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Sexual Selection and Signal Evolution:
Diversification of Peacock Spiders (Genus: *Maratus*)

by

Madeline Brown Girard

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Environmental Science, Policy, and Management

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Erica B. Rosenblum, Chair
Professor Rosemary G. Gillespie
Professor Eileen A. Lacey

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Abstract

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Professor Erica Bree Rosenblum, Chair

Across taxa, sexual communication is fundamental to an organism's reproductive fitness, and ultimately, its evolutionary success. Consequently, strong selection pressures often lead to extreme adaptations in male physiology, morphology, and behavior to increase the efficacy of signal transfer to females. Similarly, selection acts on females to detect, process, and respond to information emitted by males. By these processes, theory predicts sexual selection has the potential to drive vast and rapid diversification of some traits, and indeed empirical evidence has shown this to be the case. Of particular interest to biologists are the more extravagant radiations of sexual ornamentation, those characterized by an overwhelming amount of diversity in not just one or two sexually selected traits, but instead on whole suite of signals. While many animals use multi-modal (more than one sensory modality) displays during courtship, the majority of work on female choice has thus far focused on individual signaling elements individually, or species that produce relatively simple, quantifiable displays such as cricket calls, and house finch coloration. Comparable work on systems with extremely elaborate displays has lagged behind, and it remains unclear if the same forces driving the evolution of more basic unimodal signals are the same as those shaping complex displays.

For my dissertation research, I have used Australian endemic peacock spiders of the *Maratus* genus (Family: Salticidae) to explore the role of complex signals in mating behavior and diversification of this group. Members of this genus are ideal study organisms for such research as males use both visual and vibratory displays to attract and secure a mate. The adaptive significance (if any) of complex signaling is poorly understood, as is how females evaluate males based on these signals. Thus, my research has focused on understanding the role of sexual selection in the evolution and maintenance of such elaborate male courtship displays. Specifically, my dissertation work has aimed to: (A) describe multi-modal signal structure in peacock spiders; (B) investigate female preferences for these signals; (C) uncover how different signaling modalities act together or in isolation to affect mating and (D) elucidate patterns of signal evolution and species diversification across the genus.

In the first few chapters of this dissertation, I demonstrate that: peacock spider males produce complex multimodal courtship displays; male mating success in *M. volans* is predicted by suites of combinatorial elements; both vibrations and visual displays are important for male mating success, although visual displays seem to play a more crucial role. The latter part of this dissertation provides a molecular phylogenetic framework of *Maratus* spiders, and as such, informs our knowledge about the evolutionary history of peacock spider courtship signals. As communication underlies all social networks, this body of research is important because it enhances our understanding of broad scale links between sensory processing, decision-making, behavior, and evolution.

For the spiders…

"Sometimes [said Pooh] the smallest things take up the most room in your heart."
- A.A. Milne

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An extensive web of mentors, family and friends has been integral to all of my achievements leading up to and during my time in graduate school. There is no way for me to acknowledge every aspect of support from which I have benefitted, but I will do my best to highlight some of the most indispensible contributors.

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Chapter 1: Introduction[1]

1.1 Abstract

Natural history is the foundation of behavioral ecology research; it provides premise for the conception of both ecological and evolutionary theory, offers context for good experimental design, and also informs interpretation of all types of data collected. Here we present some natural history of *Maratus* peacock spiders and a more detailed overview of the dissertation chapters to follow.

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[1]A portion of this chapter is reprinted, with permission, from the original journal article: Girard MB, Endler JA (2014) Peacock Spiders. Current Biology 24(13): R588-R590. http://doi.org/10.1016/j.cub.2014.05.026

1.2 Background

Arachnid behavioral repertoires are ample in size and diversity, yet they are an underrepresented group in behavioral ecology (Schneider and Andrade, 2011). This is unfortunate as spiders are ideal organisms to answer questions spanning sexual selection, communication and mating systems for many reasons. For starters, spiders are often cannibalistic to conspecifics, which means that mating poses significant threat, especially to males (Andrade, 1996; Elgar 1992). Additionally, the genetalic morphology of many spiders favors intense postcopulatory sexual selection, which in turn generates the potential for wide variance in reproductive success (Elias *et al*. 2011). Moreover, paired genitalia and sperm storage organs allow for some curious copulatory patterns for both males and females and as a result, spider mating systems are often unique and complex (Elias *et al*. 2011; Uhl, 2002). Lastly, spiders offer a great wealth of opportunity to study communication as many are known to make use of visual, acoustic, chemical and/or tactile signals (Uhl and Elias, 2011). Some spiders, such as those in the family Lycosidae, have been shown to use combinations of these signaling modalities in conjunction to produce multimodal signals (Uhl, and Elias, 2011; Utez and Roberts, 2002). Even though there is a growing body of research on spider communication, much is left to be learned, few species have been explored, and the sensory world for a majority of spiders is still a complete mystery. For instance, visual system data is only available for a handful of terrestrial invertebrate groups, and any information about color-vision sensitivities is lacking in all but a few of the more than 40,000 spider species. Additionally, vibratory communication, which is ubiquitous across small invertebrates (Cocroft and Rodriguez, 2005; Hill, 2001; Hill, 2008), is one of the most poorly understood, likely because humans cannot perceive substrate-borne vibrations without the aid of specialized equipment.

1.3 Study System

1.3.1 What is a peacock spider?

Peacock spiders are small (2-6mm) jumping spiders belonging to the genus *Maratus*, a group endemic to Australia. Males generally have conspicuously colorful abdomens as well as elongated third legs that are brown/black and often tipped with white brushes (Figure 1.1). By contrast, females are cryptically colored, usually mottled brown/beige. During courtship, a male peacock spider will raise his abdomen, and wave it at a female in synchrony with his third pair of legs (Dunn, 1947). Males of many species also have lateral flaps that can be extended from their abdomen like a fan. This fan-structure, combined with remarkable ornamentation of *Maratus* males, is reminiscent of a peacock's display, hence their common name.

With more than 500 genera and over 5,000 species, jumping spiders make up the largest family (Salticidae) in the order Araneae (Maddison and Hedin, 2003a), and based on the rich array of morphology, behavior and ecology of the group, salticid diversity rivals that of birds (Hill and Richman, 2009). *Maratus* is part of the Euophryine subfamily of salticids. While the monophyly of this clade is well supported (Maddison and Hedin, 2003a; Zhang, 2012), distinguishing between *Maratus* and other closely related groups has been difficult. Within *Maratus*, relationships between species are also currently not well understood, but evidence suggests there are upwards of 60 species (Figure 1.2a; Otto and Hill, 2017b), and likely many more are yet to be discovered. At present, several morphological and behavioral species-groups

Figure 1.1. Courtship displays of three different male peacock spider species (from left to right, *M. volans, M. mungiach, and M. splendens*).
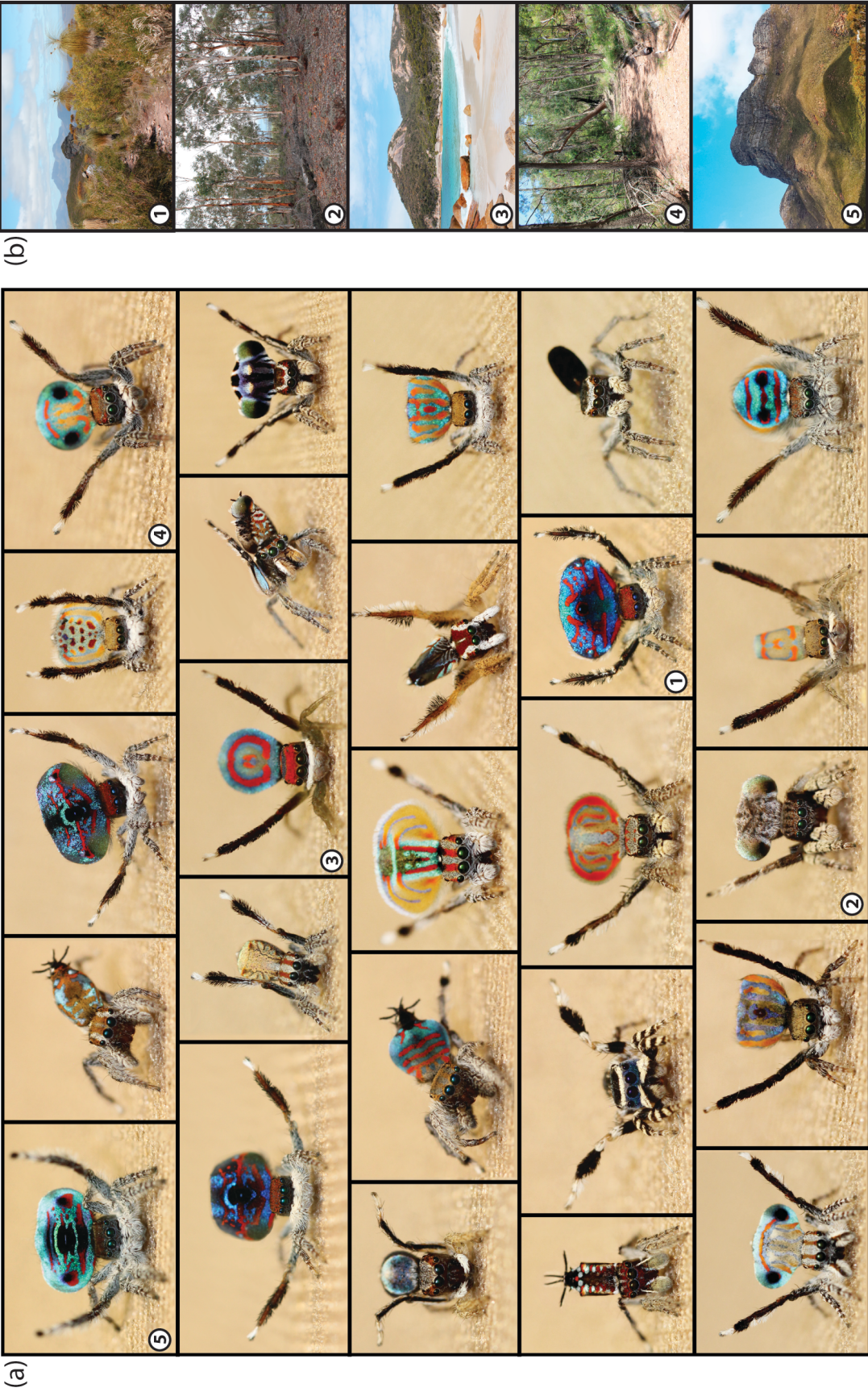
Figure 1.2. (a) A subset of *Maratus* males in the courtship posture. (b) Collection spots for five species, numbered in (a), that illustrate the range of habitat types in which these spiders are found.

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are evident (MG, pers. obs.) but again, molecular data are crucial to determine the validity of these groupings.

1.3.2 When and where are they found?

Generally, the *Maratus* breeding season occurs during the Austral Spring, but male and female activity patterns during this period seem to be species and region specific. Temperature and humidity are likely important in determining when the season commences and how long it will last, although this has not been empirically demonstrated.

Peacock spiders are widespread across the southern-half of Australia and live in a diverse range of habitats (Figure 1.2b), from sand dunes on the temperate coasts to grasslands in the semi-arid regions (J. Waldock, pers. comm.). More recent collection records indicate that peacock spiders can be found in every Australian state (QLD, NSW, SA, TAS, WA) and territory (ACT, JBT, and NT), and are not just the southern portion of the country. Like many other salticids, some *Maratus* species have a large distribution and occupy a wide array of environments (e.g. *M. volans*) whereas others are more specialized or geographically limited (e.g. *M. sarahae, which is* found exclusively in heath habitats on two peaks in the Stirling Ranges; Waldock, 2013). The majority of peacock spiders studied are ground-dwelling, predominantly found on leaf-litter under eucalypt woodlands. However, some species, such as *M. speciosus*, seem to occur more in shrubs or young grass-trees (*Xanthorrhoea*).

1.3.3 What do they eat, and how do they hunt?

Peacock spiders are diurnal cursorial hunters feeding primarily on insects and other spiders. The evolution of an acute visual system in salticids almost certainly originated as an adaptation for stalking prey (Foelix, 1996; Land, 1985). However, this development also facilitated a wandering lifestyle different from that of their sit-and-wait ancestors, enabling jumping spiders to roam and encounter many environments (Eakin and Brandenburger, 1971). Keen eyesight has probably been useful for peacock spiders in navigating, inhabiting and exploiting new types of habitats, and undoubtedly set the stage for the evolution of complex visual signals.

1.3.4 How do males produce their visual signals?

Tiny scales/hairs (Figures 1.3-1.4) produce the distinct color patterns observed across the group. Like many other salticids studied to date (Lim and Li, 2006), peacock spider scales reflect light in both the visible and/or ultraviolet range (Figure 1.5). Multilayer reflectors are responsible for producing the iridescent colors seen in several salticids (Ingram *et al.* 2011; Land *et al.* 2007; Taylor and McGraw, 2007). While only a few peacock spider species have been examined in any detail, it also appears that blue and green iridescent scales of *Maratus* males are structural, perhaps combining surface diffraction gratings with multilayer reflectors to produce interference-based colors (Foelix *et al.* 2013). The red and yellow patches of *Maratus* males instead arise from pigmented brush-like hairs (Foelix *et al.* 2013). *Maratus* males are among the most brightly colored and sexually dimorphic of the salticids. Fortunately, the detection and processing of such amazing colors isn't a problem for jumping spiders, which are among the most visually specialized of the invertebrates.
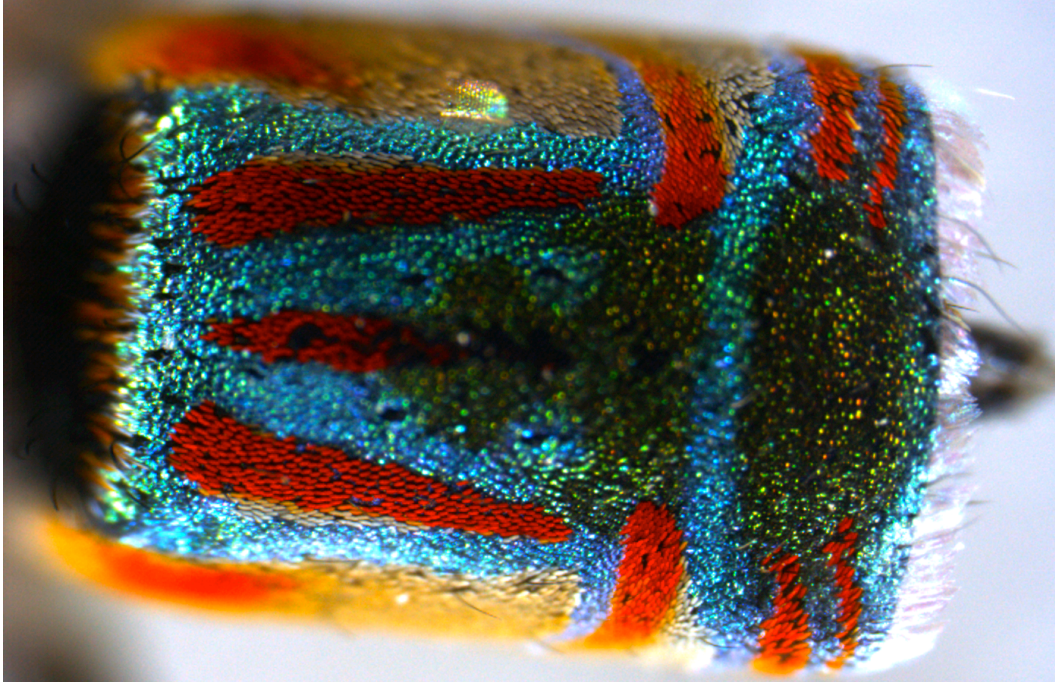
Figure 1.3. Abdominal scales of a *M. volans* male.
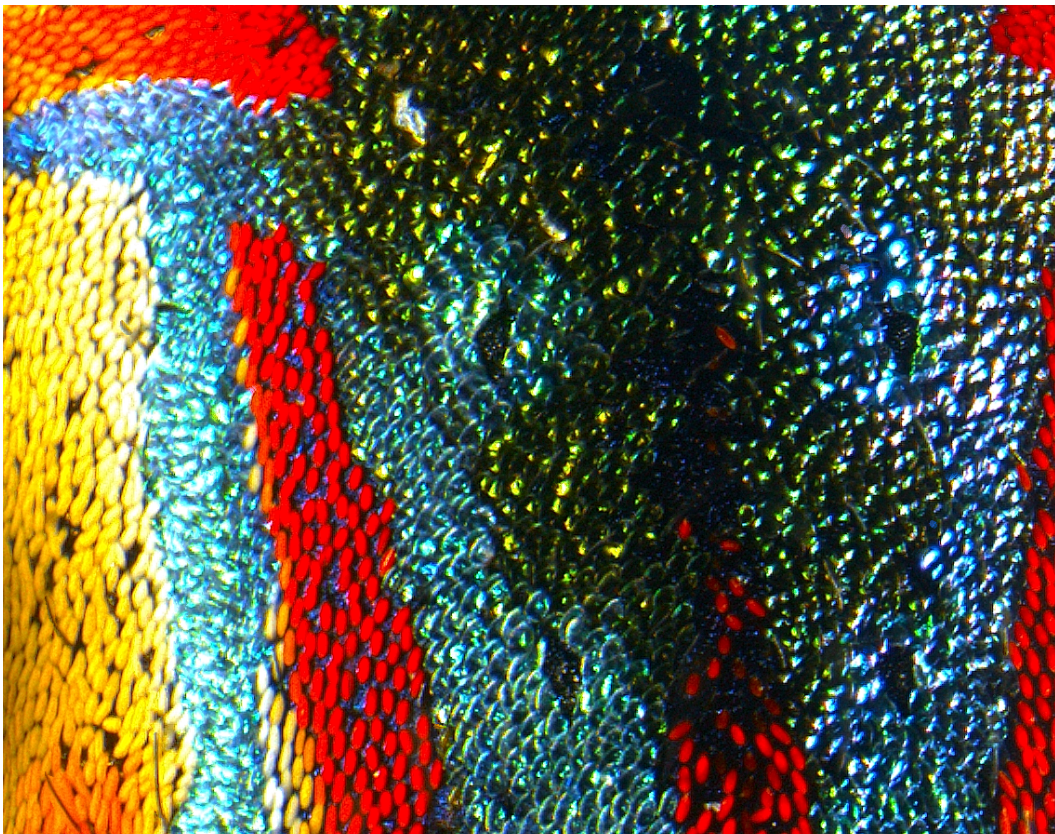
Figure 1.4. A close-up image of *M. volans* abdominal scales.

Figure 1.5. UV signalling in *M. volans.*Both images were taken using a Fujifilm Finepix IS Pro, with a quartz lens under full spectrum ligth (including UV). The image on the left was filtered with with an astronomical filter that only allowed UV light to pass, while the one on the right had no such filter. The lighter areas on the left are the patches that reflect the most UV light (see appendix A1 for methods).

1.3.5 How is their visual system special?

Using the full complement of eyes (eight), jumping spiders have exceptional abilities to perceive motion and depth (Nagata *et al.* 2012; Zurek and Nelson, 2012; Zurek *et al.* 2010). Furthermore, specialized structures in their primary eyes have allowed them to approach the physical limit of optical resolution for their compact size (Land, 1969; Land and Nilsson, 2002). Their minimum resolution angle (acuity) is about 0.04°, or about 1/13 the diameter of the sun disk; this is not much worse than ours (0.007°) and considerably better than the best insects (0.4°; Land and Nilsson, 2002; Lim and Li, 2006). Salticids are easily distinguished from other spiders by their enlarged anterior median eyes (AME). These eyes are equipped with a telephoto lens and a tiered retina, each layer containing photoreceptor cells of distinct absorption spectra (Blest *et al.* 1981; Koyanagi *et al.* 2008; Land, 1985; Land, 1969; Williams and McIntyre, 1980). Salticid color vision is much better than ours, more similar to that of birds, with as many as four evenly spaced channels, including a UV sensitive photoreceptor (humans have only three and no UV). There is strong morphological and behavioral evidence for color vision being used in both predation and sexual selection. Not surprisingly, the region of jumping spider brains used for visual processing is much larger than that of other comparably sized arthropods (Eakin and Bradenburger, 1971), and color learning has been demonstrated (Nakamura and Yamashita, 2000).

1.3.6 Do peacock spiders use other types of signals?

Substrate-borne vibrations are important for mating success in several salticids, including at least one species of peacock spider, *M. volans* (Elias *et al.* 2005; Girard *et al.* 2015; Sivalinghem *et al.* 2010), and likely many more. *Maratus* males seem to use their abdomens almost exclusively to produce vibrations. A preliminary high-speed video analysis showed that the primary form of signal production is tremulation (MG, unpub. data), simple vibrations originating from rapid movements of the abdomen. Some species also have a percussive signal that they will use intermittently throughout their display. Although males appear to be drumming their third legs on the ground, in fact, the majority of percussive energy is produced through abdominal contact with the substrate (MG, unpub. data). A third signal production mechanism, stridulation, may be used occasionally (Elias *et al.* 2003), but seems less common in this group.

In addition to visual and vibratory signals, *Maratus* males may also make use of chemical communication to locate and secure a mate. Salticids do not build webs, but they constantly produce silk as they move about their environment. Contact pheromones in salticid silk drag-lines are common, and these are detected by chemoreceptors on both the legs and palps. While visual cues alone can elicit courtship in salticids (Crane, 1949), contact pheromones can also elicit male courtship in the absence of visual cues; this has been directly observed with many species of peacock spiders (MG, unpub. data). While there is less empirical evidence for salticids using airborne pheromones (Crane, 1949; Cross and Jackson, 2009; Jackson, 1987; Nelson *et al.* 2012; but see Pollard *et al.* 1987), they may also be important. Since female peacock spiders are often very aggressive once they have already mated, it may be vital for males to identify a female's receptivity and quality from contact or airborne pheromones before risking getting close enough to court (Hoefler, 2007).

1.3.7 How does male courtship proceed?

Peacock spiders have elaborate courtship, even by salticid standards. During a search for a mate, a male will periodically pause atop a perch to wave his third pair of legs, presumably to

attract the attention of any females nearby. When a male finally spots a female, he may begin courtship by producing vibrations (Girard *et al.* 2011). If the female orients towards the male, he will raise his abdomen, extend his abdominal fan-flaps and wave the whole structure back and forth, accompanied by third leg movements that accentuate this dance.

Male courtship ranges from a few minutes to over an hour, depending on the female response. If a male is not attacked nor does the female flee, he will slowly approach her, dancing and vibrating as he does so. When he is a distance of about one body length from the female, he commences what is known as the pre-mount display, a highly conserved behavior across the genus. This display lasts until the male completes his advance and attempts to mount and mate with the female. As with courtship, copulation duration can range from several minutes to an hour or more (MG, unpub. data).

Mating trials conducted with *M. volans* indicate that *Maratus* females are very choosy, and once a female has already mated, she is unlikely to mate again (Girard *et al.* 2015). Compared to virgins, mated females are also more aggressive and generally spent a lower proportion of time attending subsequent males' displays. Overall, low mating rates, and no multiple mating, suggests that strong sexual selection is operating in this system.

