are shaped by their experience with males before they mature (Hebets 2003). Given the evidence for flexibility in courtship strategies of many species of jumping spiders (e.g., Jackson 1977; Jackson & Macnab 1991; Jackson 1992; Jackson & Willey 1995; Cross et al. 2008) and their ability to modify many aspects of their behavior through experience and learning (reviewed in Jakob et al. 2011), it would interesting to examine how female experience might create variation in male color preferences in *H. pyrrithrix*.

In this study, male *H. pyrrithrix* courted every female to some extent in every trial. Even in the field, *H. pyrrithrix* males typically court every female that they encounter, including heterospecifics (see Chapter 1). In our color manipulation experiment, we monitored male courtship effort to confirm that our treatments were not affecting a male’s motivation to court and, interestingly, we found that while male color treatment did not affect his courtship effort, males began courting virgin females more quickly than mated females and also invested more time overall to active courtship with virgins. There is growing evidence that males of some species of jumping spiders show preferences for some females over others (Cross et al. 2007; Hoefler 2007). In *Phidippus clarus*, mature males cohabit with and guard immature females before they mature; these males show a preference for larger females that mature sooner (Hoefler 2007). In *Evarcha culicivora*, both males and females actively engage in courtship, and males prefer larger females as mates (Cross et al. 2007). In the present study, because males were introduced into the cages of females, it is possible that cues from female silk provided information to males about female mating status (e.g., Hoefler 2007). Because mated female jumping spiders have been shown to be choosier than virgin females (Jackson 1981) and because males of some species deposit mating plugs over the copulatory organs of females after mating (Jackson 1980), choosing to prioritize time courting virgin, rather than mated, females may be advantageous. Even when males court indiscriminately, future work should examine subtle choices that males may make about where to focus their courtship effort.

An interesting and unexpected finding uncovered in this experiment was that females actually exhibit higher, rather than lower, levels of aggression towards males that are in better condition, and that larger males also tended to receive higher levels of aggression from females. Multiple studies have now shown that female *H. pyrrithrix* prefer to mate with bigger males in better condition (correlational study, see also Chapter 4). Why then should these presumably ‘preferable’ males elicit higher levels of aggression from females? Perhaps female aggression is simply a part of the courtship process that males must endure to progress through courtship. We show here that, regardless of color treatment, males that are able to successfully proceed to stage 2 of courtship (where they are within millimeters of the female’s face, see Figure 28b) receive higher levels of aggression than females that are not able to make it to this stage. If these attacks are attempts at cannibalism by females, this suggests that progressing through courtship might be inherently risky for males. It may be that when two males are courting simultaneously, larger males are more willing to incur such risks as they may be harder for females to cannibalize. Or perhaps these non-fatal attacks directed towards females are not missed cannibalism attempts, but are rather an additional way that females assess a male’s quality.

In summary, our data indicate that, while there is indeed variation in courtship and mating success among male *H. pyrrithrix* (i.e., not all males copulate, and there is variation in how effective males are at approaching females and proceeding through the stages of courtship), this variation was not predicted by aspects of condition-dependent red facial coloration. Throughout the honest signaling literature are numerous examples of studies in which suites of condition-dependent color variables are tested as quality-indicators (e.g., see reviews in Hill 2006; Senar 2006; Santos et al. 2011). While many of these studies report negative results alongside positive ones (e.g., see meta-analysis data in Santos et al. 2011), most discussions in the literature focus only on the specific results that support their hypotheses, glazing over the importance of the tests that do not. As a result, numerous cases of traits that are strongly condition dependent but do not affect female choice are reported, but go comparatively unnoticed in the literature. If hypotheses and predictions are indeed generated *a priori*, then non-significant results that do not support hypotheses should be just as important to our overall understanding of the importance of quality signaling as the significant results that do support them. Multiple meta-analyses have revealed that the ways in which results on a particular topic are reported (i.e., publication biases) change as paradigms in behavioral ecology shift (Alatalo et al. 1997; Simmons et al. 1999; Poulin 2000). We argue that, as the evidence for condition-dependent quality signaling continues to accumulate, we should move towards understanding why some condition-dependent traits matter to females while others are ignored by females or only matter in certain contexts. This approach may help us better understand the complex factors that shape the brilliant and often costly ornaments that males display.

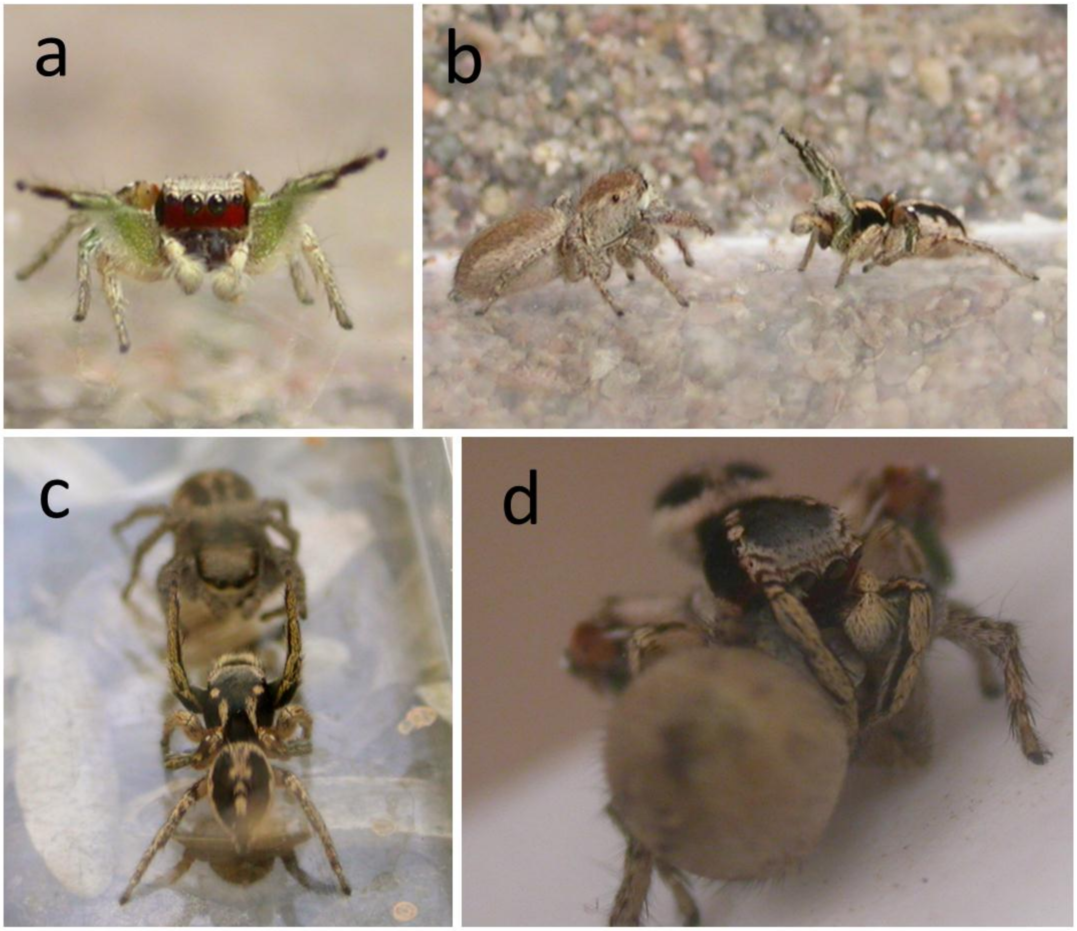

Figure 28. Stages of male courtship in *Habronattus pyrrithrix.* (a) stage 1: the male waves his legs as he approaches the female in a zigzag fashion, (b) stage 2: the male (right) has successfully approached and stopped directly in front of the female (left) and is now performing a display that consists of both visual and seismic components, (c) stage 3: the male gently taps the female's carapace with his front legs, (d) the male proceeds to mount and copulate.

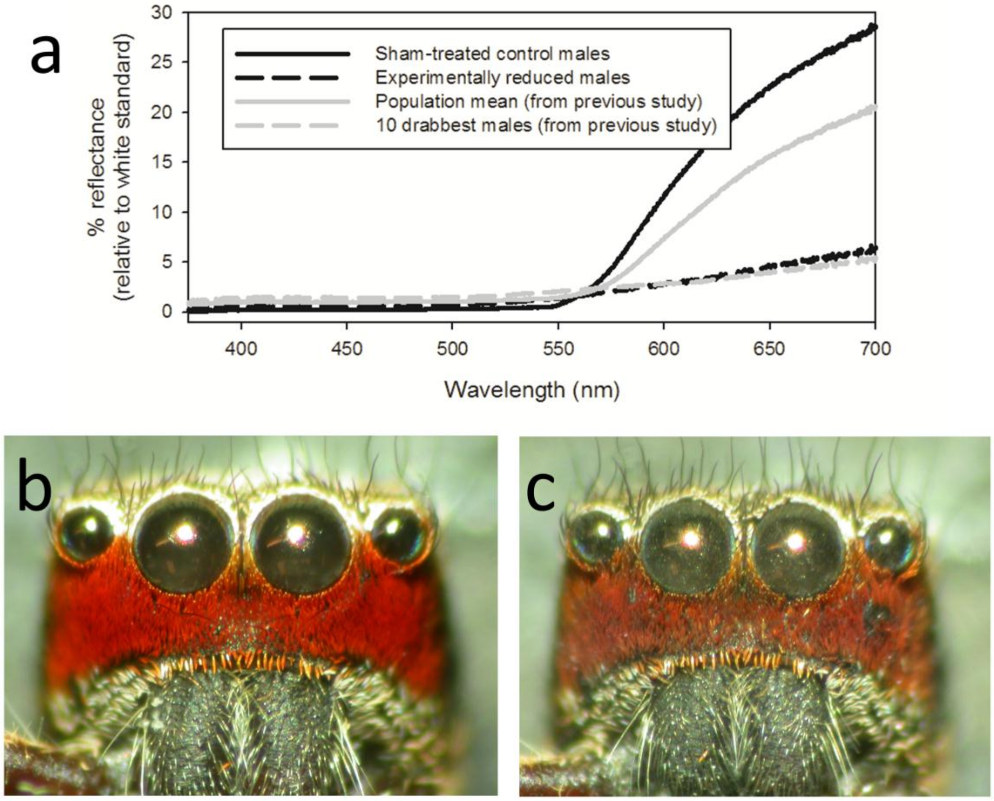

Figure 29. Experimental color manipulation used to reduce the size and color of a male's red facial patch. (a) spectral properties of sham-treated control males and color-manipulated males, compared with the population mean and the 10 drabbest males from a previous study (Taylor et al. 2011, Appendix B), (b) the intact red facial coloration of a sham-treated control male, and (c) experimental manipulation (reduction) of a male's red facial coloration.

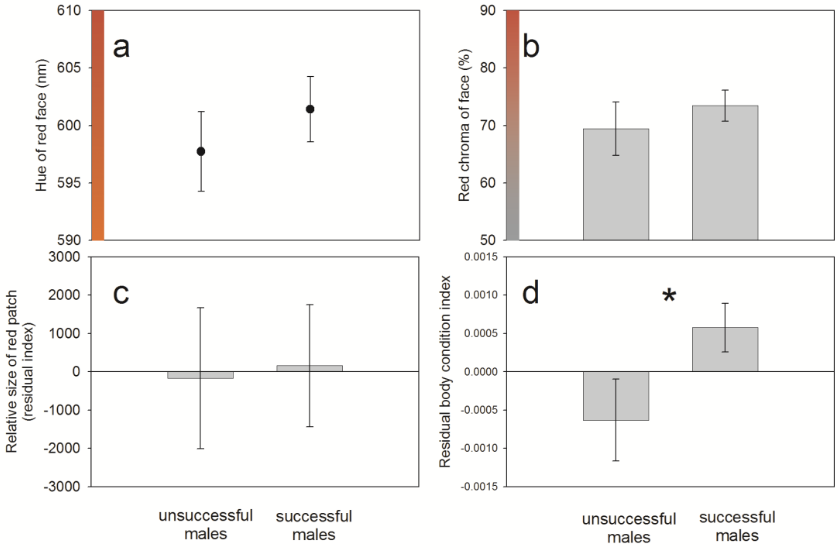

Figure 30. Comparisons of color variables (a, b, c) and body condition (d) between males that copulated successfully and those that did not copulate during the correlational mating success study. The presence of an asterisk (*) indicates a significant difference between groups.

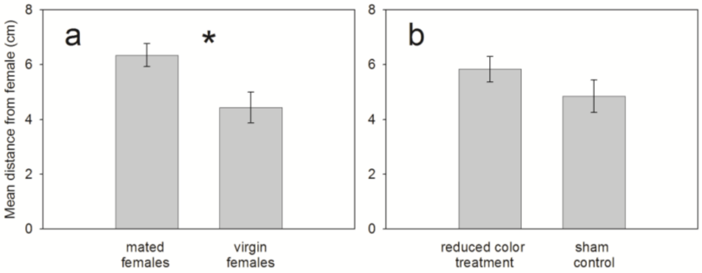

Figure 31. Courtship success (mean distance that males were able to get to females) during the color manipulation experiment. Males were more successful courting virgin compared with mated females (a). Experimental reduction of red facial coloration had no effect on male courtship success (b). The presence of an asterisk (*) indicates a significant difference between groups.

Table 8. Results of mixed model nested ANOVA and ANCOVA for color manipulation experiment examining the effect of male color manipulation and female mating status on the mean distance that males were able to get to females over the course of the trial.

ANOVA	df	*F*	*P*
Treatment	1,19	1.33	0.263
female mating status	1,19	5.55	**0.029**
treatment x female mating status	1,19	1.89	0.185
female ID (nested within female mating status)	19,19	0.43	0.962
ANCOVA	df	*F*	*P*
Treatment	1,17	0.91	0.353
female mating status	1,17	1.00	0.331
treatment x female mating status	1,17	0.99	0.177
female ID (nested within female mating status)	19,17	0.39	0.973
male body condition	1,17	2.08	0.167
male body size	1,17	0.20	0.660

Table 9. Results of mixed model nested ANOVA and ANCOVA for color manipulation experiment examining the effect of male color manipulation and female mating status on levels of female aggression (i.e., number of attacks) directed towards courting males.

ANOVA	df	*F*	*P*
Treatment	1,19	0.13	0.719
female mating status	1,19	0.01	0.941
treatment x female mating status	1,19	0.43	0.521
female ID (nested within female mating status)	19,19	0.86	0.628
ANCOVA	df	*F*	*P*
Treatment	1,17	0.27	0.616
female mating status	1,17	1.25	0.279
treatment x female mating status	1,17	0.09	0.763
female ID (nested within female mating status)	19,17	1.36	0.265
male body condition	1,17	6.40	**0.022**
male body size	1,17	3.48	0.080

Chapter 6

CONCLUDING REMARKS

In this dissertation, I addressed the question of why male jumping spiders in the genus *Habronattus* have evolved such striking and conspicuous colors compared to drab and cryptic females. I used a variety of approaches, ranging from field studies where I tracked spiders and documented natural behavior in the field to carefully controlled experiments on diet, coloration, and mate choice in the laboratory. Guided by the relevant natural history gleaned from field observations, I tested multiple hypotheses for the current function of ornate coloration, including those encompassed by both sexual selection (e.g., species recognition, quality signaling) and ecological selection (e.g., differential habitat use, different strategies of avoiding predation). While this work yielded some results consistent with patterns found in other colorful taxa, I also found several surprising results that challenge traditional views of color signaling; it is these unexpected results that offer the most novel insights into where we should direct future research efforts on elaborate coloration in spiders and other animals.

Sexual selection

A particularly surprising finding was the high rate of misdirected, heterospecific courtship documented under natural conditions in the field (Chapter 1). When examining a local community of four sympatric *Habronattus* species (none of which are closely related enough to hybridize), males courted females indiscriminately at every opportunity, regardless of the species of the female. While misdirected courtship has been documented in other taxa, it has been given little attention in the ecological literature and field studies of misdirected courtship are particularly rare (see Groening & Hochkirch 2008). Male courtship in *Habronattus* is likely to be costly; males engage in dances that combine color, motion, and seismic cues (Richman 1973; Maddison & Hedin 2003; Elias et al. 2005). Male courtship is also likely to be risky, as cannibalism by adult females made up a large proportion of the predation events that I observed in the field (Chapter 2). While the four species that I examined had slightly different habitat preferences, there was substantial overlap and high interaction rates in the field; in three of the four species, individuals were just as likely to encounter a heterospecific as they were to encounter a conspecific (Chapter 1). These findings suggest that interspecific communication is likely to be extremely important, particularly in areas where multiple *Habronattus* species co-exist.

Theoretical work, dominated by consideration of signaling in better-studied taxa such as birds, predicts that species recognition signals should not evolve to be particularly costly, conspicuous, or elaborate, as they only need to allow prospective mates to be differentiated from a limited number of other possible species (reviewed in Dale 2006). Yet male *Habronattus* might challenge this rule, given that the recognition costs subsume both survival and reproduction; the need to identify themselves clearly to females from a safe distance and to reduce their risk of cannibalism might shift the costs and benefits in favor of extremely conspicuous, elaborate species recognition signals.

Given the degree of misdirected courtship documented here, we might expect that females would pay close attention to male characteristics, such as color, that could be used to accurately identify his species. Interestingly, though, in *H. pyrrithrix*, females did not use species-specific male display color as a requirement for successful mating (Chapter 4). In an experiment under artificial full-spectrum lighting, males that had their species-specific red faces and/or green leg coloration blocked were just as likely to mate as sham-treated control males with their colors intact. Only when females were presented with simultaneously courting males in full sunlight did we find that the presence of red facial coloration improved male courtship success; when these same males were allowed to court in the shade, color treatment was unimportant and male size became the best predictor of success (Chapter 4). These findings suggest that females might hone in on different male qualities depending on the environmental conditions where courtship takes place. The transmission of color signals depends on the light environment in which they are sent; here I show that red facial coloration is more effective as a courtship signal under lighting conditions that contain more red light (i.e., sunlight compared with shade) (e.g., Endler 1993). *Habronattus* males have extremely colorful, multicomponent displays and it may be that this complexity allows them to communicate effectively in variable and unpredictable light environments. While this idea of 'backup signals' has been suggested to explain display complexity in other taxa (reviewed in Johnstone

1996; Hebets & Papaj 2005), it is surprising that in *H. pyrrithrix* such drastic color manipulations only affect male courtship in a limited context (i.e., sunlight).

In addition to species recognition signals, I also considered the hypothesis that male colors act as condition-dependent quality signals. Across three correlational studies and two diet-manipulation experiments, I found consistent patterns of condition dependence for male red facial and green leg coloration. Males in better condition had redder faces and darker green legs (Taylor et al. 2011, Appendix B, Chapter 5); follow-up experiments revealed that that red coloration is dependent on the quality of juvenile diet (Taylor et al. 2011, Appendix B) whereas green leg coloration is linked to a male's age (Chapter 3). These patterns of condition-dependence clearly fit a dominant hypothesis in the literature to explain display complexity: different colors can encode and reveal different information to potential female mates (reviewed in Hebets & Papaj 2005). Surprisingly, though, using both correlational and experimental approaches, I found no evidence that natural variation in these male colors affects any aspect of a male's courtship success and that condition was a better predictor of success than color (Chapter 5).

Throughout the honest signaling literature are studies in which suites of condition-dependent variables (e.g., multiple aspects of color) are considered as potential quality-indicating signals (e.g., see reviews in Hill 2006; Senar 2006; Santos et al. 2011). Within individual studies, negative results are often reported alongside positive ones (e.g., see meta-analysis in Santos et al. 2011), yet discussions within these studies are often centered solely on the positive results;

implications of negative results are rarely discussed and go comparatively unnoticed in the literature (pers. obs.). Rather than simply cataloguing and emphasizing the results that fit our predictions, we should move towards addressing the question of why some condition-dependent traits are consistently important to females, while other *equally* condition-dependent traits are either ignored by females or are only used in certain contexts. This emphasis may help us better understand phenomena that often puzzle behavioral ecologists, such as yearly fluctuations in mate preferences (e.g., Chaine & Lyon 2008; Lehtonen et al. 2010) or patterns of geographic variation in male ornamentation and female preference (e.g., Hill 1994; Hebets & Maddison 2005). Jumping spiders, as well as other taxa that frequently contradict our expectations, may yield new insights for the field.

Ecological selection

In addition to sexual selection, differences between the sexes can also be shaped by selection pressures that are unrelated to mating (e.g., differences in habitat, diet, etc., reviewed in Slatkin 1984). There are numerous examples across the Salticidae in which males exhibit more conspicuous dorsal patterning compared with drab and cryptic females (e.g., see images in Maddison 1995), yet to my knowledge no study has examined potential selection pressures that drive these patterns of sex differences in non-display colors. Here I tested the hypothesis that conspicuous male body patterns in three species of *Habronattus* are paired with increased leg-waving behavior, a combination that is reminiscent of the body patterns and antennation of wasps and bees. As such, this resemblance may provide protection for males by exploiting the aversions of their predators (e.g., see recent discussion in Schaefer & Ruxton 2009; Ruxton & Schaefer 2011). Furthermore, I provide data in these three species suggesting that sex differences in coloration strategies may be shaped by differences in activity patterns between males and females. Specifically, I propose that the high movement rates of males associated with mate-searching may reduce the effectiveness of crypsis (e.g., Merilaita & Tullberg 2005) and select for alternative strategies of protective coloration. Rather than fitting within the traditional paradigms of Batesian mimicry (reviewed in Ruxton et al. 2004), the coloration and behavioral strategy employed by male *Habronattus* appears to be more consistent with a recently proposed mimicry paradigm employed by tropical caterpillars that display prominent vertebrate-like eyespots (Janzen et al. 2010). In this case, the benefits of imperfect mimicry are sustained by strong, innate (rather than learned) aversions to eye-like patterns and, as such, there is no particular predator species or assemblage of species actively driving close resemblance between mimic and model (see Janzen et al. 2010).

Here again, salticids might be well-suited to provide novel insights for the field of animal coloration. In addition to the need to accommodate higher movement rates associated with mate searching, male salticids also face two additional, unique challenges that mimicry and/or perceptual exploitation might help them solve. First, males must balance the need to attract female attention from a safe distance using elaborate and complex displays (often involving leg-waving), while also avoiding predation by the females that they are courting. Because their courtship makes males so conspicuous, any effective anti-predator strategy must be one that continues to work after the male has already been detected. Second, males must be extremely focused on any female that they are courting, and must be prepared to retreat should that female attack. This need to focus their attention entirely on the female in front of them makes them particularly vulnerable from behind; color patterns that work synergistically with their already-waving legs might offer the ideal protection. These unique natural history attributes of salticids may explain why mimicry is extremely widespread across the family (e.g., Cushing 1997).