1.3.8 Is there other evidence for sexual selection in this group?

Male sexually selected traits and visually mediated displays are important during courtship of females in the majority of salticids examined (Foelix, 1996; Forster, 1982). The complex display repertoires of jumping spiders probably reflect sexual selection rather than a need for reproductive isolation or reduction of cannibalism. In peacock spiders, and other salticids using abdominal displays, the location of abdominal ornaments correspond to how and from what direction the abdomen is held and waved so that the female can see the ornaments during courtship. Peacock spider males do not develop their bright colors until they become mature, further suggesting a strong role for sexual selection in generating conspicuous male appearances. Moreover, there is direct genetic and behavioral evidence for sexual selection in jumping spiders. Numerous studies across the family show an association between species richness and the development of sexual traits, suggesting that sexual selection, supported by superb vision as a key innovation, could be an important driver of diversity in the group (Richman and Jackson, 1992).

1.3.9 Why are peacock spiders a unique biological system?

Small terrestrial arthropods are inherently different from their well-known vertebrate counterparts. As such, these organisms provide exceptional opportunities to test our understanding of fundamental biological principles. For example, are there processes that are unique to the miniature spatial scales on which salticids operate? Also, what is the cognitive architecture required for organisms of such a compact size to produce, perceive, and process complex behavioral displays? Members of the *Maratus* genus exhibit some of the most spectacular arthropod displays known. Different species vary widely in habitat as well as visual and vibrational signaling traits, making them particularly tractable for studies of multi-modal signaling. While we are only just beginning to uncover aspects of their physiology, behavior, and ecology, it is clear that peacock spiders will enrich our understanding of sensory ecology, sexual selection, and trends in diversification.

1.4 Dissertation Overview

To begin to understand complex signaling in peacock spiders, it was first necessary to categorize and describe all courtship display elements of a focal species. In chapter 2, I present data on the full signaling repertoire of the species *Maratus volans.* In this chapter, I demonstrate that males of this species use multimodal signals including visual ornaments (fan coloration), motion displays (fan dancing, third leg waving) and substrate-borne signals. Importantly, this was the first description of substrate borne signals in *Maratus,* which have been shown to be a critical component of mate choice in other spider groups. Therefore, this study not only expands our knowledge of jumping spider behavior, but also the use of multi-modal signaling in animals.

In chapter 3, I investigate female preference for courtship displays of male peacock spiders. Animal mating displays have long captured the attention of biologists, however, for most systems, the specific male traits or trait combinations underlying female mating behavior remain obscure. In order to elucidate male traits/ trait combinations that predict mating success, I conducted a series of mating trials, in a controlled laboratory set-up, with *M. volans* males and females collected from the field. Using recordings from courtship trials, I examined female response to male dances and vibrational songs and found that females use multiple, independent suites of interrelated traits to select mates across both visual and vibrational modalities; however visual displays alone describe more of the variation in mating success between males. This work also supports several of the major hypotheses for the evolution of complex displays spanning multiple modalities as many male traits are correlated, yet different elements of male signaling behavior affected distinct aspects of male mating success (copulation, latency to copulation, copulation duration, and egg laying behavior). Additionally, we observed that females only mated once, suggesting that sexual selection is strong in this system. Lastly, females reliably provide feedback to males during courtship to signify their mating receptivity, or lack thereof, which may play a role in modulating male signaling behavior.

Understanding how different aspects of multi-modal signals function together or in isolation is fundamental to our understanding of how these signals actually evolve. In chapter 4 of my dissertation I conduct an experiment where male signaling environments are artificially manipulated to elucidate the relative role of visual and vibratory displays in *M. volans* courtship. As found in chapter 3, visual modalities appear to be more important to females than vibratory signals as substrate-borne aspects of male courtship did not increase mating rates. Additionally, this study set out to investigate the importance of long wavelength signals, as red, orange and yellow color patches on male fans make up one major axis of peacock spider signaling diversity. These types of signals are prolific across the group but more rarely used as interspecific signals by other jumping spiders. As peacock spiders certainly exhibit ornamentation that imply an ability to discriminate between a wide range of colors under a diverse set of habitat parameters (background contrast, ambient light, etc.), it was surprising to find that chromatic characteristics of longer wavelength ornaments are not driving female mate choice decisions, and instead achromatic contrast and vibrations seem to be more useful for drawing attention to motion aspects of displays.

For a comprehensive study of the evolution of multi-modal signals in *Maratus* peacock spiders and a better understanding of the role of complex signals in the diversification of this group, it was important to zoom out and look at the genus at large. In particular, the goals of chapter 5 were to: (a) characterize the majority of the diversity of *Maratus*, a largely undescribed genus endemic to Australia; (b) infer and test predictions of multi-modal signal organization

across species (c) investigate if/ how explain species-level differences in complex signaling. In order to meet these goals, I constructed a molecular phylogeny for the *Maratus* genus using RAD-sequencing. Next, using videography, laser vibrometry, and hyperspectral imaging I recorded and quantified male display traits for each *Maratus* species. The data presented in chapter 5 data not only inform our knowledge of the relationships between different species, but also illuminates the evolutionary history of certain male courtship traits, and patterns of signal use that now serve as the foundation for our understanding the evolution of signal complexity in these spiders. These data also challenge the current monophyly of this group and thus have numerous implications for taxonomy of the genus.

Chapter 2: Multi-modal courtship in the peacock spider, *Maratus volans* (O.P.-Cambridge, 1874)[2]

2.1 Abstract

The peacock spider, *Maratus volans,* has one of the most elaborate courtship displays in arthropods. Using regular and high-speed video segments captured in the lab, we provide detailed descriptions of complete male courtship dances. As research on jumping spiders has demonstrated that males of some species produce vibrations concurrently with visual displays, we also used laser vibrometry to uncover such elements for this species. Our recordings reveal and describe for the first time, that *M. volans* males use vibratory signals in addition to complex body ornaments and motion displays. The peacock spider and other closely related species are outstanding study organisms for testing hypotheses about the evolution and functional significance of complex displays, thus, this descriptive study establishes a new model system for behavioral ecology, one that certainly stands to make important contributions to the field.

2.2 Introduction

Research on animal courtship has demonstrated that males of many species produce elaborate multi-component signals spanning more than one sensory modality (multi-modal signals e.g. combinations of tactile, visual, acoustic, etc. signals). The adaptive significance of multi-modal signal structure, however, is not well understood. For instance, each component of multi-modal signals may be informative to females in a different way (multiple message hypothesis; Hebets and Papaj, 2005). In contrast, different multi-modal signal components may independently reflect the same information, providing back-up for intrinsic signaling errors (redundant signal hypothesis; Hebets and Papaj, 2005). Moreover, females may evaluate only one, or a few, traits at a time with complex male signals; or instead, they may process many signal components together to facilitate the evaluation of potential mates (Condolin, 2003; Hebets and Papaj 2005; Rowe, 1999). Although complex signaling has become a recent focus of much communication research, careful dissection of signaling behavior and the signals involved in mating interactions is often missing from these studies. Additionally, biases in human senses have led to an oversimplification in the potential information contained in animal signals (Huber, 2005; Johansson and Jones, 2007), and in some instances even failed to identify the modalities and signals most involved in female choice (Elias *et al.* 2005; Scheffer *et al.* 1996; Taylor and McGraw, 2007). Comprehensive study of the signals themselves is an overlooked, yet crucial component of animal behavior research.

Jumping spiders (Family: Salticidae) are visual specialists among the arthropods (Foelix, 1996). Not surprisingly, in the majority of species examined to date, males possess sex-specific visually-mediated displays that are important during courtship (Clark and Morjan, 2001; Hill and Richman, 2009; Li *et al.* 2008; Lim *et al.* 2008; Maddison and Hedin, 2003a; Uhl and Elias, 2011). Substrate-borne vibrations, in conjunction with visual displays, have also been demonstrated to function in jumping spider courtship (Edwards, 1981; Elias *et al.* 2003; Gwynne and Dadour, 1985; Jackson, 1982; Maddison and Stratton, 1988a; Maddison and Stratton 1988b; Noordam, 2002; Sivalinghem *et al.* 2010; Uhl and Elias, 2011) and are important for mating success in a number of species (Elias *et al.* 2005; Sivalinghem *et al.* 2010; Elias *et al.* 2004; Elias *et al.* 2006a). In particular, those in the genus *Habronattus* are well known to communicate using a dynamic repertoire of both visual displays and intricate vibrational signals (Elias *et al.* 2003; Elias *et al.* 2006b; Elias *et al.* 2006c). Intense sexual selection is predicted to lead to the evolution of such complex displays (Andersson, 1994) and has also been implicated as being an important driver of diversification in jumping spiders (Maddison and Hedin, 2003b; Maddison and McMahon, 2000; Masta, 2000; Masta and Maddison, 2002).

Although male salticids are often highly ornamented relative to their female counterparts, the Australian endemic peacock spider (*Maratus volans)* stands out as an exceptional example. During courtship, a male peacock spider unfurls its brightly colored opisthosomal flaps, which are typically kept tucked around the abdomen (Hill, 2009). The whole structure, which bears resemblance to the fan of a peacock, is then waved at a female in synchrony with an ornamented 3rd pair of legs.

Despite the charismatic nature of the *Maratus* genus, virtually no work has been conducted on the displays of these species, including, *Maratus volans* (Hill, 2009). However, based on the diversity of their behavior, particularly the species-specific mating displays that are likely to exist (Otto and Hill, 2010), research on *Maratus* promises to yield important insights on patterns of signaling and signaling complexity. Accordingly, we set out to uncover the complete

repertoire of behaviors these males utilize during courtship. To characterize and quantify all male courtship displays and vibrational signals, we used regular and high-speed video as well as laser vibrometry. In this paper we describe, in detail, the remarkable courtship display of the peacock spider, *Maratus volans*. We show that males of this species make use of both visual and vibratory modalities in their courtship efforts.   Distinct components of male behavior emphasize different aspects of a male's morphology and each display element consists of a unique combination of visual and vibratory signaling. These behavioral descriptions provide the necessary foundation for future work on *M. volans* as well as the entire *Maratus* genus.

2.3 Methods

2.3.1 Ethics Statement

All necessary permits were obtained for the described field studies: New South Wales National Parks and Wildlife Service license to MMK (# S12762).

2.3.2 General Methods

Specimens were collected around the Sydney, New South Wales, area (field sites: Ku-ring-gai Chase National Park, and Cowan Field Station in the Muogamarra Reserve) during October and November of 2009. Live spiders were housed in individual containers and kept in the lab on a 12-hour on/off light cycle. Spiders were fed weekly a diet of fruit flies (*Drosophila melanogaster*) and occasionally crickets (*Acheta domesticus*).

First, live mature males (N=11) and females (N=10) were paired randomly between the hours of 09:00-15:00, and interactions between the pairs recorded on a digital VCR (Sony DVCAM DSR-20 digital VCR). Visual and vibratory courtship display elements of males were captured using a JAI CCD camera (CV-S3200) and a Polytec Scanning Laser Vibrometer (PSV-400, digitized at a 48.1 kHz sampling rate), respectively. Courtship recordings were conducted on an arena consisting of nylon fabric stretched over a circular wooden needlepoint frame (diameter: ~ 27 cm). This fabric was used as it has been shown to pass frequencies with minimal distortion (Elias *et al.* 2006c). The arena was situated on wooden dowels (height: ~7.5 cm) atop a larger rotating, circular platform (diameter: ~35 cm). The camera was stationary, so rotation of the circular platform allowed us to keep males in the recording frame as they moved around the arena after females. Several square pieces of reflective tape (area: ~1 mm²) were stuck to the surface of the nylon fabric, at the center of the arena, to serve as measurement points for laser vibrometry recordings. In between use, our arenas were cleaned with 75% ethanol to remove any chemical traces of previously run spiders.

One of the main merits of this set up was that males and females were allowed to move about freely on the arena and thus interactions would be more similar to those in the wild. In that sense, these recordings were helpful for making preliminary observations and capturing the overall progression of male visual displays and vibrational signals. However, because it is very difficult to maintain focus on moving spiders, this setup was not ideal for capturing entire courtship displays. Therefore, additional recordings were employed, similar to techniques used by Elias *et al.* (2006c).

Instead of live females, dead female models were used to elicit males to court in a more contained and determined area. Conspecific female models were prepared by attaching freshly dead females to an insect pin, using melted bee's wax on the ventral surface of the

cephalothorax. The previous arena set-up was modified so that the female models could be positioned at the center, atop the nylon fabric near the pieces of reflective tape. Glued to the top of the larger platform was a belt-pulley system with one pulley attached at the center of the platform (Figure 2.1). On top of the centrally placed pulley we attached a small piece of cork to which female models could be attached and swiveled by rotating the other pulley, which was situated at the outermost part of the larger platform. This arrangement allowed us to move female models in a "lifelike" manner, which helped to entice males to court. One at a time, males (N=11) were dropped into the arena and allowed to court freely. If males did not notice the female, mounted females were rotated until males took an interest and started to approach. Females were then positioned as if they were observing a male's activity head on. If males stopped courting for more than several minutes, female models were rotated slightly, in order to draw the male's attention. Pinned females were placed in the freezer at the end of each day for preservation, and were used for 1-2 weeks maximum.

Video and laser recordings were extracted from tapes and imported into Sony Soundforge.

2.3.3 Visual Display Characterization

Videos were assessed (resolution: 30 frames/sec), in order to record distinct behaviors. Information was compiled and displays of males (N=11) from tethered female trails were used to construct ethograms. Courtship recordings with live females could not be easily analyzed, thus they were omitted for measurement purposes. In a subset of data where we could analyze displays, we observed no differences in courtship (data not shown). Measurements of rates and durations were averaged for each individual. An Iconico screen protractor (v3.3) was used on individual video frames to find angle ranges of the 3rd legs of males (N=5).

In addition, we also recorded displays of males (N=3) with a high-speed camera (Photron fastcam SA3, 1000 frames/second) so that certain features of male movement could be more easily clarified. Using the larger rotating platform, we positioned the regular speed camera to a "female's eye view" and the high-speed camera with a side view of the courting male.

2.3.4 Vibrational Signal Analysis

Laser recordings from each individual were filtered below 80 Hz in Sony Soundforge to remove background noise. For quantification of vibratory signal elements, 5 samples of each element were randomly selected across an individual's display and averaged. Duration, dominant (peak) frequency, and bandwidth (10dB below peak frequency) were measured using custom written Matlab scripts (Mathworks Inc., v7.0.).

Only complete displays that progressed to a copulation attempt were used. In total, vibrations from 5 different males were scored. Coefficients of variation were calculated to quantify variation of signal elements within individuals and across the entire group sampled.

2.4 Results

Total courtship time of *Maratus volans* ranged from 6 -51 minutes (mean= 24.35 $\pm$19.49 min, N=5). Use of visual and vibrational signals varied although overall patterns in the sequence of mating behavior included many distinct, stereotyped behavioral elements that could be consistently identified across individuals.
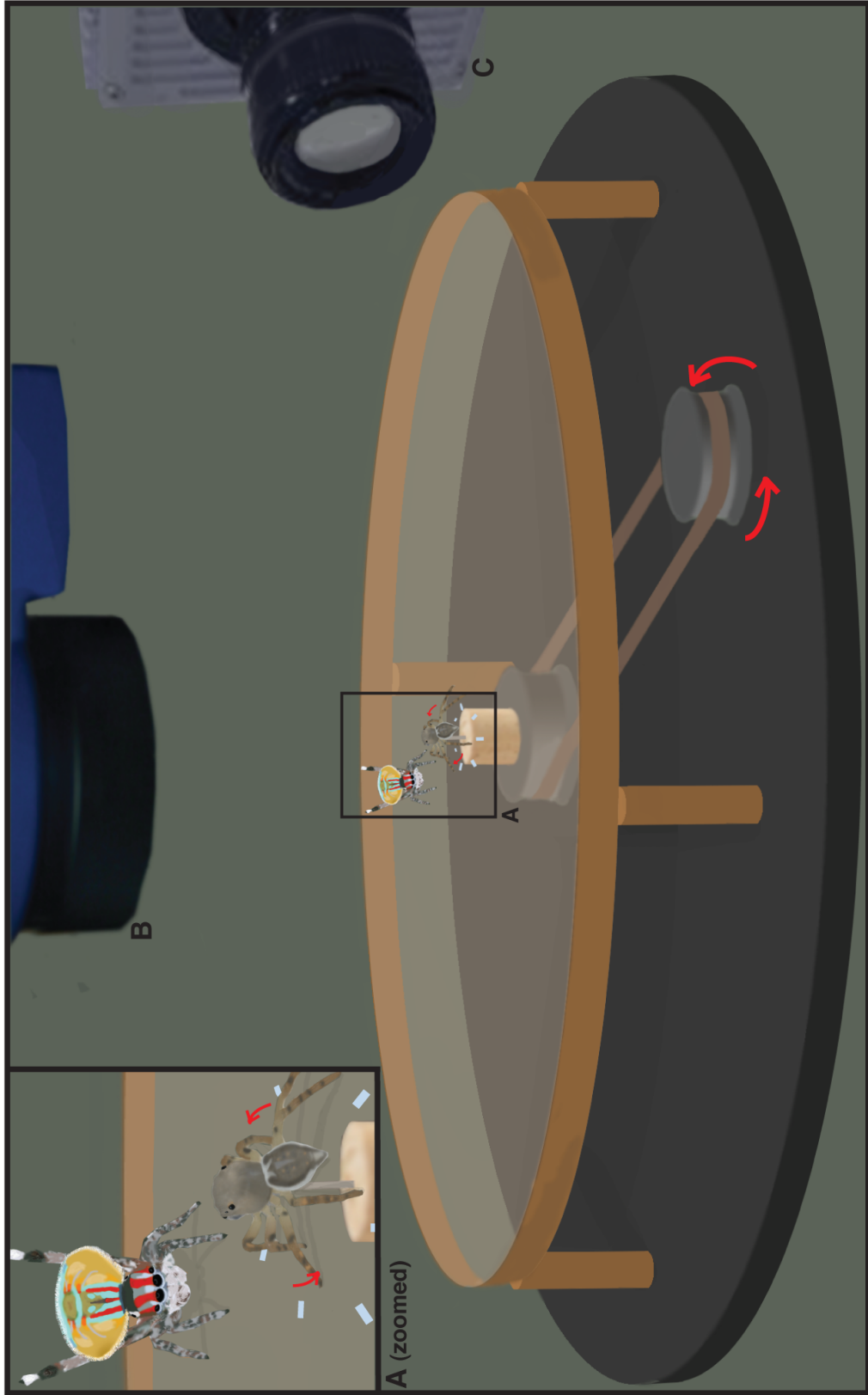
Figure 2.1. Courtship recording set-up. (A) Focal area for the regular speed camera (CV-S3200), (B) laser vibrometer (PSV-400) and (C) high-speed camera (SA3).

2.4.1 Visual Displays

For an initial review of *M. volans* displays and a comprehensive collection of images, refer to Hill (2009). Table 2.1 gives detailed descriptions, including contextual information, of distinct behaviors. Behaviors are presented in the general order of their appearance within a courtship sequence.

pedipalp flicker

Pedipalp flickers were observed to occur intermittently throughout the entire duration of courtship, alone or in conjunction with all other displays. During interactions between live individuals of both sexes, males performed pedipalp flickers even when females were not oriented in their direction; females were also observed doing this behavior. This behavior was common and occurred in other contexts (i.e. when individuals were feeding, or just moving about their individual containers alone), suggesting that pedipalp flickering may not be specific to the mating display. Regardless, it is included here as it is a prominent behavior during courtship. Pedipalp flickering ramps up in intensity immediately preceding movements, such as opisthosomal bobbing or leg waving.

opisthosomal bobbing

Male peacock spiders can move their abdomen up and down independently of expanding and retracting opisthosomal flaps, and vise-versa (Hill, 2009). Vibrational signals are associated with this movement (discussed at length in the "Vibrational Signals" section). High-speed video analysis revealed that in between "bobs", lateral movements of the abdomen sometimes occur, particularly during the pre-mount display.

3rd leg wave

The majority of a male peacock spider's courtship display is comprised of 3rd leg waves (Figure 2.2a-f). Similar to jumping spiders in the *Habronattus* genus, the 3rd legs of *Maratus* males are elongated and ornamented relative to the other pairs (Hill, 2009). Specifically, the metatarsi of *M. volans'* 3rd legs are covered with a dense tuft of black setae and a comparably thick clump of white setae adorn the tarsi (Hill, 2009). If not already in position, leg waving begins with the male raising his 3rd legs into the air rapidly. Sometimes simultaneously, or shortly after, the opisothoma is lifted and flaps are unfurled, though often, especially if the male is further from the female, leg waves precede this expansion of the "fan." Once the 3rd legs are brought upright, they are immediately lowered, while remaining extended (Figure 2.2a-e). They are only flexed at the patella when they crest the bottom of their rotation and are smoothly re-extended as they return to a vertical position. Rotation of the 3rd legs is most likely around the coxa-trochanter joint (Parry, 1957), although this could not be confirmed.