Salticids as models for future work

There are more than 5000 species of jumping spider (Platnick 2011) exhibiting a myriad of color patterns (see Figure 1, Preface), yet until recently salticids were notably absent from the field of animal coloration. To my knowledge, in only three species have colors even been quantified spectrophotometrically (e.g., Lim & Li 2006a; Li et al. 2008a; Taylor et al. 2011, Appendix B). Within the salticids, there is also a rich diversity in natural history, suggesting that the selection pressures shaping color patterns are likely to be incredibly variable. Elaborate and colorful displays are common and occur in the context of both courtship and male-male interactions (e.g., Peckham & Peckham 1889, 1890). There are species with feeding specializations on vertebrate blood (Jackson et al. 2005), plants (Meehan et al. 2009), ants (Li et al. 1996), and even other salticids (Harland & Jackson 2001). Various forms of mimicry have been described in salticids, ranging from aggressive mimicry (Jackson & Wilcox 1993) to Batesian mimicry of ants (Nelson & Jackson 2009), wasps (Edwards 1984), and even pseudoscorpions (Platnick 1984). Given this diversity in natural history, this understudied group is likely to provide a wealth of insight into the innumerable ways in which colors can evolve and continue to challenge our current thinking on the rules that apply to color evolution in other animals.

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APPENDIX A

ANIMAL COLORATION: SEXY SPIDER SCALES

Dispatches

Animal Coloration: Sexy Spider Scales

Many male jumping spiders display vibrant colors that are used in visual communication. A recent microscopic study on a jumping spider from Singapore shows that three-layered 'scale sandwiches' of chitin and air are responsible for producing their brilliant iridescent body coloration.

Lisa A. Taylor and
Kevin J. McGraw

Animals exhibit an amazing array of body colors and patterns that communicate information about themselves, such as their age, sex, mate quality and fighting ability, to other individuals. Abundant and diverse colors in groups such as butterflies [1], fish [2] and birds [3,4] have made them model systems for investigating the control, function, and information content of chromatic signals in animals. But it is important to expand studies of color signaling to other taxa, where color is not as common and where species have different life-history adaptations than these well-studied groups, so that we can test generalized principles of color evolution. Matthew Lim and colleagues have capitalized on such an opportunity with their recent investigations into the functions of the brilliant colors of jumping spiders (Araneae: Salticidae) [5–8] and the optical mechanisms that produce them [9].

Jumping spiders have been the focus of several studies on sexual selection in recent years, but primarily at the population level, where males from geographically isolated regions use various behavioral and morphological mating displays that may foster reproductive isolation and eventually speciation [10,11]. Little had been uncovered about the physical mechanisms that generate flashy, often iridescent coloration in male salticids [12] or the adaptive roles of intraspecific male color variation [13]. In other animals, from beetles to butterflies to birds, microscopy studies have shown that iridescent body colors are created by tissues containing multiple layers of material, varying in index of refraction, that reflect light differently at different incident angles [14]. Moreover, these (and other types of) colors can serve as honest indicators of the health and condition of individuals [4]. In a series of five papers published within the last year, researchers from southeast Asia and Europe have begun to test these hypotheses in *Cosmophasis umbratica*, a diminutive and iridescently colored jumping spider from Singapore (Figure 1A).

Lim and Li [5] first quantitatively described the pattern of sexual dichromatism in this species. Both sexes appear colorful to our eyes, with obvious sex-specific iridescent patterns that are brighter in males than in females. In addition, the scales of males, but not those of females, reflect ultraviolet (UV) light (Figure 1B) that is invisible to us. The authors used behavioral experiments to test the idea that this male-specific UV scale coloration serves as an important signal in male–male and male–female interactions. They took advantage of a convenient and endearing quality of male jumping spiders — their enthusiasm for interacting with every other jumping spider they encounter, including their own mirror image. In full spectrum light, males displayed aggressively to their mirror image, as we would expect one to behave towards a rival male; however, when UV light was filtered out, the males behaved differently, often courting themselves. These same patterns held up with interactions between two male spiders — in the presence of UV light, males reacted aggressively to other males, while in the absence of UV light, they courted them [6]. Females also seem to be paying attention to UV coloration in males; in experiments where UV light was filtered out, females ignored male courtship displays [7].

All of this suggests that UV color markings on males are important signals for sexual identification. Lim and Li [8] further hypothesized that variation in male color may communicate valuable information about a male's fighting ability or his quality as a mate. Using experimental manipulations, they found that younger and better-nourished male spiders developed more intense coloration, suggesting an honest signaling role for male color variability.

In their most recent work [9], this time led by Michael Land, they described the optical mechanism responsible for male coloration, which helps provide a better understanding of the challenges associated with producing such flashy colors, and the information such signals may convey. Using light and electron microscopy, they imaged the colorful body scales of males and found that each scale is composed of a sandwich structure with two outer corrugated chitin plates surrounding a thin layer of air (Figure 1C). This structure functions as a multilayer reflector, whereby the alternating layers of chitin, with a high refractive index, and air, with a low refractive index, organized at just the right periodicity with respect to the wavelength of light reflected, result in constructive interference, as light reflected off each interface emerges in phase (Figure 1C).

The result is a brilliant iridescent display by each scale, with a large reflectance peak in the human visible range — the color of which changes from green to yellow to orange depending on the viewing angle — and a smaller peak in the ultraviolet (Figure 1B). Interestingly, males also have some body scales with the same chitin plates as described above, but lacking the air gap, which results in a dull purple reflectance (with a relatively large UV contribution). Thus, some of the variation in color and pattern on

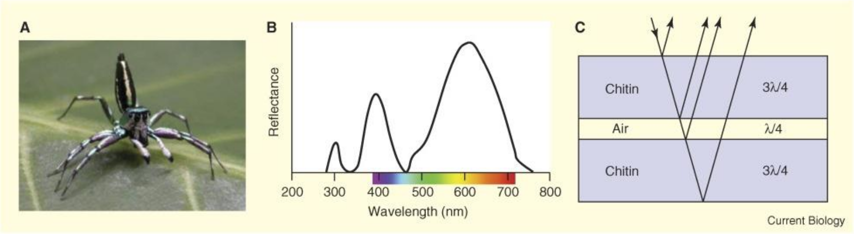

Figure 1. Male coloration in *Cosmophasis umbratica.*

(A) Male courtship display. (Photo by Matthew Lim and Daiqin Li.) (B) Spectral reflectance of iridescent scales on dorsal carapace of males, showing reflectance that spans from the human visible range into the ultraviolet. (Adapted from [9].) (C) Model of an iridescent orange-green/UV body scale showing two chitin plates separated by an air gap. Dimensions of each layer are shown as fractions of the wavelength of maximally reflected light (λ). Light reflected off of each interface interferes constructively, resulting in brilliant color that changes with viewing angle. (Adapted from [9].)

a male's body, including the relative contributions of UV and visible reflectance, can be attributed simply to a modification of the width of the air gaps in their scales. While complex multilayer reflectors are common color-producing mechanisms in a variety of iridescent animals [15], this simple three-layer sandwich structure has been described only once before, in the blue-green wing patches of an African swallowtail butterfly [16]. By demonstrating that color variation can be explained by such precise, highly organized nanostructures, which are presumably costly to produce, this work provides a mechanistic explanation for how honest information might be encoded within a male's color.

With this detailed information in hand about the control and function of ornate colors in male *C. umbratica*, the stage is now set for additional studies into the costs and benefits of color expression in other jumping spiders — a species-rich group (over 5000 species now described [17]) that exhibits an amazing diversity of male colors and patterns. Because their biology is quite different from the aforementioned groups that have dominated the field of sexual selection and coloration to date, jumping spiders may provide novel insights into the interplay of natural and sexual selection in shaping colorful male ornaments. Predation pressures are traditionally thought to limit the degree of expression of brilliant coloration in animals [18], but because of the difficulties in tracking predator behavior this phenomenon is understudied. Male jumping spiders can face predation threats from two important and tractable sources [19] — conspecific females, which are often large, voracious, and potentially cannibalistic generalist predators; and other dense-vegetation and litter-dwelling spider species (outside of the family Salticidae), with little or no color vision. Thus, the ability of a male jumping spider to develop and use color as a signal should be predictably shaped by the relative need to signal mate identity (and/or quality) to females and the relative constraints placed by interspecific predators on color detection. The fact that we can couple ecological, behavioral, and anatomical studies of jumping spiders, especially under controlled captive conditions, makes these small creatures exceptional candidates for future tests of the evolution of color communication, and of animal signaling generally.

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School of Life Sciences, Arizona State University, Tempe, Arizona 85287, USA.
E-mail: lisa.a.taylor@asu.edu

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APPENDIX B

CONDITION DEPENDENCE OF MALE DISPLAY COLORATION IN A JUMPING SPIDER

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ORIGINAL PAPER

Condition dependence of male display coloration in a jumping spider (*Habronattus pyrrithrix*)

Lisa A. Taylor · David L. Clark · Kevin J. McGraw

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Abstract In many animals, conspicuous coloration functions as a quality signal. Indicator models predict that such colors should be variable and condition dependent. In *Habronattus pyrrithrix* jumping spiders, females are inconspicuously colored, while males display brilliant red faces, green legs, and white pedipalps during courtship. We tested the predictions of the indicator model in a field study and found that male body condition was positively correlated with the size, hue, and red chroma of a male's facial patch and negatively correlated with the brightness of his green legs. These traits were more condition dependent than non-display colors. We then tested a dietary mechanism for condition dependence using two experiments. To understand how juvenile diet affects the development of coloration, we reared juvenile spiders on high- and low-quality diets and measured coloration at maturity. To understand how adult diet affects the maintenance of coloration, we fed wild-caught adults with high- or low-quality diets and compared their coloration after 45 days. In the first experiment, males fed high-quality diet had redder faces, suggesting that condition dependence is mediated by juvenile diet. In the second experiment, red coloration did not differ between treatments, suggesting that adult diet is not important for maintaining the color after it is produced at maturity. Diet had no effect on green coloration in either experiment. Our results show different degrees of condition dependence for male display colors. Because red is dependent on juvenile diet, it may signal health or foraging ability. We discuss evidence that green coloration is age dependent and alternatives to indicator models for colorful displays in jumping spiders.

Keywords Diet · Honest advertisement · Nutrition · Ornamental color · Salticidae · Sexual dichromatism · Sexual signaling

Introduction

In many animals, males are adorned with elaborate ornaments that they display to females during courtship or to other males during competitive interactions (reviewed in Andersson 1994). There are a number of hypotheses that have been proposed to explain the preponderance of such conspicuous, and potentially costly, sexual signals by males. Guilford and Dawkins (1991) provide a useful framework by distinguishing between hypotheses that are content-based and those that are efficacy-based (see also Hebets and Papaj 2005). Content-based hypotheses are those that propose that there is some sort of information being provided by the signal of interest. In the context of courtship, this could include a signal that identifies a courting male as the appropriate species (e.g., Couldridge and Alexander 2002) or provides information about the relative quality of that male as a mate (Andersson 1982). Alternatively, efficacy-based hypotheses propose that there is little or no information present in the signal, but that it has been selected simply as a mechanism to improve signal

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L. A. Taylor (✉) · K. J. McGraw
School of Life Sciences, Arizona State University,
PO Box 874601, Tempe, AZ 85287, USA
e-mail: Lisa.A.Taylor@asu.edu

D. L. Clark
Department of Biology, Alma College,
Alma, MI 48801, USA

transmission or make use of the female's sensory psychology to achieve a specific response. Examples of efficacy-based hypotheses include the use of a signal to attract a female's attention in a noisy environment (e.g., Clark and Uetz 1993), to reduce aggression or cannibalism in a potential female mate (e.g., Forster 1982; Hebets and Papaj 2005), or simply to take advantage of a female's sensory biases (e.g., Basolo 1990) or preferences for exaggerated traits that may have arisen through a Fisherian process (reviewed in Andersson 1994).

Many of these hypotheses are not mutually exclusive—a trait of interest might allow a female to recognize a courting male as the appropriate species and, if the trait is costly, it might also provide her with information about his quality as a mate. Moreover, this same trait may also be selected for its efficacy in initially attracting her attention. Despite their potential overlap, each of these hypotheses generates specific and testable predictions. Of these functional hypotheses, only content-based, indicator hypotheses explicitly predict that the color of interest should be condition dependent. Thus, examining the condition dependence of a trait is a good first step towards teasing apart competing hypotheses for signal function.