Leg waving usually occurs bilaterally, but occasionally one 3rd leg was waved completely on its own (this was observed for both right and left legs), or each side was waved asynchronously, in an alternating fashion. Bouts of leg waving occur intermittently, sometimes while a male is stationary, but often, 3rd leg waving occurs in conjunction with a side-stepping motion akin to the side-to-side motion that occurs during the fan dance of *Maratus pavonis* and *Maratus splendens* (Hill and Otto, 2011). High-speed video analysis revealed that the male takes each side step as the 3rd legs pass through the lowest part of a leg wave. A male will move in

Table 2.1. Visual ethogram for *Maratus volans*. * after behavioral descriptions similar to that of two related species, *M. pavonis* and *M. splendens* (Hill and Otto, 2011)

Behavior	Description	Occurrence	Measurement
pedipalp flicker*	The pedipalps are brought together in the front of the carapace and moved up and down in unison.	intermittently throughout courtship	**Rate:** 3.5 +1.6 flicks/sec (N=5)
opisthosomal bobbing*	The abdomen, or opisthosoma, is moved up and down in a rapid manner. It can be parallel with the substrate or more vertically oriented, with fan flaps either splayed out (expanded) or folded around opisthosoma (retracted). During production, the male is stationary, with either all leg pairs contacting the substrate, or with the 3rd legs in an erect leg wave stance.	(1) when males approached females from a distance, preceding all other courtship behaviors (excluding pedipalp flickering), (2) between bouts of leg waving/fan dancing.	**Number of bouts:** 2-11 (N=5) **Bout duration:** 75.5 + 58.6 secs
3rd leg wave	The 3rd legs are swiftly extended and raised to an approximately vertical, erect leg wave stance. Almost immediately, the 3rd legs, while still extended, are lowered and simultaneously brought back towards the abdomen slightly to form a bilateral "V". At their lowest point, the 3rd legs are flexed at the patella briefly before they are quickly rotated up and forward to their original position. This motion appears seamless and is repeated several times with no gaps between leg waves.	following several bouts of opisthosomal bobbing, while males are facing a female	**Bout duration**: 6.7 +3.2 secs (N=5) **Rate:** 3.2 +0.6 waves/sec (N=5) **Angle ranges:** (Fig. 2.2, N=5) #1= 36-71° #2= 72-120° #3= 124-168°
fan dance*	Open opisthosomal fan (flaps expanded) moves back and forth laterally, similar to a metronome, at varying speeds. Most often produced in synchrony with leg waving, but sometimes the male is stationary either with the 3rd legs in an erect leg wave stance, or in contact with the substrate.	when male is in close proximity to a female	**Rate:** 3.2 +1.5 cycles/sec **Angle range:** (Fig. 2.2, N=5) #4= 19-35°
fan-flapping	While opisthosoma is vertically oriented, fan flaps are extended and retracted several times in a sequence. The male is stationary with 3rd legs in an erect leg wave stance.	periodically during pauses in movement that follow fan dances	**Rate:** 2.5 +1.8 flaps /sec
pre-mount display	The 3rd legs are rotated forward, and the carapace is brought up over the 1st and 2nd legs. Simultaneously, opisthosomal fan flaps are retracted and the abdomen is tilted until the posterior portion is close to the substrate. Regularly spaced bouts of opisthosomal bobbing follow, each of which correspond with tremors and lowering of 3rd legs. Later, the 1st legs are flexed and raised slightly while the 3rd are rotated all the way down and behind the 1st and 2nd legs where they are held extended in an upside down "V". The 1st legs are now held erect out in front of the carapace and moved down closer to female during bouts of opisthosomal bobbing.	occurs at the end of the courtship display, immediately preceding and leading into a mounting attempt	**Duration:** 54.1 +7.4 secs **Angle range:** (Fig. 2.4, N=5) #5= 126-148°
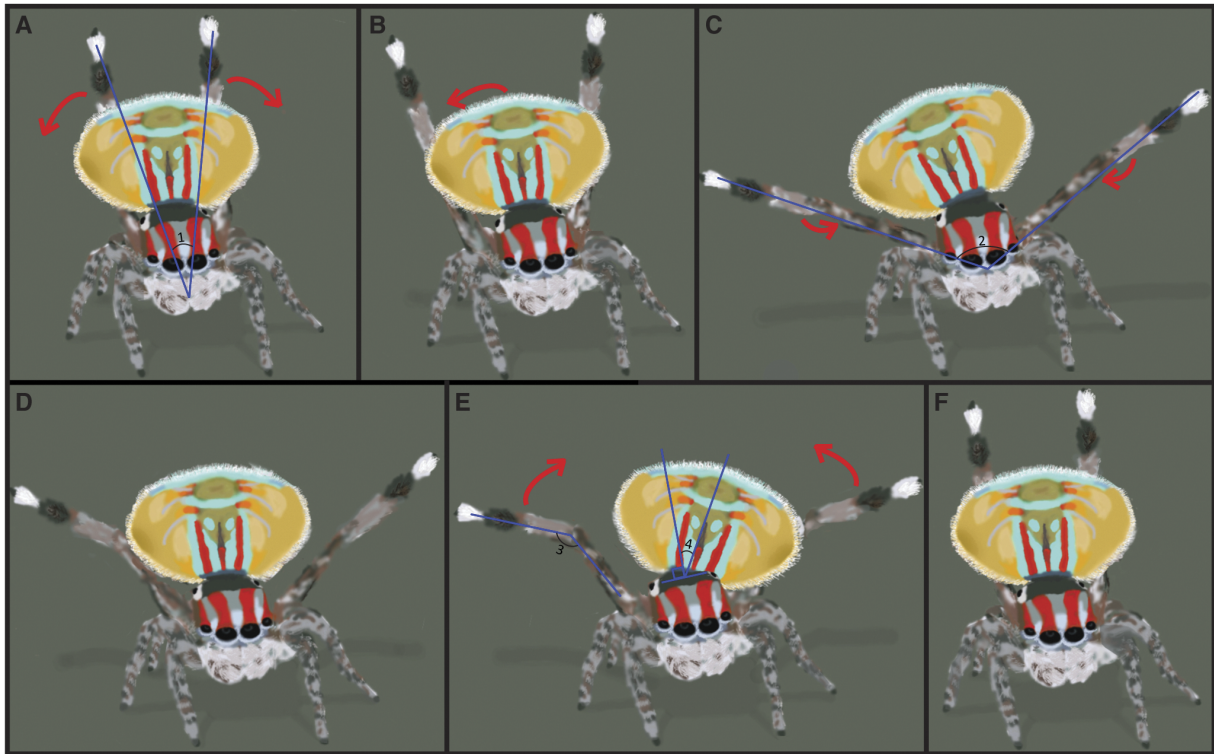
Figure 2.2. Fan dance of *Maratus volans*. (A) Males begin this display by swiftly raising the 3rd legs to an erect leg wave stance. (B) Immediately after, extended 3rd legs are lowered and (C) brought forward slightly until they are just above the top of the carapace. (D) At this point 3rd legs are slightly bent at the patella and (E) quickly raised until they are returned to their initial position. One cycle of fan dancing occurs between (B) and (F). Angle measurements (#1-4) are provided in Table 2.1.

semi circles around the female, going in one direction for a while before heading back the way he just came, getting slightly closer to where she is standing with each shift in direction. During this side stepping, the 3rd leg in the direction of movement is commonly held perpendicular to the substrate while the other leg was held slightly lower (albeit still extended) and waved much more intensely. Hill and Otto (2011) also observed this tendency in *Maratus pavonis*.

fan dance

The opisthosomal fan is the feature for which spiders in the *Maratus* genus are named (Dunn, 1947); accordingly, the fan dance is the most notable aspect of this spider's courtship. High-speed video analysis revealed that when a male is fan dancing in conjunction with leg waving (Figure 2.2c-e), each cycle of fan movement reliably corresponds with a single 3rd leg wave (Figure 2.2a-f). The closer a male is to a female, the more likely he is to adopt a "hunker-down" pose when performing a stationary fan dance. This stance is characterized by lowering of the carapace, almost to the ground, and bending of the front legs more sharply at the patella to bring them tight against the carapace; the 3rd legs remain in an erect in "V" position. While still in this pose, males will regularly follow fan dancing with some opisthosomal bobbing.

fan flapping

Males often pause after a bout of 3rd leg waving/fan dancing, seemingly to gauge female attention and/or intention. Sometimes during these pauses, especially if a female is not oriented directly in front of males any more (either by his or her movement), males will slowly flutter the portion of the opisthosomal fan that can be tucked around the abdomen for a few seconds (Figure 2.3a-d). In the context that it was seen to occur, fan flapping is potentially a means of drawing attention back to the male. Indeed in several cases, fan flapping elicited such a response by females who would reorient themselves towards the male after he performed this behavior. During courtship displays evoked using pinned dead females, a male would flap his fans until the female was swiveled slightly, in a manner to mimic a female tracking male movement, at which point the male would commence fan dancing again.

pre-mount display

In contrast with the majority of the male peacock spider's courtship display, the pre-mount display proceeds in a precisely stereotyped sequence (Figure 2.4). To begin, the 3rd legs are rotated to the front of the carapace, which is brought forward and uplifted over the 1st and 2nd legs (Figure 2.4a-b) at the same time. Simultaneously, the opisthosomal fan flaps are retracted and the abdomen is tilted until the anterior portion is level with the top of the carapace and the posterior portion is fairly close to the substrate. Regularly spaced bouts of opisthosomal bobbing follow, each of which correspond with tremors of extended 3rd legs (Figure 2.4c); this aspect of the display is discussed more thoroughly under the "Vibrational Signals" section below. During tremors, the 3rd legs are moved up and down only slightly, but very rapidly. At the end of a tremor, the 3rd legs are lowered and spread further apart than when they started. When the 3rd legs are lowered to about the top of the carapace, after approximately 3-8 tremors, the 1st legs are flexed and raised slightly off the substrate (Figure 2.4d). During the next tremor, the 3rd legs are rotated all the way down in front of the carapace and continue to be rotated back behind the 1st and 2nd legs where they are held extended in an upside down "V" (Figure 2.4e). The 1st legs are then held erect out in front of the body at carapace level almost touching the female, termed the "glider pose." Now with each bout of opisthosomal bobbing the male bends the tarsi and
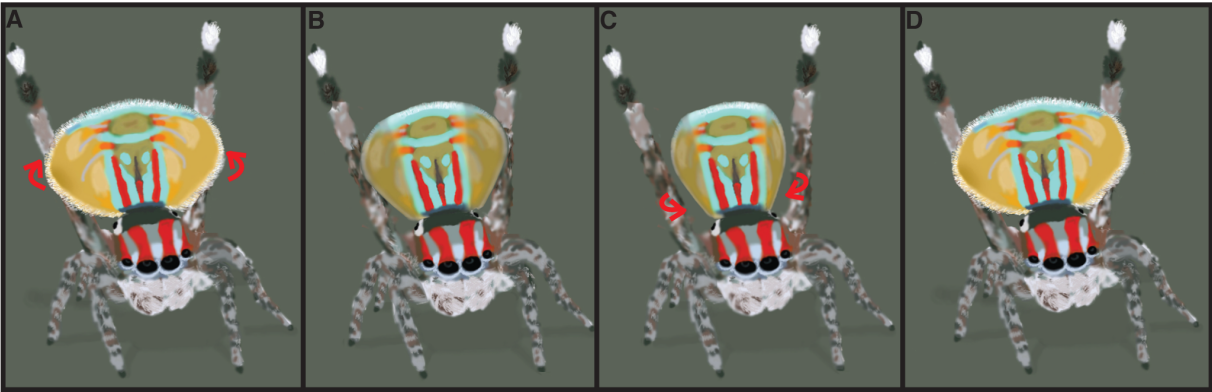
Figure 2.3. Fan-flapping of *M. volans.* (A) 3rd legs in erect leg wave stance, (B) initial retraction of the distal portion of the flaps commences. (C) Flaps are further contracted before (D) being quickly expanded again.
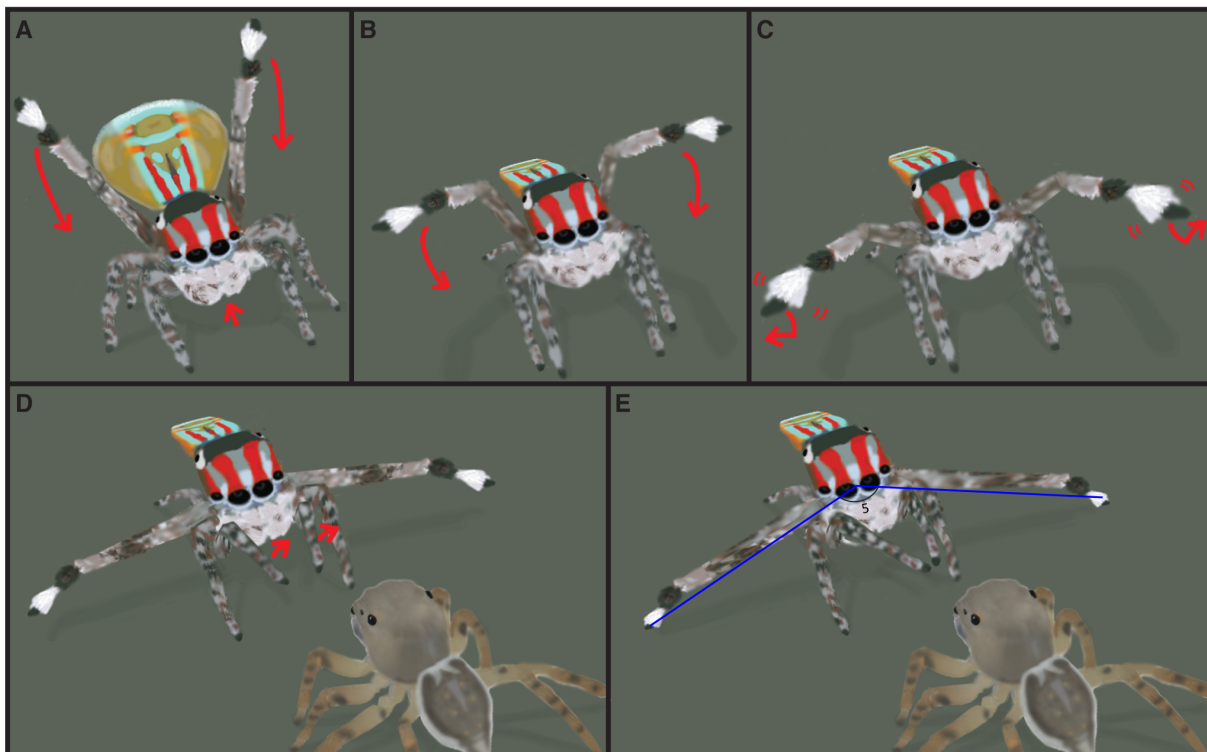
Figure 2.4. Pre-mount display of *M. volans*. (A) From an initial leg wave stance, the 3rd legs are rotated forward and (B), (C) the carapace is brought up over the 1st and 2nd legs. Simultaneously, opisthosomal fan flaps are retracted and the abdomen is tilted until the posterior portion is close to the substrate. (D) Regularly spaced tremors following paired lowering of the 3rd legs. Once legs are lowered to be approximately parallel to the substrate, the 1st legs are flexed and raised slightly while (E) the 3rd legs are rotated all the way down and held extended in an upside down "V" behind the 2nd legs. The 1st legs are now held erect out in front of the carapace and gradually moved down closer to female. Angle measurement (#5) is provided in Table 2.1.

metatarsi of the 3rd legs slightly, and moves the 1st legs down closer to the female until finally touching her carapace. It was previously inferred (Hill, 2009) that the 1st legs do not play a role in the visual courtship display of these spiders, but this is not true of the pre-mount display. The time between opisthosomal bobbing bouts continues to decrease at this point and in time, the male makes advances over the top of the female's carapace towards her abdomen.

2.4.2 Vibrational Signals

In general, vibrations are extensively utilized throughout male courtship and are often a precursor to motion displays, especially when the female was at a distance and/or not oriented directly at a male. Vibrations are caused by oscillation of the abdomen (Elias and Mason, 2011; Uhl and Elias, 2011), and indeed, analysis of video recordings demonstrated that all vibrational signals coincide with opisthosomal bobbing; vibrations were absent during any lateral movement of the abdomen between bouts of bobbing. It remains unclear which mechanisms males are using to produce vibrations though. Stridulation of paired structures on their abdomen and cephalothorax may be employed. Vibrations might also be generated by tremulation, that is, abdominal oscillations transferred directly to the substrate via the animals' legs (Uhl and Elias, 2011). Further study using synchronous high-speed video/laser vibrometry and ablation experiments are needed.

Vibrational signals can be broken down into two categories: (1) those that occur immediately and continue intermittently throughout the majority of the display, termed **rumble-rumps**, and (2) those that occur during the pre-mount display only, which include (a) **crunch-rolls**, and (b) **grind-revs**.

Some vibrational signals are comprised of several components. Not all vibrational signals correspond with visual displays. For ease of discussion, signaling elements have been given names according to the acoustic characteristics of that signal. Table 2.2 summarizes properties of each signal element and for each measure, quantifies variation seen within and between individuals. As a general trend, variation in signal elements was greater for the group than within an individual for dominant frequency. The opposite trend was observed for signal duration.

1. rumble-rumps (Rb-Ru)

Rumble-rumps are the most common signals produced during courtship, seemingly as soon as a male detects the presences of a female, and even at long-distances. Rumble-rumps are short in duration (2.44 $\pm$0.28 sec, range 2.20-2.80 sec, N = 5). Intervals between signals are usually longer than the signals themselves (3.46 $\pm$2.48 sec, range 0.19-7.17 sec, N = 5). The mean number of *Rb-Ru's* in a bout is 17.4 $\pm$12.8 (range= 4-39, N=5). *Rb-Ru* bout numbers and duration correspond with that of opisthosomal bobbing, as reported in Table 2.1.

Rumble-rumps are composed of two distinct elements (Figure 2.5b, Table 2.2), "rumbles" (*Rb*) and "rumps" (*Ru*), although, there is considerable variation in the way that rumble rumps are put together. All *Rb-Ru's* start with a *Rb*, followed by 1-5 (typically 3) *Ru's*. This is annotated as follows: *Rb Ru*$^{1-5}$. Other rumble-rump combinations observed include: *Rb Ru*$^{1-5}$ *Rb,* and *Rb Ru*$^{1-5}$ *Rb Ru*$^{1-5}$. An example of a longer *Rb-Ru* (*Rb Ru*^{4} *Rb Ru*^{5}) is shown in Figure 2.5b. Each *Rb* is made up of 3-8 "bumps" (*b*) (Figure 2.5b, Table 2.2), which occur at a mean rate of 12.1 $\pm$2.9 bumps per second (N = 5). The interval between *Ru's* in a rumble-rump ranges from 0.07-0.62 seconds (mean= 0.25 $\pm$0.26 sec, N = 5). As a general rule, *Ru's* that occur
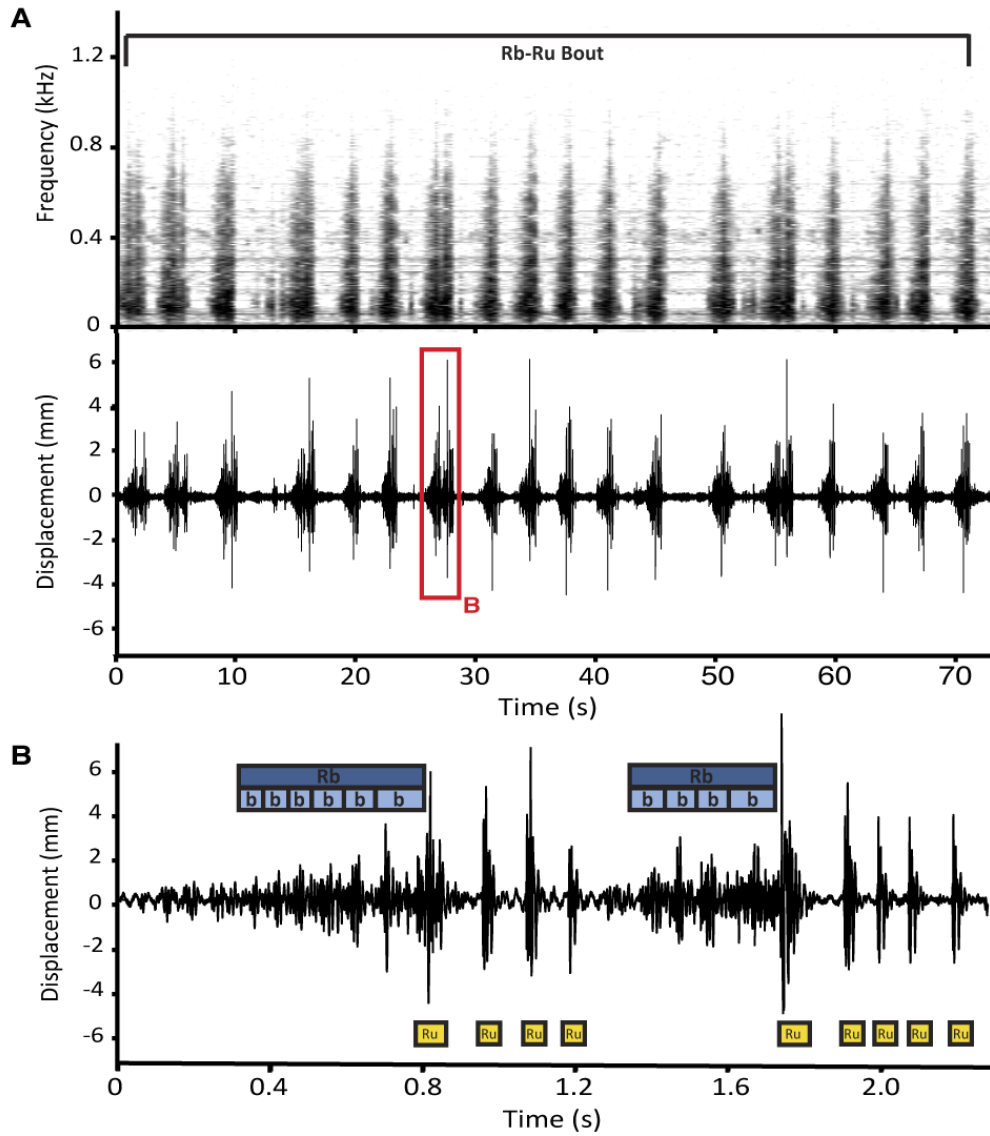
Figure 2.5. Substrate borne signals of courting male *M. volans*. (A) Spectrogram (window size= 26422) and waveform of a bout of rumble-rumps. (B) Waveform of a single rumble-rump. Substrate-borne signals occur throughout the *M. volans* display

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Table 2.2. Mean measurements of distinct vibrational signal elements of *M. volans* males (N=5), which are color coded to correspond with Figures 2.4 and 2.5. Coefficients of variation (CV) were calculated to quantify variation as it was observed within individuals (Ind.) and across the entire group sampled (Group). Frequency characteristics of *Rb's* and *Roll's* are similar to that of their components, *b's* and *r's*, respectively.

	Rb-Ru			Cr-Roll					Gr-Rev
	Rb	**b**	**Ru**	**Sw**	**Cr**	**Roll**	**r**	**Tail**	**g**
Duration (s)	.735 +.247	.113 +.033	.102 +.011	.210 +.185	.092 +.013	.668 +.085	.086 +.007	.422 +.080	.048 +.009
CV: Ind. / Group	*.373/.333*	*.439/.288*	*.310/.106*	*.278/.210*	*.146/.141*	*.384/.127*	*.156/.079*	*.131/.189*	*.187/.183*
Peak Freq. (Hz)	-	159 +64	128 +50	192 +33	165 +51	-	138 +49	157 +30	163 +47
		.213/.402	*.193/.386*	*.317/.171*	*.127/.308*		*.148/.351*	*.153/.188*	*.130/.286*
Min Freq. (Hz)	-	123 +44	78 +12	149 +19	105 +26	-	94 +36	121 +25	111 +40
		.232/.360	*.364/.150*	*.316/.128*	*.136/.247*		*.206/.380*	*.131/.208*	*.145/.362*
Max Freq. (Hz)	-	202 +86	194 +92	262 +28	254 +74	-	195 +46	222 +23	224 +64
		.127/.424	*.174/.477*	*.191/.105*	*.128/.291*		*.178/.236*	*.256/.103*	*.094/.283*
10db Bandwidth	-	79 +42	54 +28	112 +26	149 +62	-	101 +29	100 +24	113 +42
		.356/.528	*.579/.516*	*.477/.227*	*.243/.413*		*.471/.288*	*.669/.241*	*.240/.367*

immediately at the end of a *Rb* are usually highest in amplitude. *Ru-Rb's* continue to be produced during breaks in fan-dancing and leg waving until the pre-mount display begins.

2a. crunch-rolls (Cr-Roll)

The first *Cr-Roll* signal is always preceded by a very brief (mean= 0.21 ±0.19 sec) intro signal, which is just visible in the waveform in Figure 2.6a. It was difficult to determine if this intro was a signal or a byproduct of adopting the pre-mount display position (Figure 2.4a-b), when a male's legs and body are simultaneously raised. Either way, it was the lowest frequency vibration observed, with a peak frequency of 84 ±18 Hz (N=5).