Bright coloration has been studied extensively as a costly, quality-indicating trait in many animal taxa (reviewed in Andersson 1994). Indicator hypotheses to explain bright coloration predict that the expression of conspicuous coloration should: (a) be highly variable among individuals in a population, (b) be linked to an individual's condition, and (c) show heightened condition dependence compared with naturally selected (non-display) body colors (Cotton et al. 2004; Dale 2006). There is now a growing body of evidence suggesting that a variety of putative signaling colors show such condition-dependent expression in both vertebrates (fish: e.g., Boughman 2007; reptiles: e.g. Lebas and Marshal 2001; birds: reviewed in Hill 2006) and invertebrates (damselflies: e.g., Contreras-Garduno et al. 2008; butterflies: e.g., Kemp and Rutowski 2007; ambush bugs: Punzalan et al. 2008). Such condition dependence can be mediated through the production costs of color—for example, pigment acquisition or synthesis (e.g., carotenoid coloration in birds: McGraw 2006a) or arrangement and composition of highly precise nanoscale structures (e.g., structural coloration in butterflies: Kemp and Rutowski 2007). Though not well studied, it has also been demonstrated in a few systems that colors are also costly to maintain (e.g., via grooming; Zampiga et al. 2004; Lenouval et al. 2009).

There has been much interest in the evolution and function of the complex courtship displays of male jumping spiders (Family Salticidae) (e.g., Peckham and Peckham 1889, 1890). Many jumping spiders exhibit extreme sexual dichromatism, in which mature males are brightly colored while females and juveniles are dull and inconspicuous (Maddison 1995; Oxford and Gillespie 1998). Males often display their most colorful body regions to potential mates and male competitors (e.g., Peckham and Peckham 1889, 1890; Lim and Li 2004), sometimes in concert with auditory displays (e.g., Maddison and Stratton 1988; Elias et al. 2003). Studies of electrophysiology (e.g., Devoe 1975; Yamashita and Tateda 1976) and behavior (e.g., Nakamura and Yamashita 2000; Lim and Li 2006) suggest that salticids are capable of fine-tuned color discrimination. In some species, visual cues, such as male dancing (Jackson 1981) or the presence of UV light (Lim et al. 2007, 2008; Li et al. 2008) may increase a female's receptivity. In one of the most highly ornamented groups, the genus *Habronattus*, studies of geographically isolated populations suggest that sexual selection is a driving force in the diversification of male display traits (Masta and Maddison 2002). All of this evidence suggests that male color might be a good candidate as a quality signal, yet surprisingly the condition dependence of coloration has only been examined in *Cosmophasis umbratica* (Lim and Li 2007), just one of the more than 5,000 jumping spider species (Platnick 2010).

The goal of the present study was to begin to tease apart the hypotheses for the potential functions of color in male *Habronattus pyrrithrix*, a jumping spider, the adult males of which display a colorfully ornamented red facial patch, green front legs, and bright white pedipalps to females that are a drab gray and brown (Fig. 1). To understand the links between condition and color expression in these spiders, we first used a field study of wild adult male spiders within a single population during the mating season to observationally assess the relationship between body condition and display coloration. We then compared these levels of condition dependence with those of naturally selected body colors that are not displayed to females. We also ran manipulative experiments in the laboratory to explore whether there were causal relationships between display color and diet, a factor likely to affect condition. Because these spiders are generalist predators exposed to a variety of prey types in the field (LAT, personal observation), manipulative experiments can create a variation in diet quality that is biologically relevant (e.g., Mayntz et al. 2005). Furthermore, previous studies with spiders showed that manipulations of diet are effective for manipulating numerous fitness proxies (e.g., growth rate, body condition, survival; Toft and Wise 1999; Mayntz and Toft 2001). In experiment 1, we manipulated juvenile diet to determine nutritional effects on the development of adult male coloration. Because most spiders (including our study species) no longer molt after they have reached sexual maturity (Foelix 1996), males acquire their adult coloration upon their final molt. Thus, this coloration lasts a male

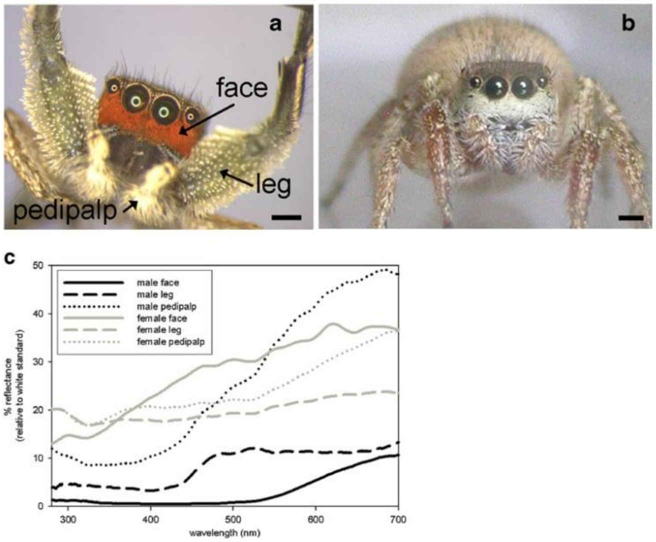

Fig. 1 Sexual dichromatism in *H. pyrrithrix*: **a** adult male, **b** adult female, and **c** representative reflectance spectra for the three potential signaling regions (on males and females) that are the focus of this study. *Scale bars* represent 0.5 mm

through the rest of the mating season and his ability to maintain this coloration may be particularly important. Spider colors are often contained on the surface of the cuticle or within fragile modified body hairs or scales (e.g., Hill 1979) and thus maintenance via grooming and prevention of scale loss, damage, or degradation may be particularly important. In experiment 2, we manipulated the diets of wild-caught adult males (that had already produced their adult colors) to determine if the quality of a male's diet affected his ability to maintain his coloration.

Materials and methods

Study species

H. pyrrithrix Chamberlin 1924 is highly sexually dichromatic. Males are adorned with bright red faces, green front legs, and bright white pedipalps, compared with dull and inconspicuous gray and brown females (Griswold 1987; Fig. 1). The red facial coloration and the white pedipalp coloration are contained within body scales (e.g., Hill 1979), while the green leg coloration is present on the surface of the cuticle of the leg, which is further adorned with white scales (LAT, personal observation). This species is distributed from southern California and Arizona south to Sinaloa, Mexico (Griswold 1987). They are common in the metropolitan area of Phoenix, AZ, where they can be found in high densities in grassy parks, backyards, and agricultural settings as well as leaf litter in more natural areas (LAT, personal observation). As in other species of *Habronattus*, males court females with complex courtship dances (e.g., Richman 1973, 1982), in which their colorful body regions are clearly displayed to females. When viewed from above or from the side, these colors are generally concealed, but during courtship males wave their green legs, expose their red face, and tap their white pedipalps (LAT, personal observation). While they also appear to have a vibrational component to their display (as in some other *Habronattus* species; see Maddison and Stratton 1988), the present study focused only on the colors involved in the visual display. In this study, we focus our discussion on color in the context of female-directed courtship rather than male–male interactions because we rarely observe males interacting with one another in either the field or lab (LAT, personal observation). When males do interact, their displays towards one another are rare and brief, lasting for only a few seconds, compared with displays towards females that can last for hours.

Correlational field study

To determine how color varies with body condition in a natural population of *H. pyrrithrix* during the mating season, we collected 57 adult males from a single population at Schnepf Farm in Queen Creek, AZ (Maricopa

County), USA (33.224744 N, 111.592825 W). The spiders were collected from areas of tall grass either using sweep nets or by hand during three collecting bouts (7 July 2006, $n=30$; 1 August 2006, $n=15$; and 25 August 2006, $n=12$) spanning the most active part of the mating season. Little is known about the synchrony of egg laying and hatching in this species, but we regularly monitor this population and 4 years of data (LAT, unpublished data) suggest that most of the adult males that are active in a given mating season hatched the previous fall and overwintered as juveniles. Immediately upon return to the lab, we weighed each individual to the nearest 0.0001 g with an electronic balance (Mettler-Toledo, Columbus, OH, USA) and photographed each spider once next to a size standard using a Nikon Coolpix 4500 digital camera (Nikon Inc., Melville, NY, USA; image resolution, 2,272 × 1,704 pixels). We measured the width of the carapace (just behind the posterior lateral eyes) from these photographs using Photoshop software (Adobe Systems Inc., San Jose, CA, USA). Because the carapace width of these spiders is fixed at maturity while the abdomen stretches as an individual gets fatter, we used the residuals of a regression of mass on carapace width as an estimate of body condition (or ‘relative fatness’) (Jakob et al. 1996). Data from a concurrent study of *H. pyrrithrix* from this same population suggests that this residual condition index is a good predictor of mating success (LAT, unpublished data), so it is likely to be a biologically relevant indicator of condition that is uncorrelated with structural size. After measurements were taken, we immediately placed the spiders in the freezer (−80°C) where they were stored until October 2006 for color analysis (described in detail below).

Captive diet experiments

For the duration of the experiments, we housed spiders individually in cylindrical plastic containers (5.5 cm tall and 2.5 cm in diameter) within an incubator at 28°C on a 14:10 light–dark cycle. For experiment 1 (manipulation of juvenile diet), we monitored the spiders daily for molt, and upon their final molt to sexual maturity we immediately weighed them, measured their carapace width, and froze them (−80°C) for later color analysis (described in detail below). For experiment 2 (manipulation of adult diet), we kept all spiders in captivity on the manipulated diets for 45 days, at which point we weighed, measured, and froze them (−80°C) for later color analysis. The time period of 45 days was chosen for two reasons. First, we wanted the duration of the diet manipulations in the two experiments to be comparable so that we could understand how the same diet manipulations would affect males over different developmental periods; 45 days was our a priori estimate of the length of time that it would take the males from experiment 1 to reach maturity. Secondly, preliminary field data from this population during the previous year indicated that there was a peak in activity of sexually mature males and females that lasted for about 45 days, and so this timeframe corresponded with the most active part of the mating season. Measuring facial coloration on these spiders involved mounting them under a microscope and focusing a full-spectrum light source on their face (described in more detail below), which could potentially blind the spiders and affect their ability to capture prey. For this reason, color measurements were only performed at the end of the diet manipulation experiments (after the spiders were euthanized).

For experiment 1, 28 juvenile male spiders were collected from the same Queen Creek population described above between 24 May and 2 July 2007. While the juveniles of many spider species cannot be reliably sexed until maturity, juvenile male *H. pyrrithrix* can be readily distinguished from females by the presence of sparse red facial scales (LAT, personal observation). Because there was considerable variation in initial body size among juveniles (carapace width = 1.006±0.238 mm (mean±SD)) and because we wanted to ensure that individuals in different size classes were distributed equally across treatment groups, we first grouped the spiders by collection date, paired them by carapace width, and then assigned individuals in each pair randomly to either a ‘high-quality’ or ‘low-quality’ daily diet regime (see more below). For experiment 2, we collected 28 sexually mature males from the same population between 24 May and 12 July 2007. We grouped the individuals by collection date and then assigned them randomly to either the ‘high-quality’ or ‘low-quality’ daily diet regime. For the adult spiders in experiment 2 (which were mature upon collection), we scored the initial adult body condition at the start of the experiment (as described above for the correlational study). Due to allometric changes as these spiders grow, the residual body condition indices of juvenile male spiders may not be comparable to those of adult males or even to juvenile males of different ages (LAT, unpublished data). Thus, throughout this study, we only used residual condition indices for mature adult spiders.