Cr-Rolls are the vibrational signals produced during the opisthosomal bobbing and leg tremors that occur at the beginning of the pre-mount display. *Cr-Roll* signals consist of "swish" (*Sw*), "crunch" (*Cr*), "roll" (*Roll*), and "tail" (*Tail*) elements (Fig 2.6b, Table 2.2). *Sw's* occur at the beginning of *Cr-Roll's* and are quickly followed by a single *Cr*. Some *Cr-Roll's* don't have *Sw's*, typically the first and last *Cr-Roll's* of a bout, but in *Cr-Roll's* that do (74.6 ±7.7% of Cr-Rolls), high-speed video revealed that the tarsi of 3rd legs are flicked down at the end of each Sw and brought back up at the beginning of each *Cr. Roll's* follow *Cr's* and are paired with the swift and shallow flick of 3rd legs at the patella. During the second or third to last *Cr-Roll*, a male lifts legs I off the substrate (Figure 2.4d). During the last *Roll* of the last *Cr-Roll*, a male would lower 3rd legs to a near upside-down "V" position, while the body was again lifted and legs I were further outstretched in front of the carapace. *Roll's* in all but the last *Cr-Roll* are made up of 3-7 (mean= 5.5 ±2.1, N=5) smaller elements (r's) (Figure 2.6b, Table 2.2), which occur at a rate of 9.6 ±0.3 *r's* per second (N=5). *Roll's* in the last *Cr-Roll* were much longer and included a mean of 17.9 ± 2.1 *r's.* Also, in contrast with the other *Cr-Roll's*, the last *Cr-Roll* signal in a bout never had a *Tail* portion.

On average, a single *Cr-Roll* was 1.32 ±0.13 seconds (N=5), and the interval between *Cr-Roll's* was 0.88 ±0.37 seconds. Bouts of *Cr-Roll's* lasted a mean of 14.13 ±5.00 (N=5) seconds and included 5-11 (mean= 7.2 +1.8, N=5) individual *Cr-Roll's.* The mean interval between the end of the last *Cr-Roll* and the first grind-rev signal was 0.71 ±0.26 seconds (N=5).

2b. grind-revs (Gr-rev)

Grind-revs are produced in the final stages of the pre-mount display and continue to occur as a male mounts and attempts to copulate with a female. As seen with *Cr-Rolls*, leg movement is highly coordinated with *Gr-rev* vibrations. When a male begins *Gr-rev* signal production, he is in the "glider pose" (Figure 2.4e), that is, 3rd legs are in a downward "V" position and the 1st legs are extended out in front of the carapace. With each successive *Gr-rev* signal, legs I are brought closer together at the most distal end and lowered (albeit still extended) nearer to the female's carapace. At this point, she is now positioned almost directly below the male's leg I tarsi. Once he is touching the female, the male begins to move over the female's carapace towards her abdomen in time with *Gr-rev's.* An average bout of *Gr-rev's* lasts for 38.77 ±19.08 seconds N = 5) and includes anywhere from 12-34 *Gr-rev's* (mean=26.9 ±6.4, N = 5). However, it is difficult to distinguish individual *Gr-rev's* approaching the finale of the pre-mount display as the interval between *Gr-rev's* becomes increasingly small (mean= 1.04 ±0.30 sec, range= 0.67-1.34 sec, N = 5). As a general rule, during the progression of the pre-mount display the duration of individual *Gr-rev's* (range= 0.16-0.81 sec, N=5) and the interval between each increases and decreases, respectively, as a male advances towards a female. The spectrogram of *Gr-rev's* included much higher frequencies than all other signals measured, (Figure 2.5a).

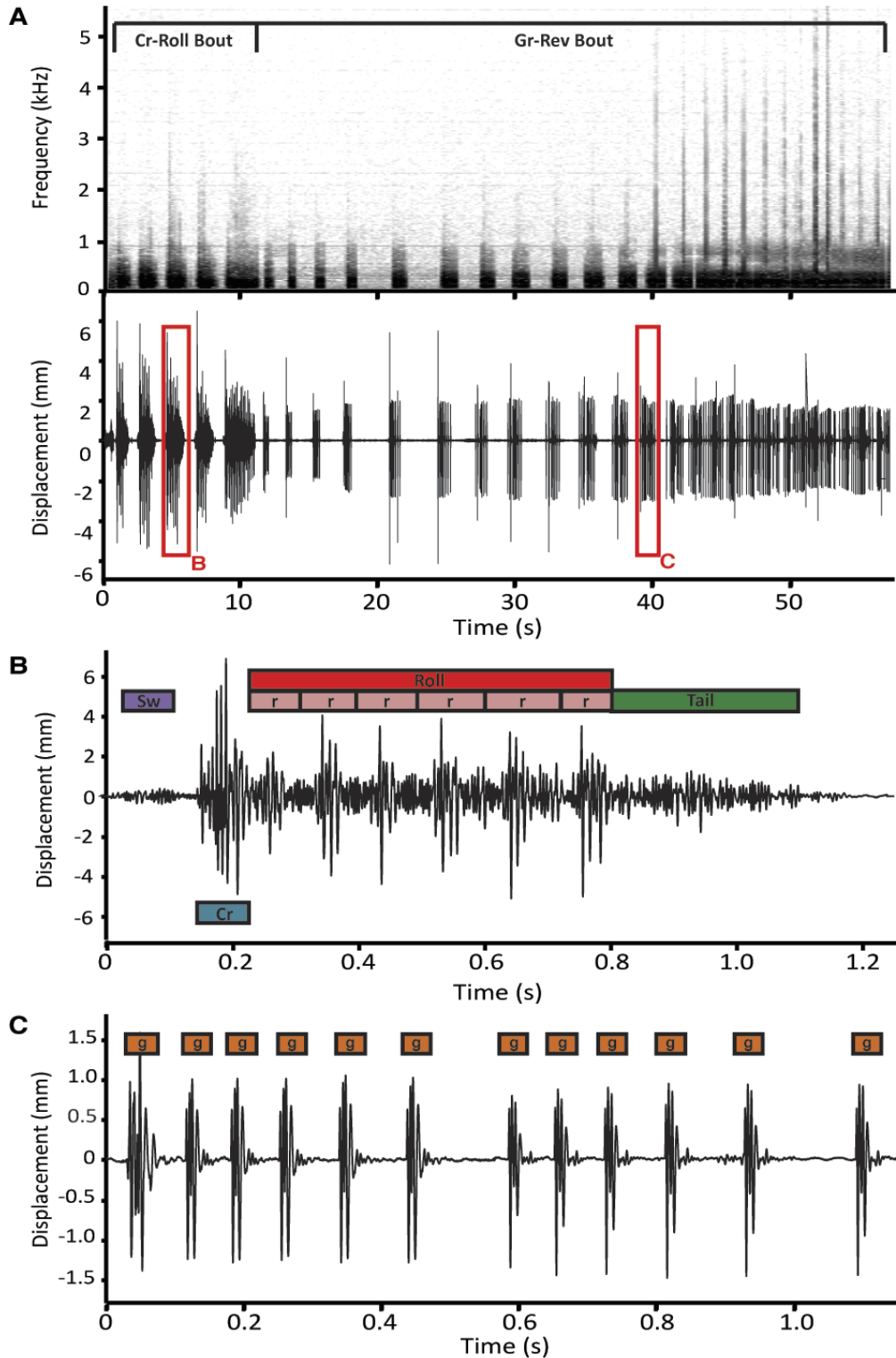**Figure 2.6.** Pre-mount display substrate-borne signals of courting male *M. volans.* (A) Spectrogram (window size= 11206) and waveform of a bout of crunch-rolls and grind-revs as they occur in sequence. (B) Waveform of a single crunch-roll. (C) Waveform of a single grind-rev. Crunch rolls and grind revs are produced exclusively during pre-mount displays.

Gr-rev's are composed of a sequence of "grinds" (*g*) (Figure 2.6c, Table 2.2), which are emitted in groups of 3-11 (mean= 8.54 $\pm$0.23, N = 5) at a rate of 12.64 $\pm$3.88 *g's* per second, N = 5); again, these groups blur at the end of the pre-mount display as the interval between *Gr-rev's* becomes increasingly small. In contrast, throughout a bout of *Gr-rev's*, intervals between *g's* remain fixed around a mean of 0.05 $\pm$0.10 seconds (N = 5). At the end of a *Gr-rev* bout, males attempted to copulate with the female (or female model).

2.5 Discussion

Our results show that peacock spiders use visual displays in conjunction with vibratory signals during courtship. The full repertoire of these males is truly remarkable, particularly the visual components. While visual and vibrational signals are variable between males, some overall patterns were evident. For instance, male courtship usually began with rumble-rump vibrations produced at a distance. When males get close to females, they begin to perform multimodal displays, primarily 3rd leg waving and fan dancing (visual) along with rumble-rumps (vibrational). Males add new elements as courtship progresses to the finale, specifically the pre-mount display and associated crunch-roll and grind-rev vibratory signals. Unfortunately, our study was limited by the number of males and complete displays we were able to capture. Larger samples are needed to assess more accurately the amount of natural variation in courtship that exists for individual males, as well as within and between wild populations of these spiders.

It should be noted that even with complete video footage of male courtship, precisely characterizing displays is still a challenge. This is especially the case when trying to pick out features that might be important to animals with sensory systems unlike our own. Display and signal elements were quantified as thoroughly as possible, but the former should only be treated as estimates. The sequence of animal behaviors is often influenced by many dynamic factors in the wild, and at this point it is difficult to accurately predict if the sequence and durations of individual behaviors observed in the lab are as similar to that which would be seen in nature. Our immobile female models offered us the freedom to easily document male displays, however, the progression of courtship is unlikely to progress in such a simple manner. In the case of these spiders, female feedback, in the form of receptivity and/ or aggression (often seen in trials between both live sexes) undoubtedly contributes greatly to the way in which males proceed in their courtship efforts.

While both visual and vibratory signals are well demonstrated as being important in mating systems of spiders (Foelix, 1996; Uhl and Elias, 2011), our understanding of complex multimodal signals is still in the very early stages (Partan and Marler, 2005). The adaptive significance of multi-modal signal structure remains poorly studied (Partan and Marler, 2005), as are mechanisms by which sexual selection operates on multi-modal signals (Candolin, 2003). Natural signaling habitats are rarely homogeneous and therefore provide a variety of signaling channels and strategies to exploit, each of which may vary contextually in their efficacy of information transfer. Not surprisingly, evidence suggests that in a mating context, males may actually use multiple different signal strategies, alone or in conjunction, in response to varying abiotic and biotic factors (Coleman *et al.* 2004; Endler, 1990; Endler, 1992; Hebets and Papaj, 2005).

We observed that *Maratus volans* males use vibratory signals over visual displays at long distances. In contrast, one well studied example from the North American genus *Habronattus*, *H.*

dossenus only uses vibrational signals at close proximities to females, mainly using visual displays when greater than 5-8mm away from females. Presumably *H. dossenus* behavior is adapted to variable vibratory environments (Elias *et al.* 2004). Our descriptions might then suggest that the visual environment of *Maratus* is more heterogeneous than the signaling substrate, and thus offers a better channel for communication over a greater distance. Future work on the ecology, habitat usage patterns, and signaling environment of *Maratus* species will test this hypothesis. Given that multimodal signal structure is a most likely a product of a combination of dynamic selection pressures (Bro- Jørgensen, 2010; Hebets and Papaj, 2005), some of the plasticity observed in *M. volans* displays could be favored by selection in order to minimize costs of signals production and maximize success in variable environments.

Although *Maratus* has evolved independently from *Habronattus,* the two genera possess several similarities in morphology, behavior, and habitat. Specifically, species of *Maratus* and *Habronattus* share elongated 3rd legs, have similarly structured genitalia, and are primarily ground-dwelling (Hill, 2009). Both genera also show a remarkable amount of interspecific morphological diversity (Elias *et al.* 2005; Elias *et al.* 2006b; Hill, 2009; Hill and Otto, 2011; Maddison and McMahon, 2000; Otto and Hill, 2010) and much diversity remains to be discovered in *Maratus* and its relatives. Direct comparisons of complex signals in these two genera will better inform our understanding of multi-modal signal structure and function. For instance, while *Maratus* and *Habronattus* both make use of multimodal signals, *Maratus* vibrational signals are relatively simple compared to those seen in some *Habronattus* species (Elias *et al.* 2003; Elias *et al*. 2006b; Elias *et al.* 2006c). Instead, *Maratus volans* males invest more in their ornamentation and visual displays (as evidenced by the evolution of the opisthosomal flaps). This pattern is predicted in several models of multiple signal evolution and has been empirically shown in several groups of birds (Badyaev *et al.* 2002; Shutler and Weatherhead, 1990; Snell-Rood and Badyaev, 2008). The opposite pattern from *Maratus volans*, where vibrations are emphasized over visual displays, is seen in some closely related species from the genus *Lycidas* (Żabka, 1987). In work on *Lycidas michaelseni* (*Saitus michaelseni* in Gwynne and Dadour, 1985)*,* behavioral observations suggested that males primarily courted females using audible substrate-borne signals. Interestingly, visual signals were deemphasized as males stridulated directly above female nests out of view (Gwynne and Dadour, 1985). This type of behavior also occurs in several other *Lycidas* species found in the same habitats as *M. volans* (Girard and Elias, unpublished observations). It is quite possible that signal complexity may be limited by evolutionary tradeoffs where investment in one modality necessitates reduction in another (Gibson and Uetz, 2008; Iwasa and Pomiankowski, 1994; Johnstone, 1996; Pomiankowski and Iwasa, 1998) and this might explain the pattern observed for greater visual complexity in *Maratus* multi-modal signals.

Despite their common occurrence in nature, multi-modal signals have received relatively little attention thus far (but see Elias *et al.* 2005; Hebets and Papaj, 2005; Partan and Marler, 2005). Female choice has long been the subject of in-depth investigations, but we are only now starting to use this framework to examine complex multimodal signals. Jumping spider communication offers an excellent system to study behavior and the role of sexual selection in the evolution of species and mating systems. Specifically, the *Maratus* genus lends itself as a perfect system for such studies. In conclusion, this study provides the foundation necessary for future research on the *Maratus* genus and the evolution of complex signals.

2.6 Acknowledgements

Chapter 3: Female preference for multi-modal courtship: multiple signals are important for male mating success in peacock spiders[3]

3.1 Abstract

A long-standing goal for biologists has been to understand how female preferences operate in systems where males have evolved numerous sexually-selected traits. Jumping spiders of the *Maratus* genus are exceptionally sexually dimorphic in appearance and signaling behavior. Presumably, strong sexual selection by females has played an important role in the evolution of complex signals displayed by males of this group, however, this has not yet been demonstrated. In fact, despite apparent widespread examples of sexual selection in nature, empirical evidence is relatively sparse, especially for species employing multiple modalities for intersexual communication. In order to elucidate whether female preference can explain the evolution of multi-modal signaling traits, we ran a series of mating trials using *Maratus volans*. We used video recordings and laser vibrometry to characterize, quantify and examine which male courtship traits predict various metrics of mating success. We found evidence for strong sexual selection on males in this system, with success contingent upon a combination of visual and vibratory displays. Additionally, independently produced, yet correlated suites of multi-modal male signals are linked to other aspects of female peacock spider behavior. Lastly, our data provide some support for both the *redundant signal* and *multiple messages* hypotheses for the evolution of multi-modal signaling.

3.2 Introduction

Decades of research exploring the effect of female preference has established that this mode of selection can lead to exaggerated traits (Andersson, 1994; Coleman *et al.* 2004; Masta and Maddison, 2002; Ritchie, 2007). Despite this, we still have a relatively poor understanding of if/how female preferences have shaped the more extreme examples of sexual ornamentation seen in the animal kingdom, specifically those characterized by an elaboration of a whole suite of signals. For example, birds of paradise (family: Paradisaeidae) are considered one of the most extravagant groups in this regard, exhibiting vocal signaling, extreme variation in coloration, and intricate dances that accompany both (Irestedt *et al.* 2009; Scholes, 2008). Although the ostentatious traits and behaviors exhibited by this and analogous systems are often attributed to sexual selection, empirical support for this idea is lacking. Moreover, studies that identify particular aspects of multi-faceted signals important for mating success are scarce.

While it is clear that selection acts on several traits simultaneously (Blows *et al.* 2003; Guilford and Dawkins, 1993; Hebets and Papaj, 2005; Hunt *et al.* 2009; Partan and Marler, 2005), previous research has primarily examined individual traits in isolation or focused on species employing simple signals for mate attraction (i.e. bird color patches, cricket calls, etc.). Thus, a potentially biased impression that females assess males based on single traits exists in the literature. Additionally, prevailing theoretical work, which predicts the evolution of female preferences for one informative signal, not multiple indicators of quality (Iwasa and Pomiankowski, 1994; Partan and Marler, 2005; Pomiankowski and Iwasa, 1993; Pomiankowski and Iwasa, 1998; but see Kuijper *et al.* 2012), has reinforced an emphasis on simple trait-choice relationships.

Australian peacock spiders of the *Maratus* genus (family: Salticidae) truly serve as excellent organisms to study complex signal evolution within a multi-dimensional framework. During courtship, male peacock spiders wave ornamented abdominal flaps and elongated third legs to nearby females (Girard and Endler, 2014; Girard *et al.* 2011; Uhl and Elias, 2011). In conjunction with visual displays, males also use vibratory signals (Girard *et al.* 2011) for intersexual communication. Preliminary data suggest this group may contain as many as 40 different species (Girard and Endler, 2014) varying widely in habitat, distribution, morphology and behavior. The main objective of our research was to examine male courtship displays and female behavior of one species, *M. volans*, in order to pinpoint if/which male traits or trait combinations predict mating success and to better understand the nature of the selective pressures acting on male peacock spiders. We expect that there will be multiple traits of importance to females, all of which will be positively correlated with various metrics of mating success, such as copulation, shorter latency to mate, longer mating duration, and egg laying. We also anticipate other aspects of female behavior; in particular, orientation and aggression toward males, will be linked, positively and negatively, respectively, with the same traits that are important for mating success.

As peacock spiders are diverse in vibrational and visual signaling traits, with repertoires rivaling those of the better-known birds of paradise, this group provides a unique system for evaluating current theories for complex signal evolution. At present, there are several non-mutually exclusive hypotheses to explain the evolution of multi-modal signalling based on: (1) the quality, type and/or amount of information conveyed in complex displays, (2) the efficacy with which these signals are transferred in response to varying biotic and abiotic factors, and (3) considerations of potential interactions between signals and how integration of signal elements

could affect signal production or reception. Another goal of this study was to explore evidence for two of the main hypotheses related to adaptive preferences for informative signals (Hebets and Papaj, 2005; Partan and Marler, 2005). The first, the *multiple messages hypothesis* proposes that each component of multi-modal signals will be informative to females in a different way. By contrast, the *redundant signal hypothesis* proposes that different multi-modal signal components will independently reflect the same information, providing back-up for intrinsic signaling error. To investigate support for either of these hypotheses, we examined patterns of correlations between different courtship traits. One simple prediction of the *redundant signal hypothesis* is that elements of multi-modal signals are expected to have tight covariance (Hebets and Papaj, 2005). Conversely, the *multiple messages hypothesis* (Hebets and Papaj, 2005; Jacob *et al*. 2011; Johnstone, 1996; Moller and Pomiankowski, 1993) predicts independence between each signal component and thus, we do not expect distinct signal elements to covary.

Given that complex multi-modal signals are used by many animals, not just peacock spiders (Narins *et al*. 2005; Vallin *et al*. 2005), major objectives for behavioral ecologists and evolutionary biologists are to elucidate both the preferences for, and function of, these signals. Such insight will not only inform what we know about the evolution of extremely exaggerated traits, but also why some species seem to use simpler modes of communication. One benefit of our study is that it examines how authentic integrated multi-modal signal structure affects mating success, rather than focusing on single traits in isolation or manipulated traits at the extreme ends of naturally occurring variation. Another advantage of this work is that we were able to measure mating success at multiple stages (i.e. latency to mate, copulation, mating duration and egg laying) and correlate these data with various courtship traits. Both aspects of this research contribute to a more complete and realistic picture of how female preferences are driving mate choice, and in turn, guiding both male and female behavior.

3.3 Methods

3.3.1 Sampling

Juvenile *Maratus volans* specimens were collected around the Sydney, NSW from August 5th to November 29th, 2011. Live spiders were brought back to the lab, where they were housed individually with leaf litter from their environment and kept on a 14:10hr light:dark cycle. Spiders were fed a diet of fruit flies (*Drosophila melanogaster*) and crickets (*Acheta domestica*).

3.3.2 First Mating Trials

Mating trials between mature males and females were conducted from October 28th - December 12th, 2011, between the hours of 09:00-16:00. Weights of all individuals were recorded prior to trials. For each trial (N=64), a unique male and female, both virgin, were paired and all interactions were recorded using a Canon EOS Kiss X4 with a 100mm macro lens. The camera was stationary and positioned directly above the arena. Concurrently, vibratory courtship was captured using a laser vibrometer (Polytec PDV100) and recorded onto a digital recorder (Sound Devices 744T, 48.1 kHz sampling rate).

Courtship recordings were conducted on an arena of nylon fabric stretched over a circular frame (diameter: ~ 10 cm). This fabric was used as it has previously been shown to pass male signaling frequencies with minimal distortion (Elias and Mason, 2014). Several pieces of reflective tape (~1 mm²) were stuck to the surface to serve as measurement points for the

vibrometer. Transparency sheets were fastened to the frame to create a 10 cm tall cylinder around the arena, which prevented spiders from escaping during trials. We used a tungsten halogen light (800W bulb) to provide broad-spectrum illumination (3200K). Lab temperature was monitored using an ibutton (Maxim DS1923), and averaged 27°C, which is well within the natural temperature range experienced in the wild.

Our set-up allowed males and females to move about freely in the arena and thus interactions would be more similar to those in the wild. Males were given 15 minutes to court and attempt a mating. After this point, if a female was not paying attention to a male, or was being aggressive toward him, the trial was terminated. If the female was still watching the male's courtship display at 15 minutes, we allowed him to continue courting until the female: (1) turned away, (2) became aggressive, or (3) copulated with the male. We cleaned the arenas with 75% ethanol between use to remove any chemical cues.

3.3.3 Second Mating Trials

To assess re-mating rates, all females that mated in the trials above (N=16), as well as six additional females that mated during preliminary trials, were tested with a second male (total N=22). For each trial, we paired a novel male with a previously-mated female two days after her initial mating. All interactions were measured and recorded in the same manner as the first mating trials.

3.3.4 Visual Display Analysis

We first constructed ethograms for male and female behaviors (Table 3.1). We next used JWatcher Video (Blumstein *et al*. 2010) to score each trial. We used proportions of time spent engaged in each behavior, rather than durations because trials varied considerably in duration depending on a male's success. We also calculated the rate of 3rd leg movement in the "third-leg-wave" display, which directly corresponded to fan movement in the "fan-dance" display as well (Girard *et al*. 2011), using an average of three distinct samples of each behavior randomly selected from the beginning, middle, and end of male courtship. A male's proximity to a female was scored using four categorical ranges measured in terms of the focal female's body length (~4mm): (1) 0-5, (2) 6-10, (3) 11-15, or (4) >15 body lengths. Lastly, for females, we tallied all occurrences of aggressive events towards males.

3.3.5 Vibrational Signal Analysis

We imported the vibrometry recordings into Sony Soundforge Pro (version 10.0e) for various signal analyses. To quantify vibratory signals, we first randomly selected continuous sequences of "rumble-rumps" (Rb-Ru's), which are the primary vibratory signal given by males early on and throughout the majority of the display (Girard *et al.* 2011). For this study, we sampled three sequences across displays, and when possible, chose sequences consisting of at least five Rb-Ru's. Rumble-rumps separated by another type of male display were not considered part of the same sequence. For each sequence, we calculated the mean Rb-Ru duration, as well as the mean number and rate of Ru's within each Rb-Ru. Overall mean durations of Rb-Ru's were calculated from the mean duration of three Rb-Ru within the sequence, averaged across three different sequences.

Using JWatcher we calculated the proportion of time males were producing bouts of Rb-Ru's by summing these bouts and dividing them by the total trial time. We also calculated the

Table 3.1. Ethogram of male and female behaviors scored using JWatcher. Rows are mutually exclusive with other like-colored, adjacent rows.

Behavior	Description
MALE:	
Away	Male is moving away from female
Toward	Male is moving toward female
Still	Male is sitting still
Side-step	Male is performing the side-step display
Pre-mount	Male is performing the pre-mount display
Mate	Male is copulating with the female
Vibrate	Male is producing vibrations
Fan-dance	Male is performing fan-waving display
None	Male is not moving abdomen in any way
Fan-raise	Male's fan is raised and fan flaps are extended
Fan-down	Fan flaps are retracted and male's fan is down
Third-leg-wave	Male is performing a third-leg display
No-leg-wave	Male's third legs are on the ground
Oriented	Male is oriented at the female
Not-oriented	Male is not oriented at the female
FEMALE:	
Away	Female is moving away from male
Toward	Female is moving toward male
Still	Female is sitting still
Oriented	Female is oriented at the male
Not-oriented	Female is not oriented at the male
Aggressive	Female is attacking male
Abdomen-wiggle	Female's abdomen is moving back and forth (sometimes circularly)
None	Female is not moving abdomen in any way
PROXIMITY:	
≤ 5	Male and female are less then or equal to 5 female-body-lengths apart
6 - 10	Male and female are between 6 and 10 female-body-lengths apart
11 - 15	Male and female are between 11 and 15 female-body-lengths apart
16 - 20+	Male and female are equal to or greater than 16 female-body-lengths apart

amount of silence taken up by gaps between rumple-rumps. This allowed us to calculate a "signal-to-silence" ratio, which we defined as the mean proportion of time males were actively producing vibrations during sequences. We calculated signal-to-silence ratios by multiplying the number of Rb-Ru signals produced in each sequence by the mean Rb-Ru signal duration within that sequence, and dividing the product by the total sequence duration. Again, when possible, these values were averaged across the three sequences taken for a given male.

Only successful males made it to the final courtship stage, the pre-mount display (Girard *et al.* 2011), during which males produce two other types of vibratory signals, "crunch-rolls" (Cr-Roll's) and "grind-revs" (Gr-Rev's). For these males, we also measured: the number of Cr-Roll's produced at the beginning of the display, duration of the Cr-Roll sequence and mean duration Cr-Roll's, duration of the first and second distinct phases of Gr-Rev production, as well as number and duration of individual Gr-Rev's in the first Gr-Rev phase. We also measured the duration of the pre-mount display.

Additionally, we examined the dominant (peak) frequency and bandwidth (10dB above and below peak frequency) for each of the signals produced using custom written Matlab scripts (Mathworks Inc., v2013b.). For Rb-Ru's, we averaged peak frequency and bandwidth for nine different signals (three signals were taken from each of the three sampled Rb-Ru sequences). As males produce only a single sequence of Cr-Roll's, and Gr-Rev's, the peak frequency and bandwidth for these signals are not means, but instead were measured from a single sample. Finally, males that successfully mate with a female produce vibrations similar to Gr-Rev's continuously throughout copulation. Although we did not examine the duration of the signal (as it is closely linked to copulation duration), we did measure the peak frequency and bandwidth for a sample of these vibrations.

3.3.6 Statistical Analyses

All statistical analyses were performed using the software JMP (version 11.1.1, SAS Institute Inc., 2013) and G*Power (version 3.1.9.2) (Faul *et al.* 2007).

male behaviors/traits that predict mating success

Two principal components analyses (PC) were performed using the correlation matrix approach to standardize data and the varimax rotation method to simplify the interpretation (Kaiser, 1958; McGarigal *et al.* 2000). Components were extracted using a scree test and variables were considered to have high loadings if they had a value of ≥ 0.5 or ≤ -0.5.

The first PCA included all male traits and behaviors, as well as the various vibrational signal components (Table 3.2). We used a Generalized Linear Model (GLM) with a binomial distribution and logit link to analyze how male mating success was related to factor scores of the retained principal components (PC's). Trial date was included as a random effect.

In order to examine if/how male traits affected latency to mate, copulation duration and success in egg laying, we ran a second PCA for the subset of males that successfully copulated (N=16). This PCA contained the original explanatory values as well as signals produced by males during the pre-mount display (Table 3.3). We used two separate GLMs, each with a normal probability distribution and identity function, to look at latency to mate and copulation duration. We used a third GLM with a binomial distribution and logit link function to examine female egg-laying. For these three tests, we used Firth-Bias adjusted estimates to correct for small sample sizes (N=16).

Table 3.2. Principal Components with Varimax Rotation: includes all males (N=64), loadings with values of ≥0.5 or ≤-0.5 are bolded.

Male Behavior/Trait	Comp. A1	Comp. A2	Comp. A3	Comp. A4	Comp. A5	Comp. A6	Comp. A7	Comp. A8	Comp. A9	Comp. A10	Behavior Groupings
Fan-dance (Prop.)*	**0.947**	0.055	0.032	0.053	0.014	-0.002	0.111	0.197	0.124	0.135	Visual Effort
Third-leg-wave (Prop.)*	**0.576**	0.056	-0.012	0.040	0.068	-0.020	0.092	0.150	0.033	0.791	
Side-step (Prop.)*	**0.950**	-0.027	0.037	0.044	-0.072	0.033	0.113	0.081	0.190	0.144	
Leg-wave Rate	0.195	0.113	0.042	0.107	-0.102	-0.115	**0.935**	0.060	0.176	0.061	Visual Vigor
Vibrate (Prop.)*	0.289	0.094	-0.043	0.174	0.021	-0.055	0.187	0.008	**0.914**	0.024	Vibrational Effort
Signal-to-silence (Prop.)*	0.262	0.149	0.153	-0.026	0.108	-0.053	0.061	**0.926**	0.008	0.099	Vibrational Vigor
Mean # of Ru's	0.017	**0.801**	**0.549**	-0.041	-0.015	0.011	0.100	0.144	0.067	-0.007	Vibrational Qualities
Mean Rb-Ru duration	0.050	-0.058	**0.961**	-0.162	-0.068	0.099	0.024	0.119	-0.050	-0.005	
Mean rate of Ru's	0.022	**0.939**	-0.260	0.125	0.065	-0.010	0.063	0.069	0.057	0.047	Early
Mean Peak Frequency	0.076	0.082	-0.164	**0.961**	-0.053	0.011	0.099	-0.023	0.151	0.025	(Rb-Ru's)
Mean bandwidth (10dB)	0.022	-0.001	0.089	0.009	-0.010	**0.989**	-0.097	-0.044	-0.044	-0.011	
Male Weight	-0.033	0.044	-0.063	-0.048	-0.010	-0.010	-0.087	0.090	0.016	0.037	Size
Variance	2.332	1.585	1.360	1.019	1.018	1.009	0.987	0.979	0.955	0.683	
% Variance	19.430	13.212	11.336	8.491	8.481	8.406	8.228	8.157	7.959	5.694	
Cumulative % Variance	19.430	32.642	43.979	52.470	60.950	69.356	77.584	85.741	93.700	99.394	

* "Prop." stands for proportion of

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Table 3.3. Principal Components with Varimax Rotation: successful males (N=16), loadings with values of ≥0.5 or ≤-0.5 are bolded.

Male Behavior/Trait	Comp. B1	Comp. B2	Comp. B3	Comp. B4	Comp. B5	Comp. B6	Comp. B7	Comp. B8	Comp. B9	Comp. B10	Comp. B11	Comp. B12	Behavior Groupings
Fan-dance (Prop.)*	**0.936**	-0.023	-0.036	-0.249	0.134	-0.023	-0.078	-0.101	0.042	-0.002	-0.117	0.062	Visual Effort
Third-leg-wave (Prop.)*	**0.681**	0.086	-0.262	-0.488	0.109	-0.083	-0.140	-0.103	-0.035	-0.328	-0.069	-0.233	
Side-step (Prop.)*	**0.926**	0.138	-0.022	-0.227	0.039	-0.021	0.018	0.056	-0.231	0.063	0.066	-0.011	
Leg-wave Rate	-0.024	0.110	**-0.609**	-0.005	0.293	-0.242	0.220	0.102	0.401	-0.044	-0.485	0.106	Visual Vigor
Vibrate (Prop.)*	**-0.917**	0.274	-0.237	0.098	0.018	-0.102	0.016	0.029	0.018	0.001	-0.042	0.050	Vibrational Effort
Signal-to-silence (Prop.)*	0.350	-0.398	-0.100	-0.130	0.416	0.029	-0.474	-0.380	0.261	-0.190	-0.131	-0.110	Vibrational Vigor
Mean # of Ru's	0.015	0.350	-0.036	0.067	**0.737**	-0.159	-0.040	-0.041	**0.518**	-0.112	-0.037	-0.022	Vibrational Qualities Early (Rb-Ru's)
Mean Rb-Ru duration	0.148	0.273	-0.069	0.022	**0.874**	-0.034	-0.071	-0.051	-0.273	-0.212	-0.055	0.052	
Mean rate of Ru's	-0.146	0.180	0.043	-0.156	-0.080	-0.075	-0.143	-0.137	**0.917**	0.107	-0.003	-0.019	
Mean Rb-Ru Peak Freq	-0.086	-0.096	-0.069	0.079	-0.286	0.250	-0.063	-0.156	0.095	**0.851**	0.229	-0.012	
Mean bandwidth (10dB)	-0.104	0.103	**0.905**	-0.054	-0.048	0.042	0.057	0.073	-0.111	0.028	-0.338	0.119	
Pre-mount duration	-0.362	0.437	0.017	0.090	-0.094	-0.268	-0.184	0.099	-0.033	0.136	-0.099	**0.715**	
# of Cr-Rolls	-0.368	-0.067	0.015	**0.860**	-0.057	0.123	-0.128	0.014	-0.042	0.007	-0.196	-0.111	
Duration of Cr-Roll sequence	-0.394	0.000	0.093	**0.875**	0.181	-0.025	-0.026	-0.069	-0.108	0.062	-0.093	0.012	
Mean Cr-Roll duration	-0.155	-0.063	0.166	0.226	0.269	-0.097	**0.674**	**0.526**	-0.017	-0.063	0.015	-0.039	
# of Gr-Rev, Phase I	0.127	**0.915**	0.056	-0.061	0.239	-0.044	-0.034	-0.102	0.169	-0.146	0.003	0.064	
Duration of Gr-Revs, Phase I	-0.180	**0.917**	-0.091	-0.039	0.136	-0.135	-0.030	-0.146	0.107	0.126	-0.095	0.122	
Mean Gr-Rev duration, Phase I	-0.014	0.380	-0.331	0.067	0.254	**-0.626**	0.078	0.452	-0.058	-0.227	-0.113	0.010	
Duration of Gr-Revs, Phase II	-0.166	0.359	0.260	-0.196	-0.444	-0.411	-0.270	-0.113	0.246	0.450	0.074	0.099	Vibrational Qualities Late (Cr-Roll's & Gr-Rev's)
Peak Freq: Cr-Rolls	-0.039	-0.050	0.139	0.154	-0.054	**0.933**	0.009	-0.020	-0.093	0.159	-0.067	-0.162	
Bandwidth (10dB): Cr-Rolls	0.176	0.163	0.092	0.191	-0.075	0.272	-0.163	0.361	-0.092	**0.644**	-0.479	-0.024	
Peak Freq: Gr-Revs, Phase I	0.155	-0.211	0.076	-0.283	0.101	**0.534**	-0.173	0.413	-0.447	0.299	-0.008	-0.185	
Bandwidth (10dB): Gr-revs, Phase I	-0.078	-0.251	-0.116	-0.102	-0.129	-0.040	-0.051	-0.145	-0.126	-0.033	-0.140	0.123	
Peak Freq: Gr-Revs, Phase II	0.244	-0.079	**0.820**	0.254	0.036	0.107	0.224	-0.145	0.237	0.041	0.098	-0.174	
Bandwidth (10dB): Gr-revs, Phase II	0.003	-0.043	0.067	-0.284	-0.142	-0.044	**0.889**	-0.167	-0.127	-0.072	0.129	-0.068	
Peak Freq: Copulation vibrations	-0.039	-0.110	**0.594**	0.141	-0.061	0.275	**0.572**	-0.080	0.134	-0.264	-0.029	-0.278	
Bandwidth (10dB): Copulation vibrations	-0.010	-0.034	-0.127	-0.172	-0.059	-0.035	0.125	-0.080	-0.006	0.096	**0.944**	-0.047	
Male Weight	0.319	0.047	-0.276	-0.473	0.183	-0.238	-0.084	0.132	0.048	-0.231	-0.060	**0.644**	Size
Variance	3.971	2.831	2.733	2.599	2.241	2.230	2.169	1.942	1.935	1.930	1.710	1.261	
% Variance	14.182	10.112	9.760	9.280	8.003	7.963	7.746	6.934	6.911	6.893	6.107	4.504	
Cumulative % Variance	14.182	24.294	34.054	43.334	51.337	59.300	67.046	73.980	80.892	87.785	93.891	98.395	

- "Prop." stands for proportion of

Finally, we ran two-sided, unpaired t-tests (assuming unequal variances) to examine whether male orientation toward a female, total courtship effort, proximity of time spent at different distances from the female, and male movement patterns (motion towards or away from the female) were different between successful and unsuccessful males. Using the subset of males that successfully mated, we also ran linear regressions and two-sided, unpaired t-tests (assuming unequal variances) to investigate whether these additional male behaviors were related to mating latency, copulation duration or egg laying behavior.

female behavior

We used a logistic regression and a one tailed Fisher's exact test to determine if greater female orientation or aggression, respectively, were correlated with mating. Then, to further examine whether greater female orientation, aggression (number of attacks) or the presence of female abdomen wiggling (another behavior we observed from some females; see Table 3.1) were related the same male qualities that predict mating success, we ran GLMs using the original explanatory variables.

In order to see if a greater number of mated females were aggressive towards males than either receptive (those that went on to mate) or unreceptive (those that did not mate) virgin females, we used a one-tailed Fisher's exact test. We also tested whether mated females performed more aggressive attacks or paid less attention towards males than either category of virgin females using a one-way ANOVA. A one-way ANOVA was also used to test for abdomen wiggling (Table 3.1) behavioral differences among receptive and unreceptive virgins as well as previously-mated females. For each ANOVA, female ID was included as a random effect, and we used Tukey's HSD to determine which means were unequal between groups.

3.7 Results

We conducted 64 mating trials with virgin females, of which 16 (25%) ended with a male successfully copulating with a female. We also conducted 22 mating trials with mated females that were each presented with a second male; none of these females re-mated.

male behaviors/traits that predict mating success

Of the ten PC scores included in our first analysis (Table 3.2), PC A1, A8 and A9 strongly predicted copulation (Table 3.4; GLM: χ^2=36.08, p<0.0001). PC A1 had positive loadings for fan-dancing, side-stepping and third-leg waving, suggesting these visual displays are important for male mating success. As these behaviors cluster together in the sense that they all measure the proportion of time that visual displays are performed, A1 was labeled "visual effort". PC A8 only had a single trait load positively, the signal-to-silence ratio, our metric to quantify differences in tempo (i.e. "vibrational vigor"). PC A9 had a positive loading for the total proportion of time males spent vibrating, suggesting that "vibrational effort" is important to females. Neither female age nor trial date had an effect on male mating success and were thus subsequently dropped from all final models reported here.

For the males that successfully mated, PC B1, B4, B6 and B11 significantly (Table 3.3) predicted the latency to copulation (Table 3.5; GLM: χ^2=21.09, p<0.05). Because the power to detect differences in latency to mate was low (power= 0.067, effect size= 0.182), our results reflect Firth-Bias adjusted estimates for small sample sizes; this is also true for our tests on

Table 3.4. GLM Parameter Estimates: Male traits that predict mating success, all males (N=64) with boxes around significant components.

Term	Estimate	Std Error	L - R $\chi 2$	Prob > $\chi 2$	Lower CL	Upper CL
Intercept	-5.283381	2.2040037	31.094176	<.0001*	-11.08309	-2.158103
Comp. A1	4.1531212	1.6269194	30.288771	<.0001***	1.8119454	8.3516613
Comp. A2	0.8838637	0.6118188	2.4868273	0.1148	-0.200186	2.3284057
Comp. A3	-0.942619	0.6462091	2.4331283	0.1188	-2.391909	0.2297004
Comp. A4	-0.208505	0.4640891	0.2066781	0.6494	-1.23398	0.6975084
Comp. A5	0.3000136	0.5315127	0.3265984	0.5677	-0.737769	1.5059431
Comp. A6	-0.350398	0.5423958	0.4359202	0.5091	-1.555979	0.6773105
Comp. A7	0.8392206	0.6495979	2.0898998	0.1483	-0.26386	2.3898163
Comp. A8	1.6516908	1.068903	4.3384291	0.0373*	0.0682	4.2362017
Comp. A9	4.0609628	1.757263	15.095356	0.0001***	1.4256057	8.5700748
Comp. A10	0.1954228	0.5124449	0.1479884	0.7005	-0.791244	1.2928092

*Significant at alpha=0.05, **significant at alpha=0.001, ***significant at alpha=0.0001

Table 3.5. GLM Parameter Estimates: Male traits that predict latency to mate, only the subset of successful males (N=16), with boxes around significant components.

Term	Estimate	Std Error	L - R $\chi 2$	Prob > $\chi 2$	Lower CL	Upper CL
Intercept	1069287.4	109870.53	29.847767	<.0001*	839393.31	1299181.5
Comp. B1	-501735.1	113726.81	12.477804	0.0004**	-739698.1	-263772.1
Comp. B2	-71024.19	113726.81	0.3850357	0.5349	-308987.2	166938.8
Comp. B3	99164.362	113726.81	0.7416597	0.3891	-138798.6	337127.36
Comp. B4	393662.66	113726.81	8.8066959	0.0030*	155699.66	631625.65
Comp. B5	44608.385	113726.81	0.1530698	0.6956	-193354.6	282571.38
Comp. B6	-251521.3	113726.81	4.2334802	0.0396*	-489484.3	-13558.32
Comp. B7	96594.125	113726.81	0.704589	0.4012	-141368.9	334557.12
Comp. B8	106227.14	113726.81	0.8480296	0.3571	-131735.9	344190.14
Comp. B9	-155786.8	113726.81	1.7680284	0.1836	-393749.8	82176.227
Comp. B10	-25186.33	113726.81	0.048966	0.8249	-263149.3	212776.67
Comp. B11	-253779.9	113726.81	4.2998741	0.0381*	-491742.9	-15816.9
Comp. B12	25804.595	113726.81	0.0513954	0.8207	-212158.4	263767.59

*Significant at alpha=0.05, **significant at alpha=0.001, ***significant at alpha=0.0001

copulation duration (power=0.067, effect size=0.282) and egg laying behavior (power=0.062, effect size=0.131) summarized below. Similar to PC A1, PC B1 had positive loadings for visual effort (fan-dancing, side-stepping and third-leg waving), but negative loading for vibrational effort. This is the opposite pattern seen for the role of vibratory effort on mating success, but was largely driven by the fact that visual and vibratory displays are performed asynchronously. Consequently, for successful males that spent a majority of their time courting, engagement in one behavior diminished time available for the other. PC B4, B6, and B11 were all related to specific vibrational qualities of crunch-rolls and grind-revs, which are the late stage vibrations produced during the pre-mount display. Essentially, shorter latencies to mate were correlated with more Cr-Rolls, increased Cr-Roll duration, shorter Gr-Rev duration, and higher Cr-Roll peak frequency.

Five PC scores (B1, B2, B5, B10, and B12) from Table 3.3 predicted copulation duration (GLM: χ^2=40.80, p<0.0001, Table 3.6). Again, PC B1 significantly predicted success indicating that visual effort was important, and negatively related to vibrational effort. PC B5 and B10 both had loadings for vibrational characteristics of early stage vibrations, and PC B2, B10 and B12 had loadings for late stage vibrations. Copulation duration was positively correlated with a greater number of rumps, increased Rb-Ru duration, higher Rb-Ru peak frequency, larger Cr-Roll bandwidth, a greater number of Gr-Rev's and a longer Gr-Rev duration in phase 1, as well as a longer pre-mount duration. Finally, PC B12 also had positive loading for male weight, suggesting that heavier males were more successful. No PC scores significantly explained whether females successfully laid eggs (GLM: χ^2=11.460, p=0.4899), although copulation duration and egg laying behavior were significantly positively related (unpaired t-test: $t(9.88)$=2.02, p=0.04).

In terms of other male behaviors, total courtship effort (the proportion of time males were engaged in any display type) was greater for successful males (Figure 3.1; unpaired t-test: $t(57.16)$=-4.91, $p < 0.0001$). Successful males also spent more time oriented at females during mating trials (Figure 3.1; unpaired t-test: $t(46.82)$=4.10, p=0.0002), and more time in the closest category of proximity, $\leq$ 5 female body lengths, (Figure 3.1; unpaired t-test: $t(16.64)$=2.72, p=0.01). Lastly unsuccessful males spent a higher proportion of time moving away from females as compared to those that were successful (Figure 3.1; unpaired t-test: $t(46.12)$=-2.87, p=0.01).

Neither latency to mate, nor copulation duration were related to any of the following male behaviors: proportion of time oriented, total courtship effort, proportion of time spent in any of the different distances categories from the female; male movement patterns (motion towards or away from the female). There was also no difference in total male courtship effort towards females that went on to lay eggs versus those that did not.

female behavior

For virgin females, greater orientation to males was positively correlated with mating (r^2=0.185, χ^2=13.33, p =0.0003). Visual effort (PC A1) and male weight (PC A5) in Table 3.2 significantly predicted greater female orientation (Table 3.7; GLM: χ^2=18.90, p=.04). Additionally, females spent a greater proportion of time oriented towards males that spent a greater proportion of time in the closest proximity category (r^2=0.12, $F_{1,62}$=7.30, p=0.009).

Unlike female orientation, female aggression was expressed more by unreceptive females (Fisher's: p=.02); only 1 out of 16 (6.3%) females that mated ever attacked the male first, whereas 17 out of 48 (35.4%) females that did not mate attacked their paired male at least once as he courted. In the GLM examining which male behaviors correlated with greater female

Table 3.6. GLM Parameter Estimates: Male traits that predict copulation duration, only the subset of successful males (N=16), with boxes around significant components.

Term	Estimate	Std Error	L - R χ2	Prob > χ2	Lower CL	Upper CL
Intercept	1124994.6	54196.206	50.880221	<.0001***	1011594	1238395.2
Comp. B1	269099.03	56098.403	13.947126	0.0002**	151718.23	386479.84
Comp. B2	517219.92	56098.403	28.457697	<.0001***	399839.11	634600.72
Comp. B3	-81436	56098.403	1.9718496	0.1603	-198816.8	35944.802
Comp. B4	45260.001	56098.403	0.6371932	0.4247	-72120.8	162640.8
Comp. B5	315761.96	56098.403	17.029768	<.0001***	198381.16	433142.76
Comp. B6	-67524.37	56098.403	1.3830705	0.2396	-184905.2	49856.43
Comp. B7	2099.0909	56098.403	0.0014	0.9702	-115281.7	119479.89
Comp. B8	77059.653	56098.403	1.7773358	0.1825	-40321.15	194440.46
Comp. B9	-89017.06	56098.403	2.3276303	0.1271	-206397.9	28363.739
Comp. B10	-394300.8	56098.403	21.856677	<.0001***	-511681.6	-276920
Comp. B11	43928.372	56098.403	0.6009801	0.4382	-73452.43	161309.18
Comp. B12	212857.13	56098.403	10.092694	0.0015*	95476.33	330237.94

*Significant at alpha=0.05, **significant at alpha=0.001, ***significant at alpha=0.0001

Table 3.7. GLM Parameter Estimates: Male traits that predict female orientation, all males (N=64), with boxes around significant components.