In both experiments, spiders receiving the ‘low-quality’ diet were fed daily with wingless *Drosophila melanogaster* reared on basic fruit fly medium only (Carolina Biological, Burlington, NC, USA), as studies with other generalist predator spiders showed this diet to provide enough nutrients for spiders to reach maturity, but with significantly lower growth rates (e.g., Toft and Wise 1999; Mayntz and Toft 2001). In contrast, the ‘high-quality’ treatment consisted of a daily feeding regime that alternated between week-old crickets (*Acheta domesticus*, approx. 3 mm in length) on one day and then wingless *Drosophila* on the following day. For the high-quality diet group, both the crickets and flies were reared on basic medium supplemented with protein, calcium, and vitamins (see Table 1 for the specific details of diet formulation and feeding regime). Again, this high-quality diet was chosen because previous studies indicated that similar manipulations increase growth rates in spiders (e.g., Mayntz and Toft 2001; Wilder and Rypstra 2008; Hebets et al. 2008). In addition to manipulating diet quality, our 'high' treatment also received a slightly higher total mass of food (Table 1). During daily feedings, we removed uneaten food from the previous day and replaced it with fresh food. At the end of the experiments, body condition was scored for each individual.

Color analysis

We used reflectance spectrophotometry to measure reflectance from three body regions that males display to females: red face, green front legs, and white pedipalps. We focused on these three body regions as potential signaling colors in this study because they are (1) sexually dichromatic in the spider's visible range, (2) displayed by males to females during courtship (LAT, personal observation), and (3) relatively large, quantifiable, single-color patches (Fig. 1). To test the prediction that these potential signaling colors show heightened condition dependence compared to naturally selected, non-signaling colors (Cotton et al. 2004), we also measured reflectance from two body regions that are not displayed to females and are therefore presumably not involved in sexual signaling: the light gray ventral abdomen, which is unlikely to be visible to females in most natural contexts (LAT, personal observation), and the dark gray dorsal carapace, which is unlikely to be visible to females once a male begins courting (LAT, personal observation).

Depending on the area to be measured, we used one of two spectrophotometer setups: either a standard spectrophotometer or a microspectrophotometer (details of each are described below). Because the color patches on the dorsal abdomen and ventral carapace were relatively large (both approximately 1.6 mm), we were able to take reflectance measures using a standard UV–vis spectrophotometer (USB2000 with PX-2 pulsed xenon light source, Ocean Optics, Dunedin, FL, USA) that can precisely collect reflectance data on areas as small as 1 mm in diameter over the range of 275–700 nm. Reflectance measures were taken in a dark room with the probe positioned perpendicular to the colored surface and were measured relative to a Spectralon diffuse reflectance white standard (Labsphere, Inc., North Sutton, NH, USA).

Because the diameters of the other color patches (red face, green front legs, and white pedipalps) were smaller than 1 mm (ca. 0.4, 0.5, and 0.6 mm, respectively), we were unable to use this same method to accurately and repeatably measure color on these areas. Instead, for these body regions, we used a microspectrophotometer which consisted of a standard spectrophotometer (USB2000, Ocean Optics, Dunedin, FL, USA) coupled to a modified Leica DMLB2 fluorescence light microscope fitted with a ×40 quartz objective lens (Leica Microsystems, Wetzlar, Germany) and illuminated with a full-spectrum Leica 75 W xenon arc lamp (Leica Microsystems, Wetzlar, Germany). Again, reflectance measures were taken in a dark room, relative to the same Spectralon white standard described above and with the probe positioned perpendicular to the colored surface. This setup allowed us to collect precise data within a circular sampling area that was 0.4 mm in diameter. Unfortunately, the optics of the microscope cut out a portion of the UV spectrum and so this instrument only provides data within the range of 375–700 nm for these smaller body regions. In some jumping spider species, UV reflectance appears to be important in signaling (Li et al. 2008; Lim et al. 2008, 2007), so we

Table 1 Feeding regimes of male spiders in juvenile and adult diet manipulation experiments

Experimental diet treatment	Prey species and feeding frequency	Prey diet
High-quality diet	Fed daily, alternating between three wingless *Drosophila melanogaster* on 1 day and three 1-week-old crickets (*Acheta domesticus*) on the following day	High-quality *Drosophila* diet: 3:1:1:1 ratio of Carolina basic fruit fly medium (Carolina Biological, Burlington, NC), high-calcium cricket feed (Fluker Laboratories, Baton Rouge, LA), tropical fish flakes (TetraMin, Blacksburg, VA), and crushed Total cereal (General Mills, Minneapolis, MN)
	Approximate (wet) mass of daily diet=0.480± 0.152 mg (*n*=10 samples, mean ± SD)	High quality cricket diet: 1:1:1:1 ratio of organic cornmeal (Arrowhead Mills, Melville, NY), high-calcium cricket feed (Fluker Laboratories, Baton Rouge, LA), tropical fish flakes (TetraMin, Blacksburg, VA), and crushed Total cereal (General Mills, Minneapolis, MN)
Low-quality diet	Fed three wingless *Drosophila melanogaster* daily	
	Approximate (wet) mass of daily diet=0.359± 0.069 mg (*n*=10 samples, mean ± SD)	Low-quality *Drosophila* diet: Carolina basic fruit fly medium (Carolina Biological, Burlington, NC)

must use caution in excluding UV reflectance from our analyses on these small body regions. In pilot studies, to ensure that we were not missing potentially important information in the UV range of the spectrum, we coarsely measured the reflectance on the smaller body regions with the full-spectrum spectrophotometer (without the microscope) described above and we were able to confirm qualitatively that, while reflectance does extend into the UV for the green legs and white pedipalps of males, there are no UV peaks in these spectra (Fig. 1c). Thus, the benefit of using the microspectrophotometer setup to get accurate and repeatable measures in the longer wavelengths (where most of the reflectance and variation are found in this species) far outweighed the disadvantage of not being able to obtain UV data for these small body regions.

For the correlational study, we took three reflectance measurements on each of the following body regions of each spider: face, left leg, right leg, left palp, right palp, ventral abdomen, and dorsal carapace. For the diet manipulation experiments, we took two measurements on each of these regions. All color measurements were completed by the same person (LAT) blind to the treatment group of the specimen being measured. We calculated the overall brightness of the mean spectral curve of each body region, as well as hue and chroma, where relevant. For achromatic regions (gray regions with a flat reflectance), there were no relevant hue or chroma values, and thus we calculated brightness only. For the putative signaling colors, this amounted to seven color variables: (1) hue of the red face (the wavelength corresponding to the inflection point of the red curve), (2) red chroma of the face (the proportion of total reflectance between 600 and 700 nm), (3) brightness of the red face (mean reflectance), (4) green chroma of the front legs (the proportion of the total reflectance between 450 and 700 nm), (5) brightness of the front legs (mean reflectance), (6) brown chroma of the pedipalps (the proportion of the total reflectance between 500 and 700 nm), and (7) brightness of the white pedipalps (mean reflectance) (color metrics reviewed in Montgomerie 2006). For each of the non-signaling regions (abdomen and carapace), which were gray, we calculated brightness (mean reflectance).

Because the face of the spider is a relatively large surface that is only partially ornamented with red scales (unlike the legs and pedipalps in which the entire surface of the structure is covered with scales), we also measured the size of this red patch. We digitally photographed the face of each spider through a Leica MZ 125 stereo microscope at ×50 magnification using a Spot Insight 11.2 digital camera (Diagnostic Instruments, Sterling Heights, MI, USA; image resolution 1,600×1,200 pixels) and Image-Pro Express software (Media Cybernetics, Silver Spring, MD, USA). All photos were taken with the same light and camera settings. We calculated the total area of red scale coverage using Photoshop software (Adobe Systems Inc., San Jose, CA, USA). Not surprisingly, larger spiders (with wider carapaces and thus larger faces) had significantly larger red patches (regression, R^2=0.222, $F_{1,56}$=15.77, P=0.0002). Thus we calculated a ‘relative patch size index’ from the residuals of a regression of patch area on carapace width (see Jakob et al. 1996 for a description of this method for estimating relative body condition).

Statistical analysis

For the correlational field study, we examined all possible intercorrelations among the color parameters for the putative signaling traits. Because we were interested in broad patterns of intercorrelations rather than the significance of any individual relationship, we did not employ a Bonferroni correction (e.g., Cohen et al. 2008). We then went on to examine the condition dependence of color using linear regression to determine if there were relationships between color traits and body condition. Because the main goal of our study was to assess the condition dependence (and potential signal functions) of each color variable separately, we used univariate, rather than multivariate, tests. Because this correlational data on condition dependence was used to identify potentially important biological relationships that we would explore further with manipulative experiments, we wanted to avoid an excessively high type II error rate (e.g., Moran 2003; Nakagawa 2004), and therefore we again did not employ a Bonferroni correction.

To compare the levels of condition dependence in these display traits with naturally selected traits, we also used regression to assess the condition dependence of the abdominal and carapace colors that are not displayed to females (Cotton et al. 2004).

Because the spiders used in this study were collected during three bouts (from 7 July to 25 August), we examined relationships between collection date and condition using ANOVA and Tukey–Kramer pairwise comparisons (with an alpha level of 0.05). These analyses indicated that condition declined sharply across the mating season (see “Results”). Because condition and collection date were highly correlated, we only included condition in our regression models. This tight relationship between condition and collection date prevents us from being able to tease apart the individual effect of each on color, which was the goal of our diet manipulation experiments.

To assess whether we effectively manipulated condition, we used one-tailed *t*-tests to determine if individuals in the high-quality diet group were in better body condition at the end of the experiments than those in the low-quality diet group. In experiment 1, where the spiders were still growing and molting over the course of the experiment, we also used a one-tailed *t*-test to determine if the individuals in the high-quality diet group matured with a larger carapace width than those in the low-quality group. In addition, for experiment 2, where adult body condition could be scored both at the start and end of the experiment, we used a one-tailed *t*-test to determine if the change in body condition over the course of the experiment was greater in high- versus low-quality diet treatments. In all of these cases, we used one-tailed tests because we were specifically interested in testing the directional prediction that our diet manipulations were effective (i.e., diet supplementation increased body condition or body size). To ensure that we were not manipulating condition outside of the natural range of variation, we confirmed that the final body condition in each treatment group of our experiments fell within the natural range of variation observed in this population between 2006 and 2008 (LAT, unpublished data).

Color variables that were condition dependent in the field study (hue, red chroma, and size of the red face patch and brightness of the green front legs; see "Results") were compared in the manipulative experiments between animals fed high- and low-quality diets using analyses of covariance (ANCOVA). For experiment 1, there was considerable variation in the initial size of the juvenile spiders (carapace width = 1.006±0.238 mm (mean±SD)), as well as in the total time spent on the experimental diets before reaching maturity (35.8±24.6 days (mean±SD)). Thus, we included these two variables as covariates in the model. In experiment 2, all spiders were on the diet manipulation for the same amount of time (45 days); thus, we included only initial body size (carapace width, 1.77±0.247 mm (mean±SD)) as a covariate. Because of our ability to calculate initial body condition on the adult spiders in experiment 2 (but not in experiment 1—see above), we also ran a second set of analyses on the data from experiment 2 with initial body condition as a covariate. These results were qualitatively similar, and thus only the first set of analyses is presented. Again, for comparison, we ran the same statistical tests on the data for the presumably naturally selected abdominal and carapace coloration. Spiders that died over the course of the experiment (three in experiment 1 and two in experiment 2) were excluded from the analyses.

All data met assumptions of parametric statistics, with two exceptions. For red chroma and relative face patch size data in experiment 1, variances were unequal between treatment groups (Brown–Forsythe test, red chroma: $F=10.61$, $P=0.0035$; relative face size: $F=5.46$, $P=0.029$), and thus a reciprocal transformation was employed to achieve homogeneity. All statistical analyses were conducted using SAS 9.1 and JMP 8.0 for Windows (SAS Institute, Cary, NC, USA).