Term	Estimate	Std Error	L - R χ2	Prob > χ2	Lower CL	Upper CL
Intercept	0.3637511	0.0231574	96.221613	<.0001***	0.3176014	0.4099009
Comp. A1	0.0718696	0.0228397	9.1418591	0.0025*	0.0263531	0.117386
Comp. A2	-0.02665	0.0226594	1.3669661	0.2423	-0.071807	0.0185076
Comp. A3	-0.001184	0.0226452	0.0027316	0.9583	-0.046313	0.0439454
Comp. A4	0.033258	0.022713	2.1054179	0.1468	-0.012006	0.078522
Comp. A5	-0.049076	0.0237599	4.1166872	0.0425*	-0.096426	-0.001726
Comp. A6	-0.012063	0.0227255	0.2810921	0.5960	-0.057352	0.0332258
Comp. A7	0.0346012	0.022626	2.2927536	0.1300	-0.010489	0.0796918
Comp. A8	-0.004197	0.0228581	0.0336988	0.8543	-0.04975	0.0413565
Comp. A9	0.0297378	0.0246705	1.4350834	0.2309	-0.019427	0.0789029
Comp. A10	-0.00884	0.0225799	0.1530733	0.6956	-0.053839	0.0361587

*Significant at alpha=0.05, **significant at alpha=0.001, ***significant at alpha=0.0001
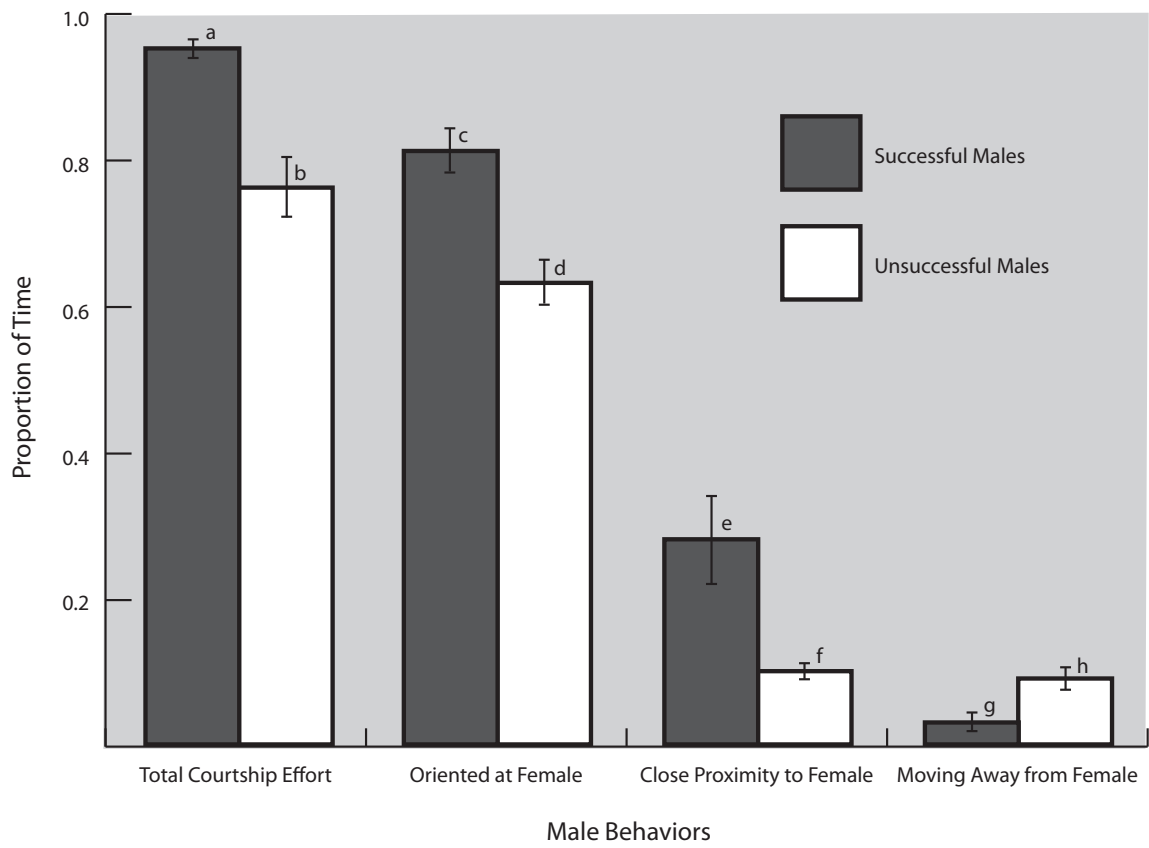
Figure 3.1. Proportion of time successful and unsuccessful males were engaged in various behaviors.

aggression, only two PC scores were significant and negatively correlated with female aggression; PC A7 and A9, which had positive loadings for leg waving rates and vibrational effort respectively (Table 3.8; GLM: χ^2=18.74, p=0.044), were significant and negatively correlated with female aggression. Males also spent less time oriented (unpaired t-test: $t(41.10)$=-4.10, p=0.0002) to more aggressive females and their total courtship effort towards these females was lower (unpaired t-test: $t(24.01)$=-3.34, $p < 0.0027$).

Females' abdomen wiggling behavior was similar to aggression in that unreceptive females were more likely to perform the display (Fisher's: p=0.04); we never saw this behavior from virgin females that went on to mate, whereas 10 out of 48 (20.8%) unreceptive virgin females abdomen-wiggled. In the GLM examining whether abdomen wiggling was associated with any male traits, we found that visual effort (PC A1), and vibrational effort (A9) were positively correlated with abdomen wiggling, similar to mating success. Additionally, PCA3 and PC A6 both had positive loadings on specific vibrational characteristics related to Ru-Rb's and were negatively (A3) and positively (A6) correlated with female abdomen wiggling (Table 3.9; GLM: χ^2=23.57, p=0.009).

Lastly, we found that previously-mated and virgin females differed in their response to male displays during our trials. When looking at female orientation across mated and both receptive and unreceptive virgin females, we found significant differences across the three groups (3.2; $F_{2,84} = 7.22$, p, 0.0015). Receptive virgin females spent a greater proportion of time oriented towards males than unreceptive virgin or mated females. However, compared to virgin females, mated females were much more aggressive; during trials, 20 out of 22 (90.9%) mated females attacked the male at least once, compared to 35.4% of unreceptive virgin females (Fisher's: p<.0001) and 6.3% receptive virgin females (Fisher's: p<.0001). Additionally the number of aggressive attacks differed significantly across these same three groups (Figure 3.2; $F_{2,84}$=37.582, p= 0.0001) with mated females performing significantly more attacks on males than either receptive or unreceptive virgins. Lastly, a greater number of mated females (14/22, 63.6%), performed abdomen wiggle displays compared to receptive (20.8%; Fisher's: p<.0001) and unreceptive virgins (0%; Fisher's: p=.0007). The proportion of time that females spent abdomen wiggling differed significantly across the three groups (Figure 3.2; $F_{2,84}$=8.46, p=0.0005) with mated females spending a significantly higher proportion of time abdomen wiggling than both receptive and unreceptive virgins.

3.8 Discussion

One of the greatest challenges in mating behavior studies is to elucidate which male traits are important to female mating decisions, especially when complex displays spanning many modalities are involved. The main objective of this research was to explore both visual motion and vibratory courtship traits of one species of peacock spider, *Maratus volans,* to better understand multi-dimensional female preferences in this system. We found that *M. volans* males use a combination of visual and vibratory signaling, and our data indicate that each modality is important for mating success (Table 3.10). Females were more likely to mate with males that put forth more visual effort, those that spent the largest proportion of time engaged in these displays. The production of vibrational signals, specifically, the proportion of time males spent vibrating and the vigor with which they signaled was also linked, although less strongly, with mating success. For females that mated, increased visual courtship effort by males was also strongly

Table 3.8. GLM Parameter Estimates: Male traits that predict female aggressive events, all males (N=64), with boxes around significant components.

Term	Estimate	Std Error	L - R $\chi2$	Prob > $\chi2$	Lower CL	Upper CL
Intercept	0.320378	0.0671341	19.210312	<.0001***	0.1865886	0.4541674
Comp. A1	-0.091587	0.0662128	1.8824153	0.1701	-0.22354	0.0403667
Comp. A2	-0.113884	0.0656903	2.9302815	0.0869	-0.244797	0.0170277
Comp. A3	0.0257648	0.0656491	0.1538227	0.6949	-0.105065	0.1565948
Comp. A4	-0.006182	0.0658455	0.0088131	0.9252	-0.137403	0.1250399
Comp. A5	0.1112117	0.0688805	2.54992	0.1103	-0.026058	0.2484815
Comp. A6	-0.100039	0.065882	2.2610726	0.1327	-0.231333	0.031255
Comp. A7	-0.132809	0.0655933	3.9611784	0.0466*	-0.263528	-0.002091
Comp. A8	0.0560247	0.0662663	0.7104127	0.3993	-0.076035	0.1880847
Comp. A9	-0.193744	0.0715205	6.9098709	0.0086*	-0.336275	-0.051213
Comp. A10	0.0258367	0.0654599	0.155576	0.6933	-0.104616	0.1562897

*Significant at alpha=0.05, **significant at alpha=0.001, ***significant at alpha=0.0001

Table 3.9. GLM Parameter Estimates: Male traits that predict female abdomen waving behavior, all males (N=64), with boxes around significant components.

Term	Estimate	Std Error	L - R $\chi2$	Prob > $\chi2$	Lower CL	Upper CL
Intercept	3.2558057	1.0233853	37.148576	<.0001***	1.7476828	3.2558057
Comp. A1	1.5116427	0.8469098	5.700619	0.0170*	0.205224	1.5116427
Comp. A2	0.9901666	0.6175398	3.5753365	0.0586	-0.031296	0.9901666
Comp. A3	-1.458135	0.8466997	5.634737	0.0176*	-3.601345	-1.458135
Comp. A4	0.4648346	0.6708322	0.5169507	0.4721	-0.760071	0.4648346
Comp. A5	-0.967048	0.639816	2.2923621	0.1300	-2.413485	-0.967048
Comp. A6	1.4503601	0.7631433	5.9666013	0.0146*	0.2399728	1.4503601
Comp. A7	1.057427	0.6580455	3.7160175	0.0539	-0.015631	1.057427
Comp. A8	-0.765539	0.6526607	1.5897149	0.2074	-2.30548	-0.765539
Comp. A9	1.0958743	0.5959337	4.0862189	0.0432*	0.0315818	1.0958743
Comp. A10	0.3098178	0.5351152	0.3770522	0.5392	-0.623716	0.3098178

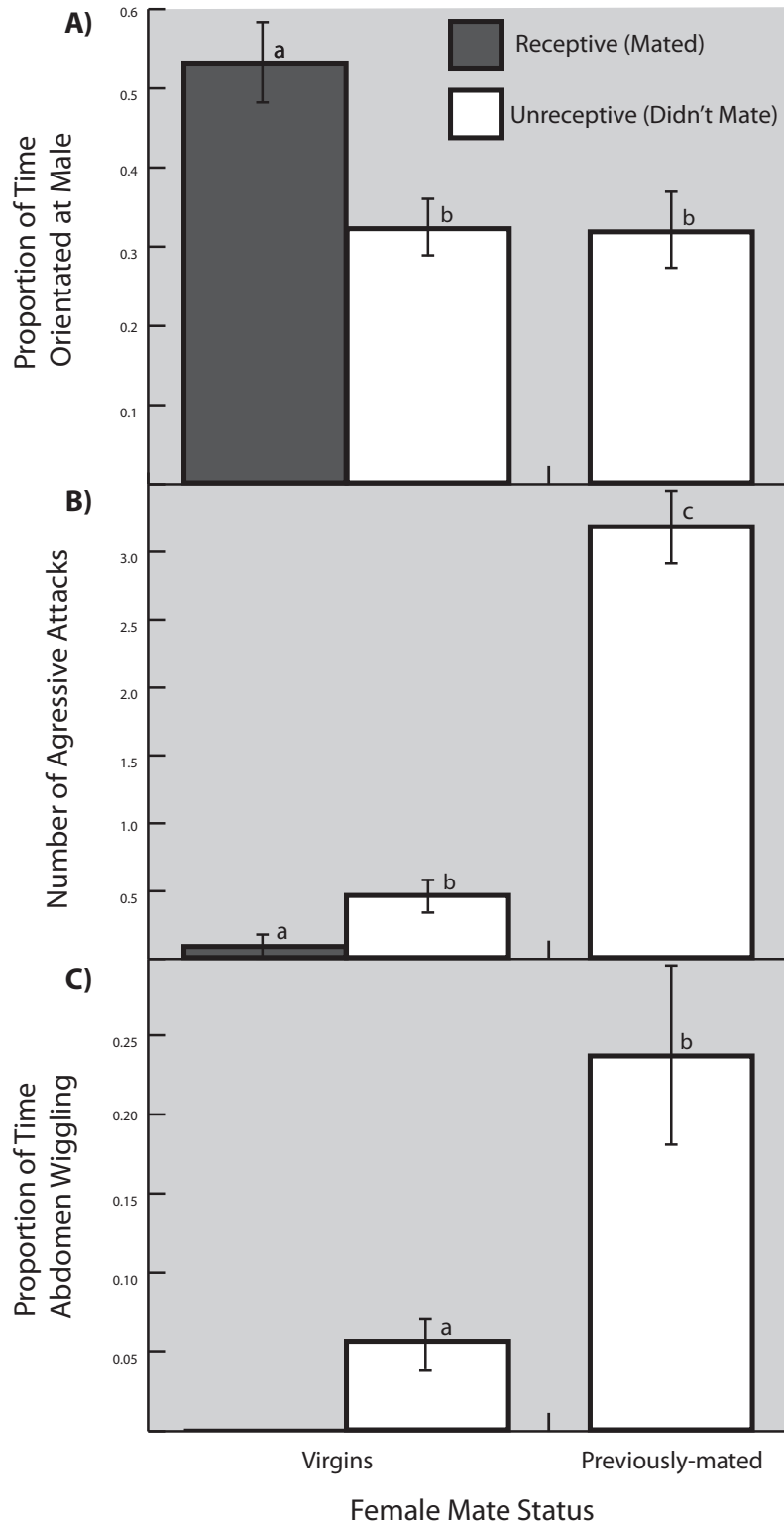*Significant at alpha=0.05, **significant at alpha=0.001, ***significant at alpha=0.0001

Figure 3.2. Female (A) orientation, (B) aggression and (C) abdomen wiggling towards males based on mate-status (virgin vs. previously-mated).

Table 3.10. A summary: various aspects of male courtship significantly affect female mating and associated behaviors. Positive and negative correlations between male traits and female behaviors are denoted with a "+" and "-" sign, respectively. The first column shows behavioral groupings of male traits according to clusters that were revealed by the PCA in Table 3.2.

	Mating Success	Mating Latency	Copulation Duration	Female Orientation	Female Aggression	Female Abd. Wave
Visual Effort	+	-	+	+		+
Visual Vigor					-	
Vibrational Effort	+	+	-		-	
Vibrational Vigor	+					+
Size			+	-		

correlated with reduced latency to mate, and increased copulation durations. Although we found no relationship between visual courtship effort and egg production, copulation duration and egg laying were highly positively correlated.

The finding that visual effort explained more than twice the variance in male mating success than either vibrational effort or vigor suggests that in *M. volans* visual signaling modalities are more important for success (e.g. Papke *et al*. 2007). Overall though, females prefer males that excelled at multiple aspects of their performance (total courtship effort). Successful males were also more persistent, continuously moving toward the female to stay in close proximity and maintain constant visual contact with her (Figure 3.1). These results support previous research that courtship effort and/or motor performance may be better indicators of male quality than individual trait elements (Barske *et al*. 2011; Byers *et al*. 2010; Shamble *et al*. 2009) Alternatively, it may be the male's ability to keep a female interested that matters most and greater courtship effort across modalities prevents habituation to male displays.

For peacock spiders, female orientation was especially informative as a metric of female preference. Many of the same characteristics that predicted male mating success also predicted the attention males garnered from females. Females demonstrated aggressive behaviors when unreceptive, particularly when males made less vibratory effort and performed leg waving at lower rates. The other female behavior we scored, abdomen wiggling, was exclusively performed by females that did not mate, thus we think this female display is an anti-receptivity signal to males, akin to that of other taxa (Blackenhorn *et al*. 2000; Rowe, 1992; Schnell *et al*. 2015). A female may benefit from deterring unworthy mates from continuing their efforts as displaying males are potentially much more conspicuous to predators, drawing unwanted attention her way (Su and Li, 2006). This form of feedback is perhaps also important to males, in that they may tailor their behavior to better avoid costs associated with courting an unreceptive female (Baena and Eberhard, 2007; Linley and Hinds, 1975), specifically, wasted time, lost energy and in some cases, death.

Aspects of both major hypotheses for the evolution of multi-modal signaling are consistent with our results. As we found many suites of correlated traits across males, our study provides at least partial evidence for the *redundant signal hypothesis*. Some of these correlations were unsurprising because certain male displays are often produced in conjunction (i.e. third-leg waves, fan-dancing and side-stepping), albeit using different independent morphological structures. Beyond these correlations though, there appear to be several other traits with tight covariance across modalities (i.e. vibrational effort with visual vigor). On the other hand, even though many of the visual and vibrational traits we looked at were highly correlated, when separated into disjoint sets using varimax rotation, traits primarily segregated by modality, and each modality independently predicted mating success. The independence of each modality may suggest that each offers unique information about distinct aspects of male quality as predicted by the *multiple messages hypothesis* (Hebets and Papaj, 2005; Jacob *et al*. 2011; Johnstone, 1996; Moller and Pomiankowski, 1993). Further support for this hypothesis comes from the fact that individual vibrational signaling qualities affected each stage of the mating process (copulation, latency, duration, egg laying) in a different way.

For this study, we were unable to measure the complex abdominal fan ornamentation of *M. volans* males (i.e. size, shape, reflectance). This is because the small size of the fans and complex color patches precluded the use of a traditional spectrophotometer. Future work using a hyperspectral camera (Garcia *et al*. 2015) will avoid these problems and will allow us to (1)

investigate how variation in color traits affects female preferences, (2) explore patterns of correlation between color and other aspects of courtship, and (3) examine if ornamentation patterns act as independent signals or if they serve predominantly as amplifiers for other elements of courtship displays, as observed in the more simply ornamented *Schizocosa* wolf spiders (Hebets, 2005). In peacock spiders, males are much more likely to perform vibratory displays when females are not looking at them (Girard *et al*. 2011), suggesting that vibrations may serve to capture a female's attention, and direct her focus toward other more salient visual signals.

Our research demonstrates that, in peacock spiders, sexual selection in the form of female preference acts on complex groupings of correlated and non-correlated suites of male traits. At present, our data best support theoretical models that predict the optimal coding strategy for receivers is a combination of redundancy and multiple messages, as this allows for robust yet efficient processing of complex information (Ay *et al*. 2007). In this study, low mating rates and no evidence for multiple mating in *Maratus volans* suggest that selection on males of this group is strong. Many *Maratus* species are found in sympatry and robustness may be especially vital for these spiders, or in other systems where mating errors are likely to come at a high cost. There is already at least some evidence from insects that multi-modal signals facilitate more quick and reliable decision-making (Balkenius and Dacke, 2013; Kulachi *et al*. 2008; van Doorn, and Weissing, 2004). In a mate selection context, these types of benefits may outweigh potential costs associated with having multiple signals (Partan and Marler, 2005; Roberts *et al*. 2007). However, experimental manipulations of male signals and work on male quality are still needed in order to more accurately evaluate and distinguish between existing hypotheses related to the evolution of complex displays. Across taxa, surprisingly few studies exist quantifying trait combinations that predict mating success in semi-natural contexts. We advocate for an increase in these types of studies in order to better place empirical manipulations of signaling behavior into their proper context and to help address hypotheses on signal evolution and function.

3.9 Acknowledgements

Chapter 4: What makes males red-hot? Longer wavelengths may mean doodly-squat for mating success in peacock spiders[4]

4.1 Abstract

Research on animal signaling enhances our understanding of broad scale links between sensory processing, decision-making, behavior, and evolution. Studies of sexually-selected signals may be particularly informative as mate choice provides access to decision patterns in the way that male courtship leads to an easily observable behavioral output in females, i.e. mating. Male peacock spiders have some of the most elaborate and varied courtship displays known among animals. Particularly striking to human observers is the diversity of red, orange and yellow ornaments that males exhibit across the genus. The primary objective of our research was to investigate how visual ornaments interact with vibratory displays to affect female mating behavior of one species, *Maratus volans*. Accordingly, we conducted mating trials under a series of experimentally manipulated vibratory and lighting conditions. Contrary to expectation, chromatic characteristics of longer wavelength ornaments are not driving female mate choice decisions, despite their extensive presence on male fans. Instead, our results suggest that achromatic contrast may be important to females. This study illustrates, as researchers, we must be careful not to inflate the importance of particular animal signals according to our own biases, their prominence and/or prevalence. Additionally, we found that vibratory signals were not necessary and did not increase mating rates. Our study demonstrates the intricacies inherent in complex signaling systems and suggests that individual signaling elements may evolve to serve different functions inherent to the process of mating.

4.2 Introduction

For many animal systems, the manner in which females evaluate male courtship signals is unknown. Empirical work is needed to improve upon existing models for decision-making in mate choice, but designing experiments to elucidate male traits of interest to females is difficult. This is especially true for complex signals spanning multiple modalities (multi-modal signals), where it is difficult to tease apart the role of each type of signal. However, in systems where male courtship displays are characterized by multi-modal signals, determining how females assess different signaling modalities is critical to understanding how sexual selection shapes male phenotypes.

The challenges of studying multimodal signals are further compounded by the inherent subjectivity of observing animal signals through a human lens, especially when our knowledge of the perceptual capabilities of the animal in question is limited. Displays and features that stimulate our eyes and ears grab our attention such that we forget prominent features to human observers might not be those most salient to the focal organism. In fact, the most relevant components of signals may escape our notice entirely. For example, many animals utilize visual features that are invisible to us, such as polarization (Chiou *et al*. 2008; Foster *et al*. 2014), and UV coloration (Cronin and Bok, 2016; Hausmann *et al*. 2003; Hogg *et al*. 2011; Xu and Fincke, 20015). Additionally, the bulk of our planet's animal diversity is composed of species that utilize modes of communication completely imperceptible to humans, including: substrate-borne signals (Aiken, 1985; Cocroft and Rodriguez, 2005; Ladich, 2015; Sueur *et al*. 2011), water-borne signals (Parmentier *et al*. 2017; Patek and Oakley, 2003; Popper *et al*. 2001), near field particle signals (Cator *et al*. 2009; Heidelbach *et al*. 1991; Santer and Hebets, 2008; Shamble *et al*. 2016), and/or chemical signals (Cardé and Millar, 2004).