Results

Correlational study

We found several significant correlations among color parameters of the putative signaling traits (Table 2). In general, variables associated with facial color were positively correlated (with the exception of face brightness), as were variables associated with leg color. In general, red and green color variables were negatively correlated with one another. Variables associated with the pedipalps were not correlated and generally were not correlated with either face or leg coloration.

Field-caught male *H. pyrrithrix* in better body condition during the mating season had redder faces (i.e., higher hue values and higher red chroma values; hue: $R^2=0.144$, $F_{1,56}=9.24$, $P=0.0036$; red chroma: $R^2=0.084$, $F_{1,56}=5.04$, $P=0.0289$; Fig. 2a, b) and more red scale coverage on their faces ($R^2=0.113$, $F_{1,56}=7.02$, $P=0.0105$; Fig. 2d). However, there was no relationship between condition and face brightness ($R^2=0.004$, $F_{1,56}=0.24$, $P=0.6227$; Fig. 2c). Males in better condition also had darker green front legs ($R^2=0.069$, $F_{1,56}=4.07$, $P=0.0486$; Fig. 2f), but there was no significant relationship between condition and green chroma of the legs ($R^2=0.054$, $F_{1,56}=3.15$, $P=0.0813$). There was no relationship between condition and any aspect of pedipalp coloration (brown chroma of pedipalps: $R^2=0.017$, $F_{1,56}=0.96$, $P=0.3301$; Fig. 2g; brightness of the pedipalps: $R^2=0.051$, $F_{1,56}=2.98$, $P=0.0898$; Fig. 2e, h).

For comparison with non-signaling traits, there was no significant relationship between condition and gray abdomen coloration (brightness = 21.386±9.040% (mean±SD), $R^2=0.0120$, $F_{1,56}=0.666$. $P=0.418$) or gray carapace coloration (brightness=11.618±9.309% (mean±SD), $R^2=0.050$, $F_{1,56}=2.896$. $P=0.094$).

Body condition varied among collection bouts ($F_{2,54}=15.73$, $P<0.001$); males collected later in the season (during the last collection date, 25 Aug) had lower body condition indices than the males collected earlier in the season (7 July, 1 Aug).

Experimental studies

In experiment 1, males reared on the high-quality diet matured with a higher body condition index than those on the low-quality diet ($t_{21}=1.737$, $P=0.0484$; Fig. 3a), suggesting that our diet manipulation was effective. Final carapace width was not greater on the high-quality diet than the low-quality diet (high-quality carapace width= 2.043±0.0965 (mean±SD), low-quality carapace width= 1.973±0.122 (mean±SD), $t_{21}=0.544$, $P=0.295$). Faces were redder (with higher hue and red chroma values) in

Table 2 Pairwise correlations between putative signaling color variables for males in the correlational field study

	Face hue	Face red chroma	Face brightness	Face patch size	Leg green chroma	Leg brightness	Palp brown chroma	Palp brightness
Face hue	–	0.719**	−0.133	0.475**	−0.328*	−0.342**	0.011	0.076
Face red chroma		–	−0.227	0.623**	−0.312*	−0.368**	−0.061	0.140
Face brightness			–	−0.173	0.335*	0.361**	-0.018	−0.140
Face patch size				–	−0.281*	−0.291*	−0.084	0.251
Leg green chroma					–	0.717**	−0.086	−0.294*
Leg brightness						–	−0.143	−0.294*
Palp brown chroma							–	0.166
Palp brightness								–

*$p<0.05$; **$p<0.01$

males from the high-quality diet group compared to the low-quality group (hue: $F_{3,21}=4.61$, $P=0.0436$; red chroma: $F_{3,21}=5.21$, $P=0.0329$; Fig. 4a, b). The relative size of the red color patch was larger in the high-quality diet group compared to the low-quality diet group, but this relationship was not statistically significant ($F_{3,21}=3.83$, $P=0.0638$; Fig. 4c). Brightness of the green legs was unaffected by diet ($F_{3,21}=0.37$, $P=0.550$; Fig. 4d). For comparison with non-signaling traits, diet treatment had no effect on either gray abdominal coloration (high-quality brightness=31.386±18.044% (mean±SD), low-quality brightness=27.384±16.356% (mean±SD), $F_{3,21}=0.19$, $P=0.666$) or gray carapace coloration (high-quality brightness=13.567±7.380% (mean±SD), low quality brightness=11.639±6.998% (mean±SD), $F_{3,21}=0.16$, $P=0.697$). Full ANCOVA tables are provided as "Electronic supplementary material".

In experiment 2, there was no difference in final body condition between the two treatment groups ($t_{24}=0.391$, $P=0.650$; Fig. 3b), nor was there any difference in the change in body condition between the treatment groups over the course of the experiment (change in condition index for high-quality group=0.001±0.003 (mean±SD), change in condition index for low-quality group=0.0008±0.004 (mean±SD), $t_{24}=1.28$, $P=0.893$). Adult diet had no effect on any aspect of red or green coloration (hue of red face: $F_{2,23}=0.23$, $P=0.634$; red chroma: $F_{2,23}=0.59$, $P=0.449$; size of red facial patch: $F_{2,23}=1.83$, $P=0.189$; brightness of green legs: $F_{2,23}=0.43$, $P=0.519$; Fig. 4e–h). Adult diet also had no effect on the non-signaling gray abdomen coloration (high-quality brightness=25.686±12.807% (mean±SD), low-quality brightness=37.759±22.093% (mean±SD), $F_{2,23}=2.65$, $P=0.117$) or gray carapace coloration (high-quality brightness=11.800±7.505% (mean±SD), low-quality brightness=13.658±8.545% (mean±SD), $F_{2,23}=0.22$, $P=0.645$). Again, full ANCOVA tables are provided as "Electronic supplementary material".

Discussion

We used field observational and lab experimental approaches to examine the condition dependence of three separate colored body regions that male *H. pyrrithrix* display to females during courtship: a red facial patch, green legs, and white pedipalps. We compared this with the condition dependence of two colors that are not displayed to females: gray abdomen and carapace coloration. Our correlational field data suggest that aspects of both red facial and green leg coloration (but not white pedipalp coloration) are both variable and condition dependent in a natural population within the most active part of the mating season and that these colors show heightened condition dependence compared with the abdominal and carapace colors not displayed to females. The condition-dependent red and green color variables (but not white pedipalp color variables) were also generally intercorrelated with one another (Table 2), suggesting that these two traits contain information about a male's quality (e.g., Møller and Pomiankowski 1993; Hebets and Papaj 2005).

In addition to assessing condition dependence of color, it is often useful to directly compare the levels of variation among the different color patches to test the prediction that the putative quality-signaling colors are more variable than naturally selected colors (Dale 2006). This was not possible in the current study because the color patches of interest were different colors (red, green, gray) that are quantified on different scales and thus cannot be directly compared (see Dale 2006; Delhey and Peters 2008 for a discussion on the problems associated with comparing variation in different color traits).

To further explore the potential information content of leg and facial colors, we went on to experimentally examine a dietary mechanism underlying their condition dependence and found that juvenile diet affected the development of the adult red facial coloration but had no effect on green leg coloration. Again, in the diet

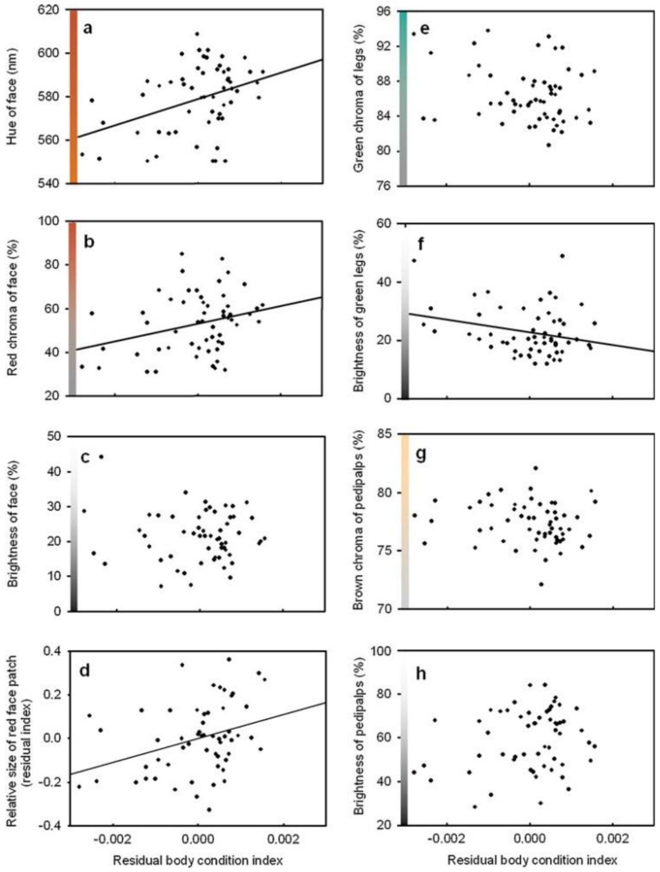

Fig. 2 **a**–**h** Relationships between body condition (residual body condition index) and the different forms of display coloration in wild-caught male *H. pyrrithrix*. *Regression lines* indicate significant relationships between body condition and coloration

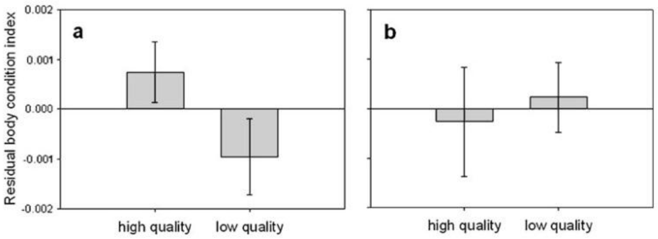

Fig. 3 Effects of diet manipulation on body condition (mean ± SEM). **a** Experiment 1 (manipulation of juvenile diet). **b** Experiment 2 (manipulation of adult diet)

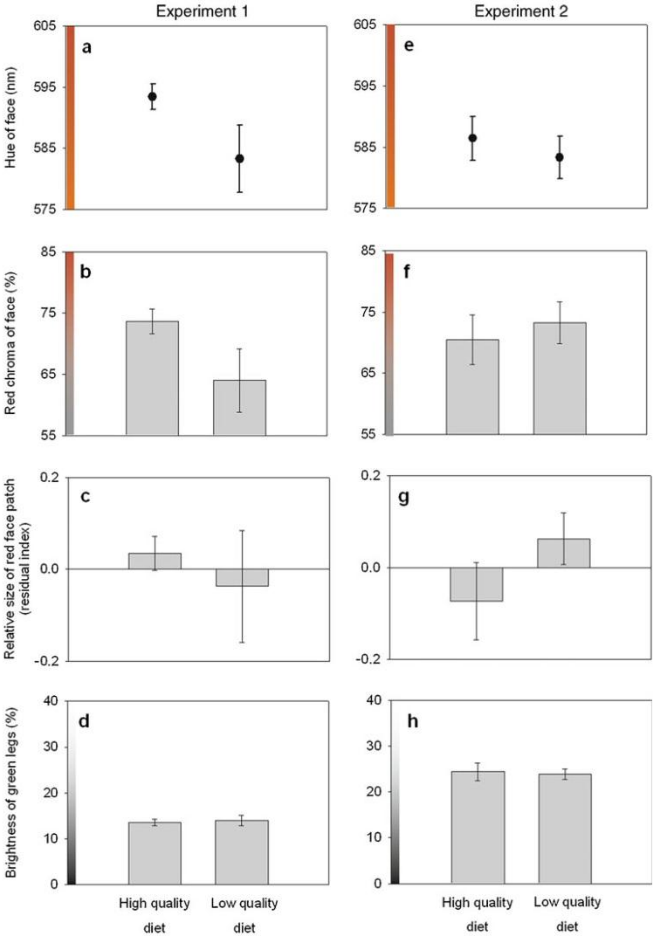

Fig. 4 a–h Effect of juvenile diet manipulation (*Experiment 1*) and adult diet manipulation (*Experiment 2*) on putative signaling colors in mature male *H. pyrrithrix* (mean ± SEM). As discussed in "Materials and methods", variances were unequal and thus data were transformed in **b** and **c**

manipulation experiment, red coloration showed heightened condition dependence relative to non-signaling colors (abdomen and carapace). However, once this red coloration is produced, manipulation of adult diet did not seem to have any effect on color maintenance. These results suggest that, while facial and leg coloration were both condition dependent and intercorrelated in the field, they may actually contain slightly different information about a male's quality (discussed in more detail below).

The condition dependence of putative intraspecific signaling coloration has only been examined in one other of the more than 5,000 species (Platnick 2010) of jumping spider (*C. umbratica* from Singapore, Lim and Li 2007). Male *C. umbratica* have sexually dimorphic color patterns that are required for eliciting the attention of the opposite sex, and aspects of these colors were found to be dependent on both diet and age (Lim et al. 2007, 2008). However, while our study and theirs both uncovered patterns of condition dependence in coloration, both the experimental approaches and the details of the findings were quite different (reviewed in more detail below).

Condition and diet dependence of red facial coloration

The red facial coloration of adult males in our experiment was positively correlated with body condition in the field and dependent upon juvenile diet in the lab, and thus this coloration has the potential to be an honest indicator of a male's health and foraging ability. Such patterns of diet dependence are common for many well-studied colorful traits in male animals that are both displayed to and preferred by females (e.g. Kodric-Brown 1989; Hill 1990; Kemp and Rutowski 2007).

Specific to jumping spiders, Lim and Li (2007) found that diet affected the male display coloration in *C. umbratica*; however, they manipulated diet quantity, while we predominantly manipulated diet *quality* (with only subtle differences in diet quantity; Table 1). Furthermore, we found treatment effects on the *development* of male coloration, while Lim and Li (2007) found treatment effects on the *maintenance* of adult coloration. As a result, the putative mechanism for the treatment effects of diet on color was very different in the two studies. Lim and Li (2007) showed that a period of fasting in mature males affected their abdominal coloration, presumably as a result of the colored body scales being more spread out on the stretched abdomens of fatter males. In our study, in contrast, the color patch of interest is on a body region (the face) that does not stretch with feeding as the abdomen does and so any diet-related changes in color in *H. pyrrithrix* will presumably be due to the effects on the development or maintenance of coloration within the individual body scales where it is produced (e.g. Hill 1979) rather than to the mechanical stretching of the body surface. In this way, the control of facial coloration in *H. pyrrithrix* may be much like the control of color production within the wing scales of butterflies (e.g., Morehouse et al. 2007; Giraldo and Stavenga 2008) and the feathers of birds (e.g., McGraw 2006a, b; Prum 2006).

We found no effect of diet on the maintenance of adult male red facial coloration. Interestingly, our diet manipulations had different effects on the body condition of the juvenile spiders (experiment 1) than they did on the adult spiders (experiment 2), despite the fact that the diet manipulations were identical and the two experiments were run in the lab concurrently. The finding that our diet manipulation was more effective at manipulating the condition of juveniles than adults may indeed explain the lack of a treatment effect on the maintenance of adult coloration. If so, this difference in treatment effect on juvenile and adult spiders might suggest that, in the field, juvenile diet may be more likely than adult diet to affect a male's colors. Alternatively, it could also be that because a male's facial coloration is fully produced within body scales prior his final molt, any effect of diet post-maturity is indeed minimal. However, the maintenance of coloration in other animals often requires grooming (e.g., Zampiga et al. 2004; Lenouval et al. 2009), which is also a common behavior in *H. pyrrithrix* (LAT, personal observation). More investigation into the mechanisms of color maintenance post-maturity is clearly warranted in *H. pyrrithrix*.

Condition dependence and potential age-related changes in green leg coloration

Despite the finding that the green leg coloration of males was condition dependent in the correlational field study, neither of our diet manipulation experiments had any effect on its development or maintenance. Due to the patterns of condition dependence that we saw in the field, it would be worthwhile to further explore other environmental and physiological parameters that are linked to condition (such as parasites, health, and age). Males attain their full ornamental coloration at sexual maturity, and throughout the mating season the scales that produce the colors may undergo natural wear and degradation, which may result in predictable, post-maturity, age-related changes (e.g., Kemp 2006; Kemp and Macedonia 2006). If so, male color variation might signal viability, in which case we would expect females to choose older males as mates (reviewed in Kokko and Lindstrom 1996). Alternatively, if older individuals are more likely to carry disease or parasite infection (e.g., Tarling and Cuzin-Roudy 2008), we might expect females to choose younger males as mates.

Interestingly, a post-hoc comparison of the results of the two diet manipulation experiments suggests that age may indeed play a role in this coloration. Males in experiment 1 (which were measured immediately at maturity) had much darker leg coloration than the males in experiment 2 (which were at least 45 days post-maturity) (post-hoc *t*-test, $t_{49}=8.30$, $P<0.001$, compare Fig. 4d and h) but showed no differences in aspects of red facial coloration (post-hoc *t*-tests, hue: $t_{49}=0.981$, $P=0.332$, compare Fig. 4a and e; chroma: $t_{49}=0.778$, $P=0.441$, compare Fig. 4b and f; relative size of red patch: $t_{49}<0.0001$, $P>0.999$, compare Fig. 4c and g). These results were similar to those found by Lim and Li (2007), where the color profiles of older males differed from those of younger males and may be typical of invertebrate structural colors (e.g., butterflies: Kemp 2006). In our study species, *H. pyrrithrix*, prey capture specifically involves the green ornamented front legs (LAT, personal observation) and this may lead to natural wear and degradation of the structures that produce the green coloration as males become older.

Although our two experiments with different-aged males were not designed to assess the effect of age on coloration, they were run in the lab concurrently, and specimens from the two experiments were combined and randomized before color measurement; thus, the differences in the green leg coloration of males in the two experiments are likely to be a result of the different ages of the two groups of spiders. We must use caution in this interpretation, however, because the males in experiment 1 spent much of their juvenile development under lab conditions, while the males in experiment 2 spent their entire juvenile development in the field. Clearly, age-related changes in leg coloration should be examined more closely in *H. pyrrithrix*, while eliminating these confounding factors of juvenile diet by using males raised entirely in the lab from eggs.

Functions of jumping spider display coloration

One goal of our study was to begin to disentangle content-based, quality-indicating hypotheses for elaborate male coloration, which explicitly predict condition dependence, from other functional hypotheses that typically do not. Despite the overwhelming support for quality-indicating functions in other taxa (reviewed in Andersson 1994), many have argued that the brilliant and diverse colors of some species of male jumping spiders are likely to function as species recognition signals rather than as indicators of a male's individual quality (e.g., Richman 1977; Crane 1949). Courting male spiders have to deal with the risk of cannibalism, as females of most species are generalist predators that may attack and eat anything that is perceived as a potential prey item. For this reason, immediate and unambiguous species recognition may be more important to male jumping spiders than it is to males of other taxa where the potential costs associated with courtship are not as high. Given the high cost of cannibalism, we might also expect the elaborate colors of males to be more likely to have efficacy-based functions, such as reducing female aggression (e.g., Forster 1982; Hebets and Papaj 2005) or attracting a female's attention from a safe distance (Clark and Uetz 1993).

Here we present correlational and experimental data that support quality-indicating hypotheses for the red facial coloration of male *H. pyrrithrix*. However, these patterns of condition dependence did not hold up for the other two display colors that we examined (green leg color and white pedipalp color). We provide evidence that the green leg color is correlated with condition in the field and may be related to male age. The white pedipalp coloration showed no evidence of condition dependence. These different patterns of condition dependence for each of the different colored regions that males display to females suggest that each may serve different functions or multiple functions (or perhaps no function at all). With an understanding of the patterns of condition dependence for these three colored body regions in male *H. pyrrithrix*, we can now create informed and well-designed behavioral experiments to further test the hypotheses about potential signal functions of these colors both independently and in concert with one another as part of a complex display (e.g., Hebets and Papaj 2005).

Much of our understanding of the functions and control of elaborate coloration in animals comes from a small set of well-studied groups (e.g., birds, fish, butterflies—reviewed in Andersson 1994). Spiders differ in aspects of their biology that might provide additional insights into the evolution of color. Because females are often larger than males and are voracious and potentially cannibalistic generalist predators, courting males have to treat females not only as potential mates but also as potential predators. In addition, in many habitats such as leaf litter and dense vegetation, jumping spiders may face a suite of predators that is quite different from the predators faced by other groups of animals. These two factors are very likely to affect the costs and benefits of being colorful. Given the amazing diversity of colors among jumping spiders (Maddison 1995; Oxford and Gillespie 1998), there is likely to be as much diversity in the way individuals use color as there is for the most thoroughly studied groups of colorful animals. It may be that understudied groups such as spiders will provide novel insights for the field of animal coloration.

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APPENDIX C

SUPPLEMENTARY MATERIAL FOR CHAPTER 2

Supplementary material for Chapter 2

(Sex differences in deception: can activity patterns or microhabitat use explain sexually dimorphic dorsal color patterns in *Habronattus* jumping spiders?)

Supplementary Table 1. Results of Kruskal-Wallis tests examining sex-differences in microhabitat use in four species of *Habronattus* jumping spiders

Species	df	χ^2	*P*
H. clypeatus			
cottonwood leaf litter	1	0.184	0.668
willow leaf litter	1	0.037	0.847
cottonwood vegetation	1	1.560	0.212
willow vegetation	1	0.336	0.562
grass	1	1.400	0.237
dirt/rock	1	3.050	0.081
H. hallani			
cottonwood leaf litter	1	<0.001	>0.999
willow leaf litter	1	*	*
cottonwood vegetation	1	<0.001	>0.999
willow vegetation	1	*	*
grass	1	*	*
dirt/rock	1	*	*
H. pyrrithrix			
cottonwood leaf litter	1	2.200	0.138
willow leaf litter	1	2.350	0.126
cottonwood vegetation	1	0.065	0.799
willow vegetation	1	0.046	0.830
grass	1	1.430	0.232
dirt/rock	1	0.587	0.443
H. hirsutus			
cottonwood leaf litter	1	0.479	0.489
willow leaf litter	1	1.700	0.192
cottonwood vegetation	1	0.674	0.411
willow vegetation	1	2.570	0.109
grass	1	1.700	0.192
dirt/rock	1	3.530	0.060