Even in systems where we understand something about the physiological capabilities of animals and can measure the pertinent signals, it is still easy to draw erroneous conclusions about how different aspects of these signals are utilized and processed. For example, recent work on mantis shrimp overturned the existing assumption that these exceptionally colorful stomatopods also have remarkable color vision. Despite having four times the number of photoreceptor types as humans, the way mantis shrimp process color is different from our color opponency system, and only allows for coarse color discrimination at best (Thoen *et al*. 2014). Another example comes from research on the fly *Ormia ochracea*, which revealed they use very different mechanism of sound localizing than was expected based on how other, larger auditory animals accomplish such a task (Miles *et al*. 1995; Robert *et al*. 1996). These studies, like so many others (Mason *et al*. 2001) highlight the necessity of behavioral data to test hypotheses about signal use across taxa.

Peacock jumping spiders of the species-rich *Maratus* genus present an ideal system for examining the role of complex signaling traits in female choice of mates. During courtship, a male peacock spider will unfurl colorfully-patterned flaps attached to his abdomen, which he waves at a female in coordination with an ornamented third pair of legs (Girard *et al*. 2011). The extensive use of visual signals by male peacock spiders is not surprising because jumping spiders (Family: Salticidae) are widely considered visual specialists and have many adaptations that allow them to approach the physical limit of optical resolution for their compact size (Land, 1985; Land, 1969; Nagata *et al*. 2012; Zurek *et al*. 2012; Zurek *et al*. 2010). Behaviorally, visual traits have been demonstrated to play an important role in courtship of many jumping spiders (Forster, 1982; Jackson, 1981; Uhl and Elias, 2011). In *M. volans*, aspects of male displays were

shown to predict mating success (Girard *et al*. 2015), although it is not known if male coloration contributes to this outcome. Studies of the effects of color in other species of jumping spiders have generated equivocal results (Lim *et al*. 2007; Taylor and McGraw, 2013). Moreover, physiological studies investigating color vision in salticids indicate that there is interspecific variation in the number and spectral sensitivities of photoreceptors, although most are believed to be UV-green sensitive dichromats (Blest *et al*. 1981; DeVoe, 1975; Nagata *et al*. 2012).

 In addition to complex ornaments and displays, *Maratus* males employ vibratory signals during courtship, as do males in several other groups of jumping spiders. Some genera use simple substrate-borne vibrations (*Phidippus;* Elias *et al*. 2014; Elias *et al*. 2008) while others evolved more complex vibratory displays (*Habronattus:* Elias *et al*. 2012; Elias *et al*. 2003; *Saitus:* Gwynne and Dadour, 1985; and *Maratus:* Girard *et al*. 2011*)*. The few studies that have examined the impact of vibratory signals on female mate choice determined that these signals are essential for males to achieve copulation (Elias *et al*. 2016; Elias *et al*. 2005; Sivalinghem *et al*. 2010). Although previous work on *M. volans* identified several aspects of male vibrational signals that predict mating success, elements of motion displays by males explain more than twice the variance in mating success as any aspect of vibrational signals, suggesting that visual signaling modalities are dominant in this species (Girard *et al*. 2015). To date, however, no studies of *Maratus* have examined the impacts of vibrational and visual signals in isolation or how these two courtship elements interact in combination to affect mating. Is the presence of both modalities required for, or possibly serve to amplify, mating success? Instead, perhaps each modality can compensate for the other in diverse signaling environments.

 Here, we examine how interactions between different signal modalities during courtship affect mate choice by female *M. volans*. In particular, we focus on vibrations and long wavelength ornaments (575-700 nm), which exemplify the major axes of visual signaling diversity across this genus. Unlike most other jumping spiders studied thus far, *Maratus* appears to have evolved sensitivity to long wavelengths (Morehouse *et al*., *in prep*.), but the role of this sensitivity in mate choice remains untested. To explore the impact of long wavelength visual signals on female mate choice to assess the relative contributions of visual and vibratory signals to male fitness, we compared patterns of male mating success under various vibratory and lighting conditions. In addition to generating new insights into the mate choice decisions of females, our analyses reveal how interactions between signaling modalities may vary across taxa, thereby highlighting the importance of assessing multiple sensory modalities when evaluating the impact of female choice on signal evolution.

4.3 Methods

4.3.1 Study animals

 M. volans specimens were collected from four locations around Sydney, New South Wales from 15 September to 11 November, 2015 and, 13 September to 11 November, 2016. Live spiders were brought back to the laboratory, where they were housed individually with leaf litter from their environment and kept on a natural 14:10 light:dark cycle. Spiders were fed a diet of fruit flies (*Drosophila melanogaster*) and occasionally pinhead crickets (*Acheta domestica*). Only females that were collected as juveniles and that matured in the lab were used in this experiment so that we could ensure their virgin mating status.

4.3.2 Experimental Design

To assess the effects of vibratory and visual cues on female mating decisions, we used a 2x2 factorial design to conduct mating trials under variable vibratory and lighting conditions. In order to modify the chromatic properties of male signals, trials were conducted in chambers with one of two illumination types (Figures 4.1 & 4.2): broadband (full spectrum light: **FS,** 400-700nm) or lighting with longer wavelengths removed (minus red light: **MR,** 400-575nm); a more detailed description of the illumination chambers is provided in the "experimental manipulations" section below. Under each type of lighting, we also varied the vibratory environment of our mating arenas (again, see "experimental manipulations" section below for more details). The two mating arena types used in our trials either propagated all male signaling frequencies (vibration: **V)** or attenuated all vibrations (no vibration: **NV)**. Our four treatments were thus labeled as follows: full spectrum-vibration (**FS-V)**, full spectrum-no vibration (**FL-NV)**, minus red-vibration (**MR-V)**, and minus red-no vibration (**MR-NV)**. This design allowed us to distinguish between the importance of vibratory signals and chromatic signals in dictating female mating behavior.

By truncating the longer wavelengths of the visible spectrum for our **MR** treatments, we greatly reduced the total level of lighting in the arenas compared to the **FS** treatments (Figure 4.2); the latter were 1.76 times brighter. To address this potential confounding factor, once initial mating trials were complete, we conducted a fifth manipulation to test whether the differences in mating rates detected in the first experiment were related to the color composition of the lighting environment or the overall brightness of the chamber illumination. In this additional treatment, the **MR** condition was the same in terms of wavelength range (400-575nm) but the brightness (area under the curve) was adjusted to be similar to that of the full spectrum treatment (Figure 4.2, increased brightness: **+MR**); vibrations were not manipulated and thus we labeled this treatment **+MR-V**.

4.3.3 Mating Trials

Mating trials proceeded as follows: two to three weeks post maturation, each female was randomly paired with a mature male and assigned to one of the treatment groups. Immediately prior to each trial, vibratory arenas were placed inside the cylinders, which were then covered with blackout cloth to ensure that our setups were completely sheltered from all other ambient light. All mating trials (N=175, 35 for each treatment) were conducted between 09:00–17:00 hrs; temperature in the arena was monitored using ibuttons (Maxim Integrate Thermochron iButton) to ensure that all experiments were run under approximately the same ambient temperature (26 ± 1.0412 °C). Trials were allowed to run for 15 minutes (we ensured that all males made courtship attempts during this time) and we recorded interactions between the pairs using a Go Pro (Hero 4) mounted to the top of each lighting chamber. In between use, our arenas and chambers were cleaned with 85% ethanol to remove any potential chemical cues remaining from previous trials. Whenever possible, females were paired with males collected from the same population (only 16/175 females were paired with males from a different collection location).

4.3.4 Experimental Manipulations

We used irradiance measurements (obtained with a Jaz, *Ocean Optics* Inc.) from the shady understory of Eucalyptus forests, *Maratus volans'* native habitat, to reconstruct the approximate spectrum and brightness of natural illumination conditions. Light treatments were created using Radion XR15w Pro lamps which consisted of tunable arrays of LED's across a
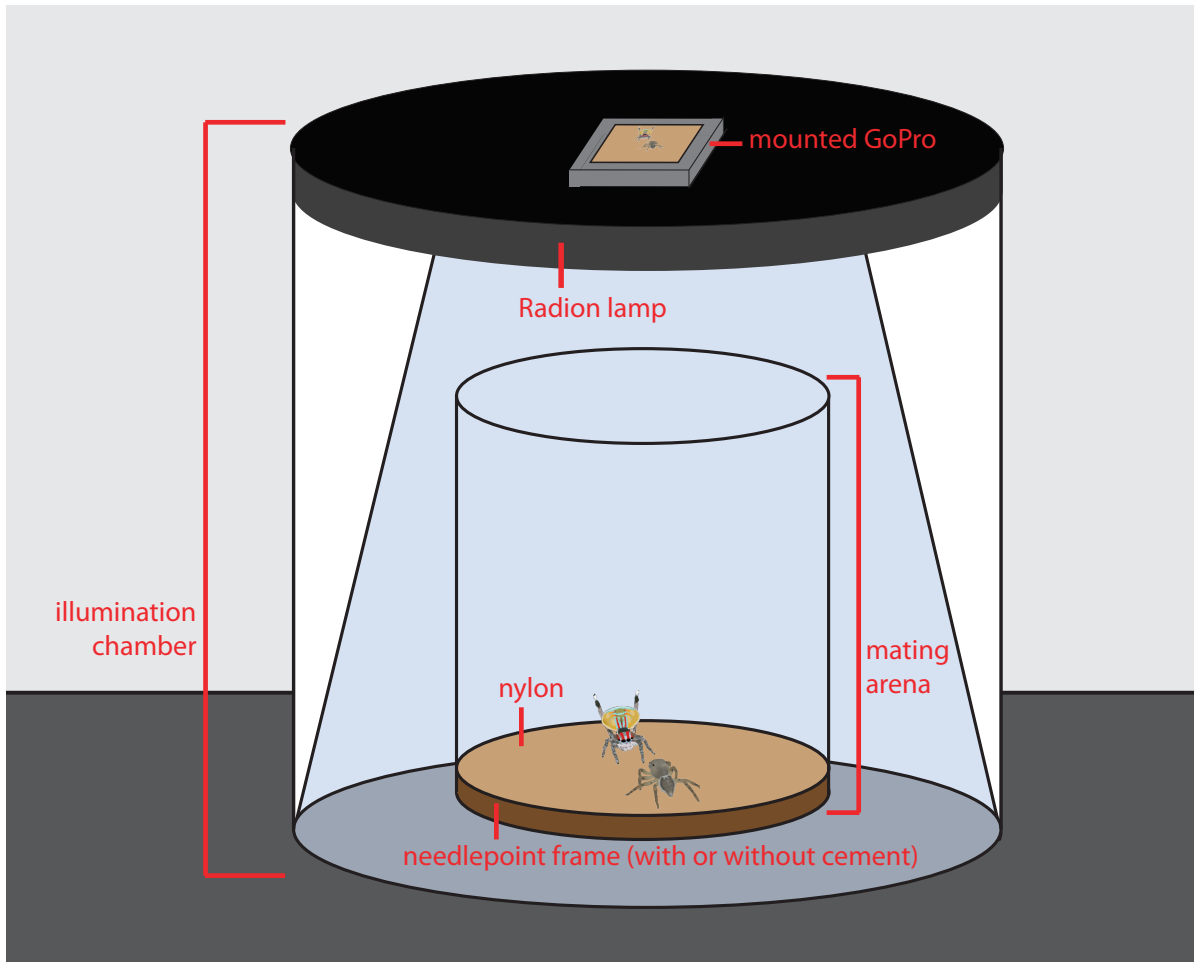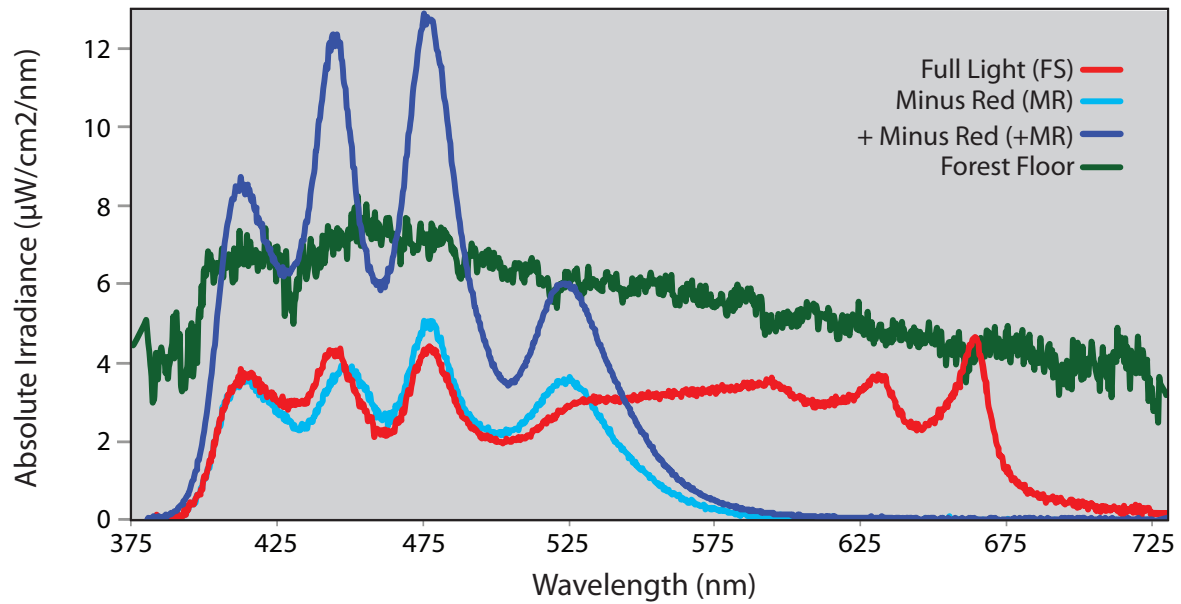
Figure 4.1. Experimental set-up.

Figure 4.2. Irradiance spectra across natural and experimental conditions: lighting conditions (at noon) of the natural environment of *M. volans* (green)*;* experimental lighting condition for the Full Spectrum (**FS**) light treatment (red); experimental lighting condition for the Minus Red (**MR**) light treatment (light blue); experimental lighting condition for augmented brightness Minus Red (**+MR**) light treatment (dark blue).

broad spectrum of wavelengths. Six cyan (490-520nm) LED's were used to augment the light provided by these lamps and help smooth the middle (green) part of each spectrum. LED arrays were mounted aerially inside lighting chambers made from cardboard cylinders (r= 12.5cm, h=30.48cm) spray painted matte white. The removal of the long wavelengths from ambient illumination affected color patches that reflect light in the 575-700 nm portion of visible spectrum (yellow, orange and red ornaments; Figure 4.3), presumably reducing the ability of females to detect these signals or to distinguish differences in these patches between individual males. While we were not able to recreate the UV portion of sunlight, all treatments were equal in this regard and thus should have affected female behavior equally in all trials.

In the treatments that allowed vibratory communication by males (**FS-V** and **MR-V, +MR-V**), our arenas consisted of nylon fabric stretched over a circular wooden needlepoint frame (diameter: 10 cm) and surrounded with white Teflon sheets to prevent spiders from leaving the arena. Nylon was used as the signaling substrate because it passes relevant frequencies with minimal distortion (Elias and Mason, 2014). The arena for our non-vibration treatment groups (**FL-NV** and **MR-NV**) used wooden needlepoint frames filled with cement. Nylon fabric was also stretched over these frames to replicate the background color and texture of the vibratory arenas; because the nylon was directly in contact with the cement, however, it was unable to move freely and thus drastically attenuated vibrational signals (Figure 4.4: muted/non-muted spectral display and video demonstrating audio difference as well as). To remove any effects of potential vibrational noise in the room during our trials, we always ran similar vibration treatments concurrently. For example, **FS-NV** trials were run simultaneously with **MR-NV** trials and **FS-V** trials were run simultaneously with **MR-V** trials.

4.3.5 Measuring Male Color

In order to understand which color patches of male *M. volans* would be affected by our lighting manipulations, we used a SOC710 hyperspectral imaging system (Surface Optics Co., USA) with 128 channels (bands) to collect spectral data from their fans. Pinned specimens of dead male *M. volans* were imaged under a broadband 800W tungsten halogen photographic light (StudioPRO). The camera was operated using a PC laptop and the *Lumenera* software v.6.3.0 provided by the manufacturer. The integration time for image capture was 300 ms, which was determined to work best with our illumination levels. The raw hyperspectral image cube (hypercube) generated by the system's CCD sensor was calibrated to express camera responses for each pixel in radiance units ($mW \cdot cm^{-2} \cdot nm^{-1} \cdot sr^{-1}$) using a dark image reference file (recorded immediately after photographing each spider), and a calibration file provided by Surface Optics. Absolute reflectance data (this imaging system detected wavelengths between 380-750nm) for each pixel location was then reconstructed using the manufacturer's spectral radiance analysis software with an additional light reference calibration step. The light reference consisted of a pixel sample area from a Munsell grey N5 panel that was positioned next to the focal spider in every photo. See Garcia *et al*. (2015) for similar methods. For all individuals imaged (N=21), two non-overlapping pixel samples within each color patch on a male (Figure 4.3a) were randomly selected for analysis. When possible, the samples analyzed were sized to be approximately 10 X 10 pixels, although some samples (e.g., patch F in Figure 4.3a) were necessarily smaller. Finally, all spectral data were exported into excel where samples were averaged within and then across individuals (N=21) to generate Figure 4.3b.
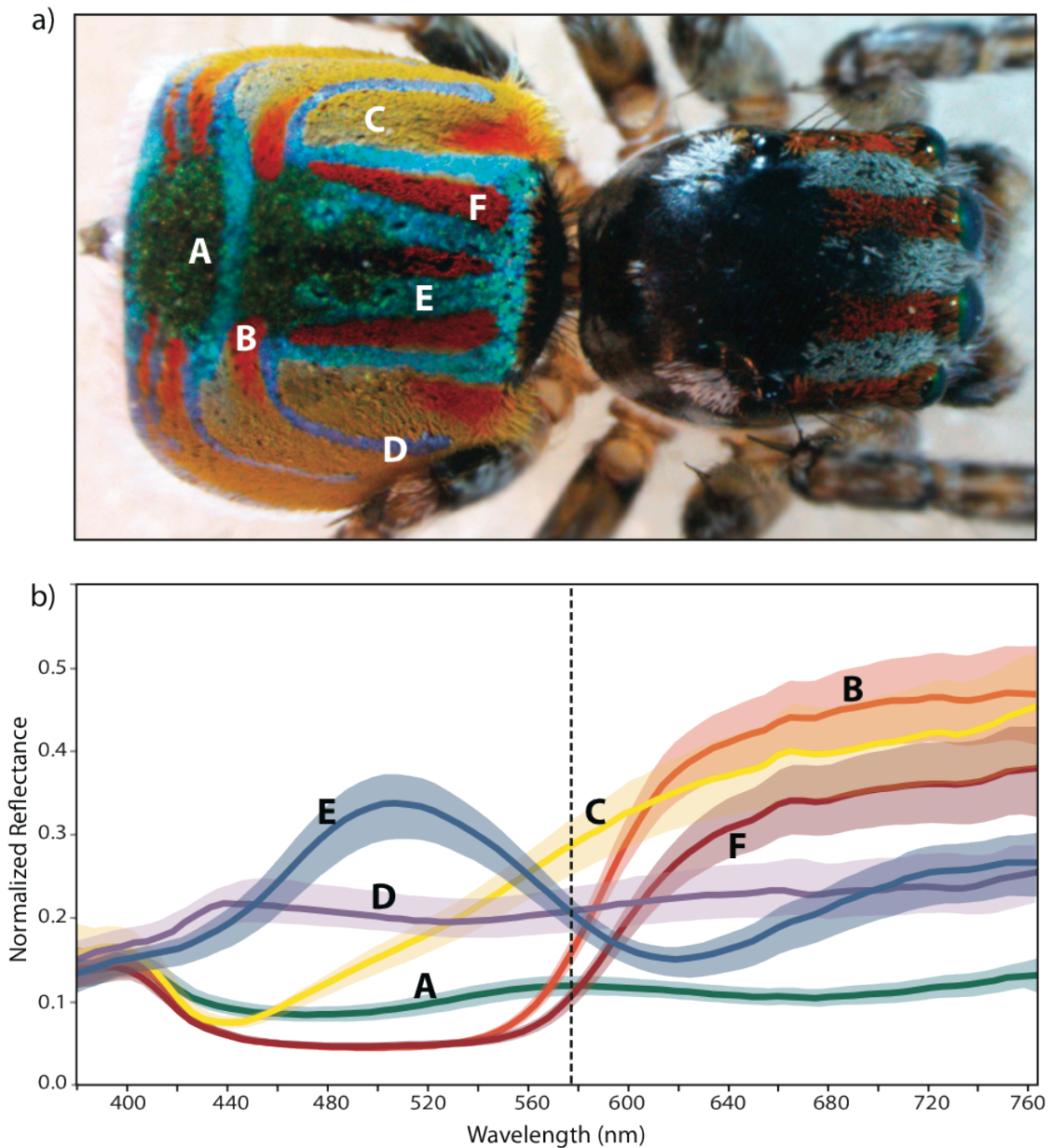
Figure 4.3. *M. volans* fan ornamentation coloration. (a) color patches lettered A-F correspond to reflectance curves A-F in (b). Wavelengths to the right of the dotted line in (b) are those that were removed in our MR treatments. Data shown is averaged for N=21 individuals, and the shaded region around each curve reflects standard error for each wavelength.
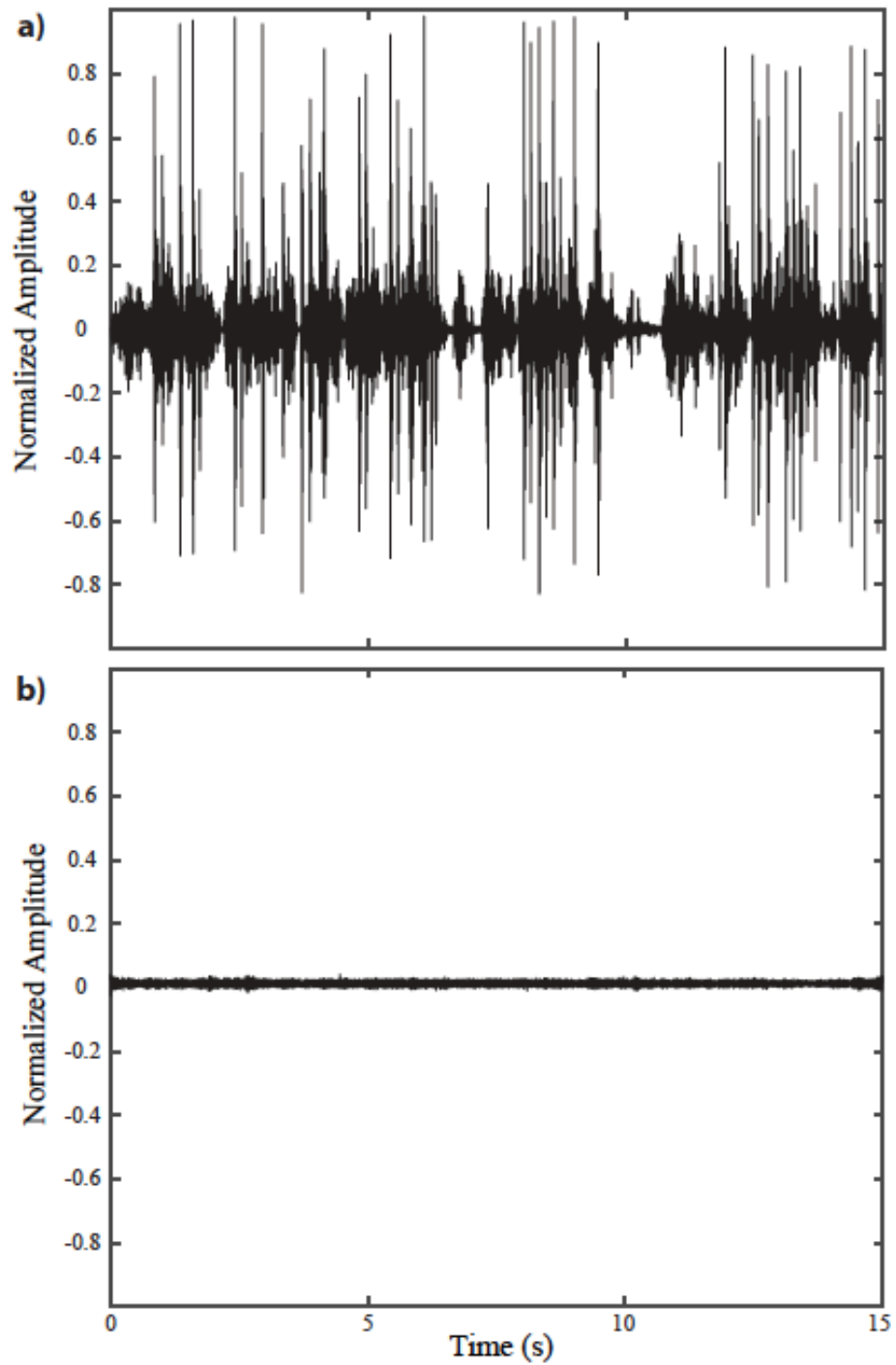
Figure 4.4. Waveforms of *M. volans* vibratory displays in both the a) Vibration treatment and b) No-Vibration treatment arenas. These waveforms accompany videos S1 and S2 provided in the supplementary data section.