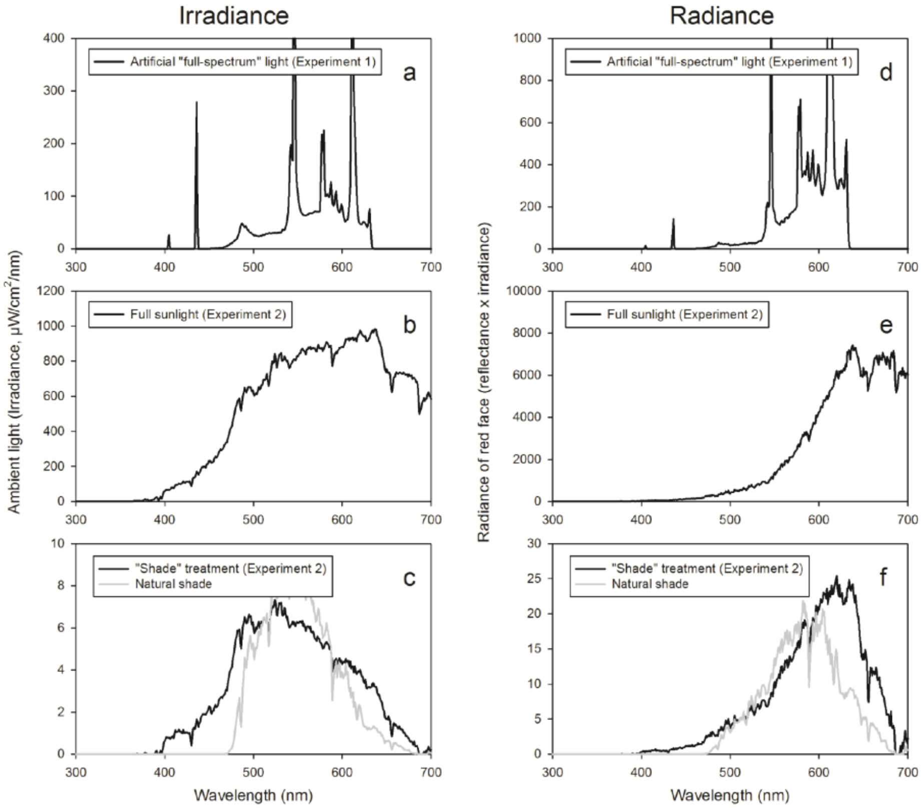
Asterisks (*) denote microhabitat types in which a given species was never found

APPENDIX D

SUPPLEMENTARY MATERIAL FOR CHAPTER 4

Supplementary material for Chapter 4

(Colorful ornaments increase courtship success in a jumping spider, but only in the sunlight)



Supplementary Figure 1. Ambient light (irradiance spectra) of light environments used in this study (a-c) and the estimated radiance of this light when reflected from the red face of a male *Habronattus pyrrithrix* (d-f). Artificial "full-spectrum" lights (a, d), full sunlight (b, e), and artificial and natural shade (c, f). Irradiance data were collected using a portable spectrometer (USB 2000+) fitted with a cosine corrected irradiance probe (CC-3) and calibrated with a tungsten halogen light source (LS-1-CAL, all from Ocean Optics, Dunedin, FL, USA). Radiance was estimated by multiplying each irradiance spectrum by the reflectance spectrum of the red face of male *H. pyrrithrix* (e.g., see Macedonia 2001; Macedonia et al. 2003). Note that in natural sunlight, the radiance in the red portion of the spectrum (600-700nm) is higher than in either the shade or artificial light treatments.

Supplementary Table 1. Results of repeated measures ANCOVA for Experiment 2 examining the effect of male color manipulation, body size, body condition, and light environment on the number of aggressive attacks received from females

within subjects effects	df	*F*	*P*
light	1,7	0.380	0.559
light*treatment	1,7	<0.001	0.976
light*size	1,7	0.370	0.564
light*body condition	1,7	0.360	0.565
light*female ID	9,7	1.930	0.200
between subjects effects	df	*F*	*P*
treatment	1,7	2.470	0.160
size	1,7	2.700	0.145
body condition	1,7	1.890	0.211
female ID	9,7	2.480	0.122

Supplementary Table 2. Results of repeated measures ANCOVA for Experiment 2 examining the effect of male color manipulation, body size, body condition, and light environment on the number of aggressive attacks received from the partner male

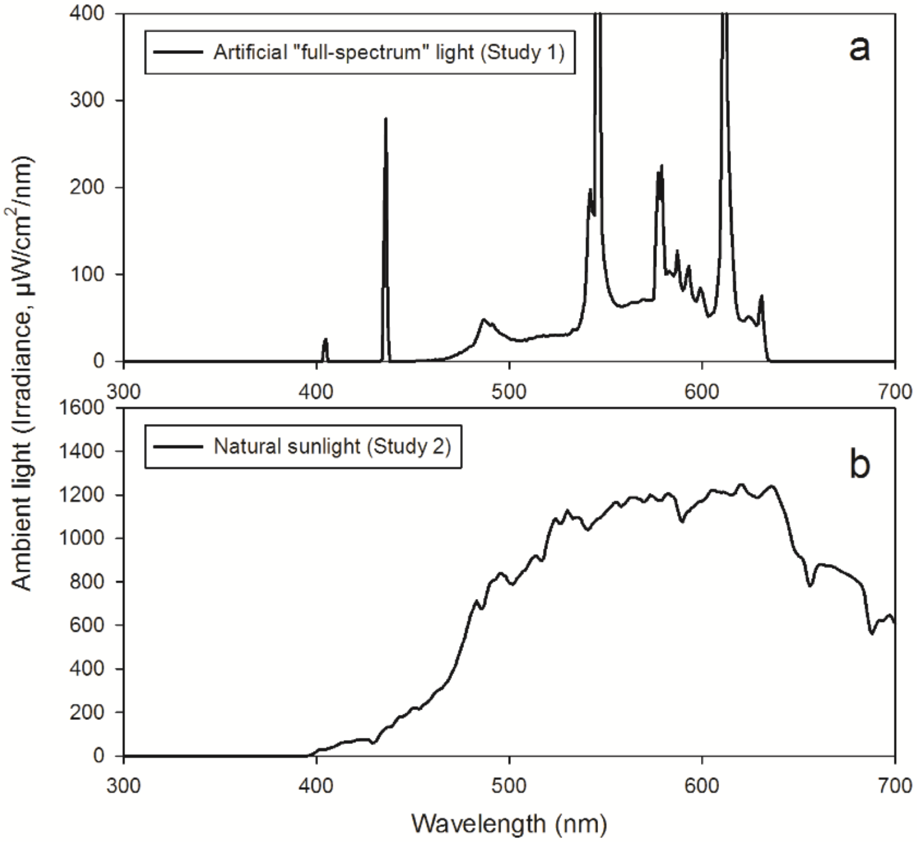
within subjects effects	df	*F*	*P*
light	1,7	0.050	0.832
light*treatment	1,7	0.020	0.889
light*size	1,7	0.040	0.845
light*body condition	1,7	0.440	0.527
light*female ID	9,7	0.920	0.558
between subjects effects	df	*F*	*P*
treatment	1,7	1.660	0.238
size	1,7	0.830	0.392
body condition	1,7	0.600	0.465
female ID	9,7	1.470	0.313

APPENDIX E

SUPPLEMENTARY MATERIAL FOR CHAPTER 5

Supplementary material for Chapter 5

(Natural variation in condition-dependent display coloration does not predict male courtship success in a jumping spider)



Supplementary Figure 1. Ambient light (irradiance spectra) of light environments used in this study. Artificial "full-spectrum" lights (a), natural sunlight (b). Data were collected using a portable spectrometer (USB 2000+) fitted with a cosine corrected irradiance probe (CC-3) and calibrated with a tungsten halogen light source (LS-1-CAL, all from Ocean Optics, Dunedin, FL, USA).

APPENDIX F

COAUTHOR PERMISSIONS FOR INCLUSION OF PUBLISHED WORKS

January 4th, 2012

To whom it may concern,

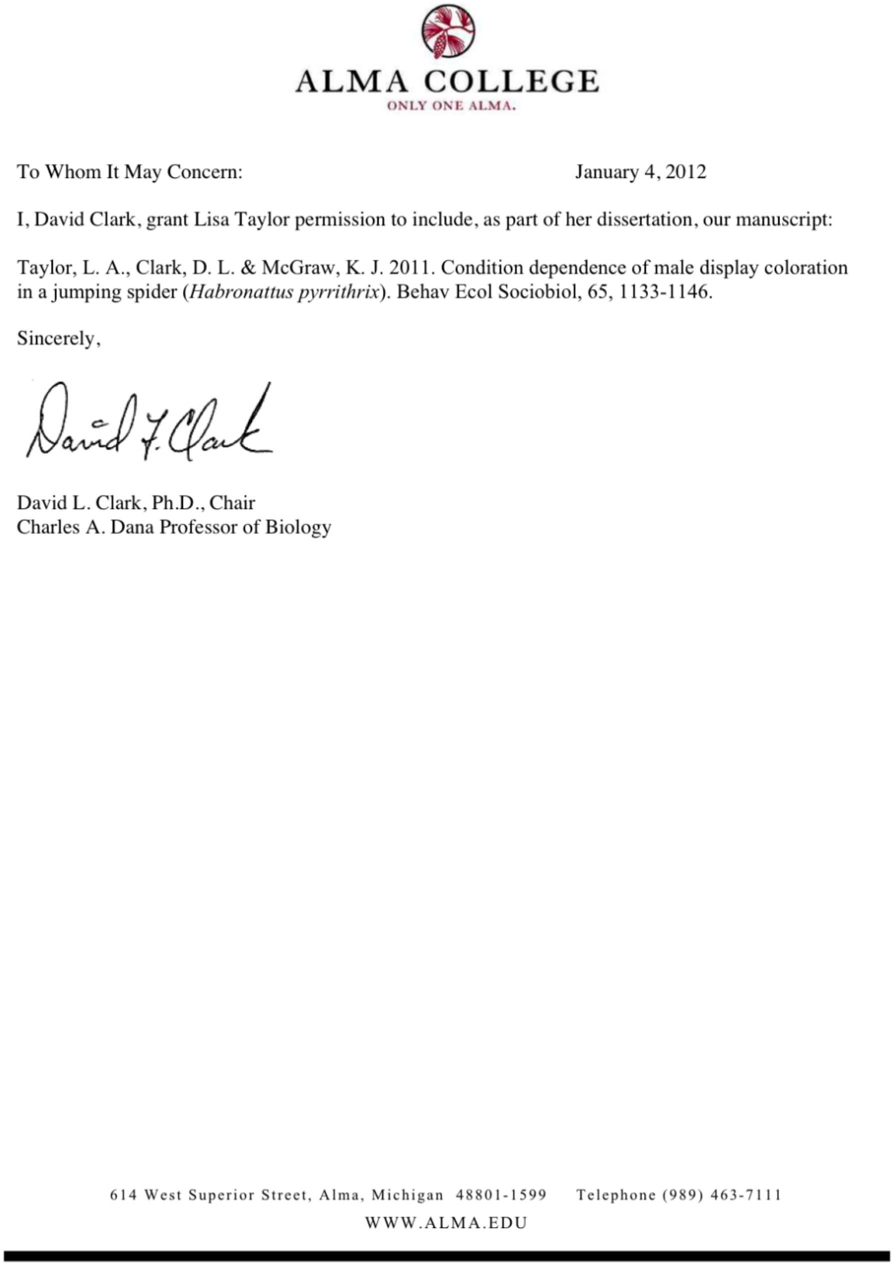
I, Kevin McGraw, grant Lisa Taylor permission to include, as part of her dissertation, our manuscripts:

Taylor, L. A., Clark, D. L., and McGraw, K. J. 2011. Condition-dependence of male display coloration in a jumping spider (*Habronattus pyrrithrix*). Behav. Ecol. Sociobiol. 65:1133-1146.

Taylor, L. A. and K. J. McGraw. 2007. Animal coloration: sexy spider scales. Curr. Biol. 17:R592-R593.

Sincerely,

Kevin J. McGraw



ALMA COLLEGE
ONLY ONE ALMA.

To Whom It May Concern: January 4, 2012

I, David Clark, grant Lisa Taylor permission to include, as part of her dissertation, our manuscript:

Taylor, L. A., Clark, D. L. & McGraw, K. J. 2011. Condition dependence of male display coloration in a jumping spider (*Habronattus pyrrithrix*). Behav Ecol Sociobiol, 65, 1133-1146.

Sincerely,

David L. Clark

David L. Clark, Ph.D., Chair
Charles A. Dana Professor of Biology

614 West Superior Street, Alma, Michigan 48801-1599 Telephone (989) 463-7111
WWW.ALMA.EDU