4.3.6 Scoring Behavior

We constructed ethograms for both males and females. We then used the JWatcher Video software package (Blumstein *et al.* 2010) to score each trial from the original four treatments. For males, we recorded the proportion of time spent engaged in visual displays such as fan dancing or leg waving (Girard *et al.* 2011). To quantify the proportion of time that males spent vibrating, we identified this behavior based on the abdominal movements of males (Girard *et al.* 2011) that were visible on recordings. Both visual and vibratory courtship displays are important for male mating success (Girard *et al.* 2015), thus we wanted to examine whether male behavior was similar across treatment groups or if there were any differences in male courtship under separate lighting and vibratory conditions that would account for any differences we saw in mating rates. For females, we recorded the proportion of time spent oriented at males, as well as any instances of aggression and abdomen wiggling. All three of these female behaviors correlate with female mating receptivity, positively in the case of orientation, and negatively with aggressive attacks or abdomen waiving (Girard *et al.* 2015). For pairs of spiders that mated, we also scored latency to mate and mating duration.

4.3.7 Statistical analyses

Statistical analyses were performed using JMP (v.13.0.0, SAS Institute Inc., 2016). To examine differences in mating rates across treatments, we used a nominal logistic regression, with population, temperature, trial date and time as random effects. For pairs that mated during one of the original four treatment groups, we used one-way ANOVAs to determine if treatment type affected latency to mate or copulation duration. We also used one-way ANOVAs to determine if male behavior was consistent across lighting and vibrational regimes and to assess whether female behavior changed under different treatments. Finally, we used unpaired *t*-tests (assuming unequal variances) to investigate whether the same aspects of male and female behavior that were found previously to be correlated with mating success (Girard *et al.* 2015) were important in our study. Similarly we used two separate GLMs, each with a normal probability distribution and identity function, to examine whether latency to mate or copulation duration predicted by male behavior.

4.4 Results

We completed a total of 175 mating trials, 86 (49.1%) of which ended with a male successfully copulating with a female. Population of origin, temperature, trial date and time had no effect on mating rates and thus these parameters were dropped from all subsequent analyses.

As predicted, the number of successful copulations differed significantly among the five treatment groups (Figure 4.5; χ^2=10.760, df=4, *p*=0.0294). Of the original four treatment groups, **FS-V** had the greatest mating rate (65.7%); this rate was significantly higher than that for both reduced spectrum (**MR**) treatment groups (**MR-V**: χ^2=7.033, *p*=0.0080; **MR-NV**: χ^2=5.780, *p*=0.0160), which had mating rates of 34.3% and 37.1%, respectively. Between MR treatments, there was no significant difference in mating rate for trials with and without vibratory signals (MR-V versus MR-NV; χ^2=0.062, *p*=0.8030). The final treatment group (**FS-NV**) did not differ significantly from any of the other treatment groups (**MR-NV**: χ^2=0.936, *p*=0.3334; **FS-V**: χ^2=2.112, *p*=0.1462; **MR-V***: χ^2=1.478, *p*=0.2241). Interestingly though, this **FS-NV** treatment groups, **FS-V** had the greatest mating rate (65.7%); this rate was significantly higher than that for
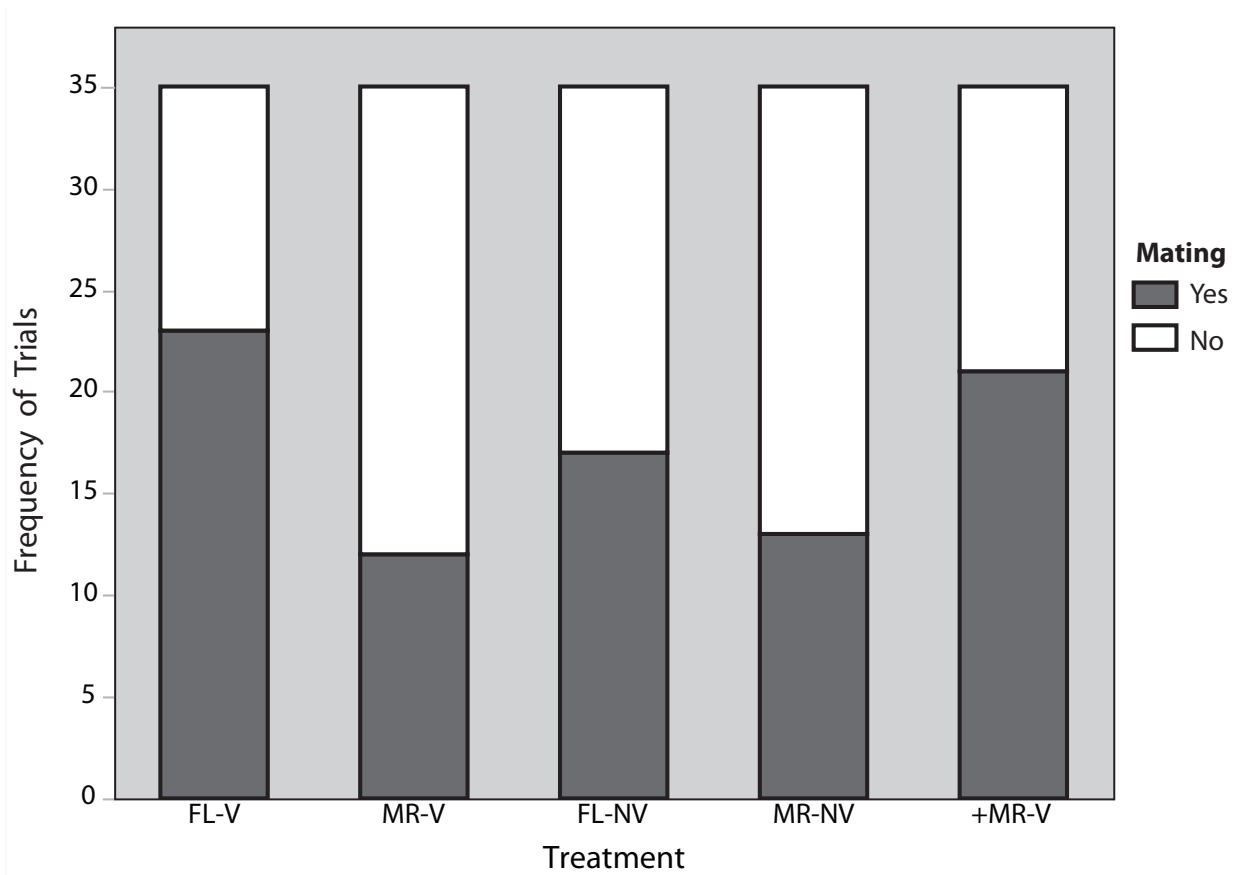
Figure 4.5. Number of males that mated in each experimental treatment: Succesful matings (dark grey) vs. non-successful matings (white) for each of the five treatments groups (Full spectrum–Vibration, Minus Red–Vibration, Full spectrum–No Vibration, Minus Red–No Vibration, Augmented Brightness Minus Red–Vibration). Letters above bars indicate significant statistical differences between treatment groups.

both reduced spectrum (**MR**) treatment groups (**MR-V**: χ^2=7.033, p=0.0080; **MR-NV**: χ^2=5.780, p=0.0160), which had mating rates of 34.3% and 37.1%, respectively. Between MR treatments, there was no significant difference in mating rate for trials with and without vibratory signals (MR-V versus MR-NV; χ^2=0.062, p=0.8030). The final treatment group (**FS-NV**) did not differ significantly from any of the other treatment groups (**MR-NV**: χ^2=0.936, p=0.3334; **FS-V**: χ^2=2.112, p=0.1462; **MR-V**: χ^2=1.478, p=0.2241). Interestingly though, this **FS-NV** treatment had a mating rate (48.6%) that was intermediate to the **FS-V** and both **MR** treatments, suggesting that there may be some reduction in female receptivity when vibrational signals are reduced.

Surprisingly, overall brightness levels appeared to be the major factor impacting mating rates during our trials, and not the specific chromatic characteristics of the ambient illumination. Specifically, the increased brightness of the **+MR-V** treatment versus the **MR-V** treatment resulted in a significantly greater number of matings (χ^2=4.697, p=0.0302). We observed a 60% mating rate in our **+MR-V** treatment, which is comparable to the mating rate we observed in the **FS-V** treatment group.

For the subset of spiders that did mate during trials, we found no difference across the original four treatments with respect to the latency to mate ($F_{3,62}$=1.694, p=0.1780) or the duration of copulations ($F_{3,61}$=0.159, p=0.9232). However, regardless of treatment we found that both the proportion of time that males spent displaying visually and the proportion of time spent vibrating (including the pre-mount display) strongly predicted mating latency (χ^2=39.854, df=59, p<0.0001), with a negative relationship between the amount of time engaged in both signal types and the interval to mating. The amount of time that males spent dancing also predicted copulation ($F_{3,134}$=17.269, p<0.0001). Together, these data suggest that successful males spend a greater amount of time engaged in courtship displays, which is consistent with previous data for this species (Girard *et al*. 2015).

We found no difference in the proportion of time that males engaged in visual ($F_{3,134}$=0.302, p=0.8240) or vibrational ($F_{3,134}$=1.282, p=0.2832) signaling across the original four treatment groups (Figure 4.6). Looking at only the short wavelength lighting treatments (**MR-V** and **MR-NV**), however, revealed a significant difference in the proportion of time that males spent vibrating (Figure 4.7: $F_{1,67}$=3.099, p=0.0415), with males vibrating more on arenas with the unmanipulated nylon substrate than on those that dampened vibrations using concrete. This suggests males may alter their activity in response to their substrate or in response to female behavior when vibrations are present.

Lastly, the proportion of time that females spent oriented ($F_{3,134}$=1.0646, p=0.3666) in the male's direction, the number of aggressive responses toward males ($F_{3,134}$=0.897, p=0.4449), and the occurrence of abdomen wiggling ($F_{3,134}$=1.653, p=0.1805) did not differ with respect to treatment. However, across the original four treatments, females that mated during our trials performed significantly fewer aggressive attacks than females that did not mate (Figure 4.8: $F_{3,134}$=17.029, p<0.0001).

4.5 Discussion

In systems where complex displays spanning many modalities are involved, it remains a great challenge to elucidate which male traits are important to female mating decisions. Contrary to expectation collectively our data indicate that chromatic aspects of long wavelength (LW)
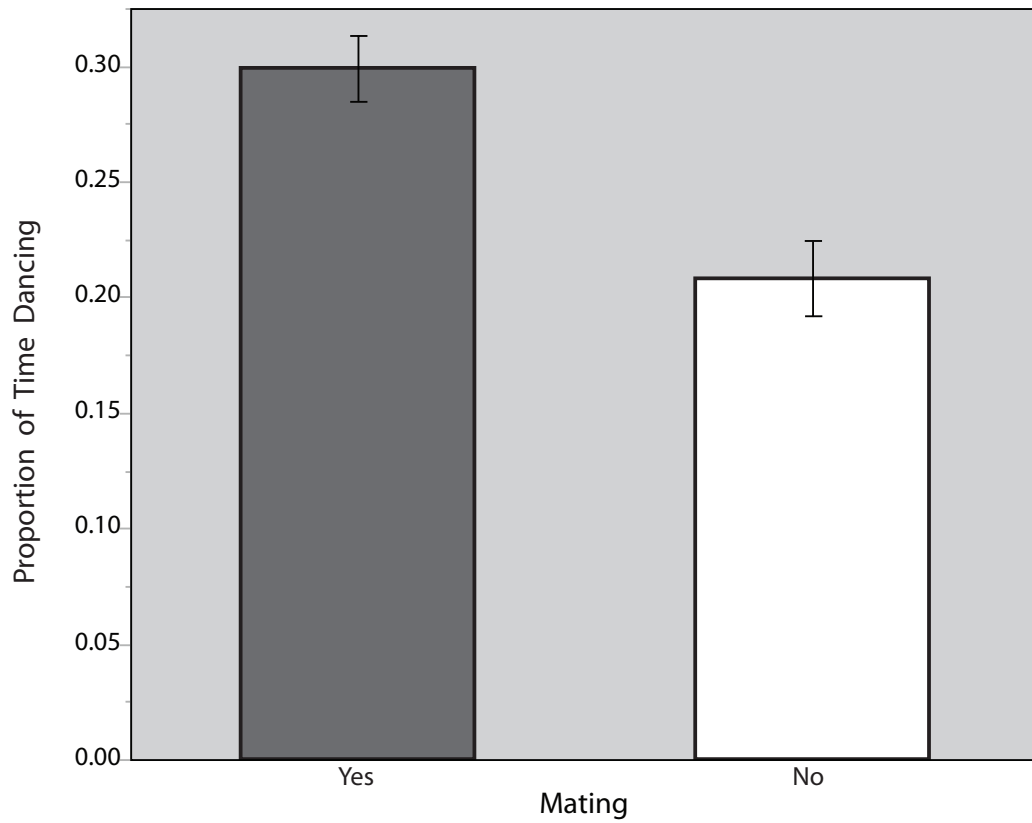
Figure 4.6. Proportion of time males spent dancing during mating trials relative to copulation success: Across treatments (**FS-V**, **FS-NV**, **MR-V**, and **MR-NV**), males that mated (dark grey) spent more time dancing than males that did not (white).
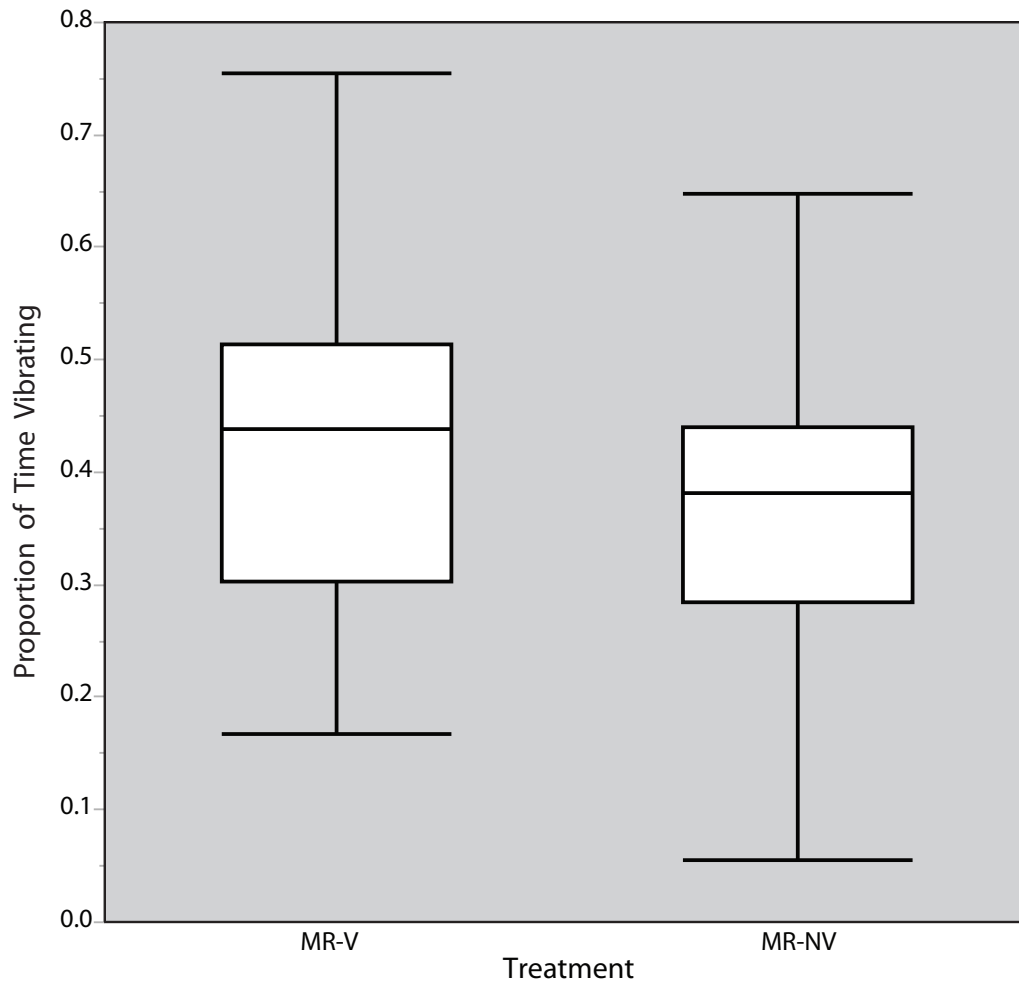
Figure 4.7. Proportion of time males spent vibrating in Minus Red (MR) treatments. Males in the vibration treatment spent significantly more time vibrating than those in the No-Vibration treatment.
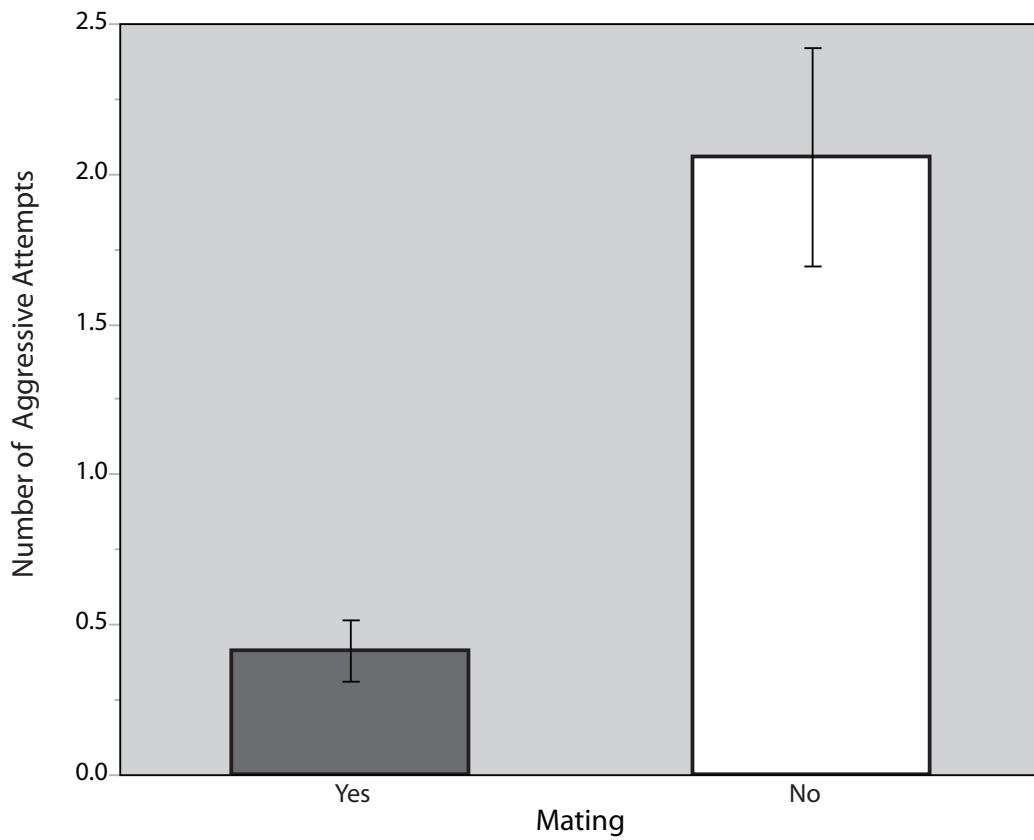
Figure 4.8. Number of aggressive attempts made by females relative to mating success: across the treatments (**FS-V**, **FS-NV**, **MR-V**, and **MR-NV**), females that mated (dark grey) were less likely than females that did not (white) to make aggressive lunges at males.

signals do not play a role in female preference for male courtship signals. While there was a significant decrease in mating rates in our minus red (**MR**) treatment groups as compared to our full spectrum (**FS**) treatments, the increase in mating we saw with our **+MR-V** treatment suggests that elements of male visual signals other than long-wavelength colors are the focus of female attention. We also observed that attenuating vibratory signals did not significantly affect mating rates. However, we found evidence that vibrations may be useful in some contexts, supporting the idea that vibratory signals may serve to complement visual signals. The proportion of time that males invested in courtship did not differ across our treatment groups, suggesting that differences in mating rates were based on female responses to male signals.

4.5.1 Why do males have long-wavelength visual signals?

We hypothesized that chromatic characteristics of LW fan ornaments (e.g. hue, chroma, saturation, chromatic contrast) were likely important to female peacock spiders, as they are to females of some other species (Blows *et al.* 2003; Hill, 1990; Houde, 1987; Kodric-Brown and Nicoletto, 2001), because LW signals make up the immense diversity of male ornamentation across *Maratus,* and are fairly unique across jumping spiders. If the long-wave color signals (e.g., reds, oranges, yellows) that are so prominent on male peacock spider fans are not used by females in mate choice, why do males invest in the production of these signals? One possibility is that females are evaluating overall fan pattern and not the specific color components of the fan. Under this hypothesis, the long-wave components of fan color are not themselves important but instead function to establish the contrast between adjacent ornament patches (achromatic contrast). Our treatment with enhanced overall illumination (**+MR** treatment) provides compelling support for this achromatic contrast hypothesis, as do data from other species (Barry *et al.* 2015; Cole and Endler, 2015; Fuller, 2002; Gaskett *et al.* 2017) Enhanced contrast may serve to capture and then perhaps maintain a female's attention. Lighting in natural environments is heterogeneous (Endler, 1993; Warrant and Johnsen, 2013) and red, yellow and orange signal components may be important to create a contrast with the mottled brown/green backdrop of the forest floor (Lovell *et al.* 2005; Maria *et al.* 2014). Contrast between LW signals and green/blue backgrounds have been used to explain the prevalence of these ornaments in aposematic coloration across taxa (Arenas, 2014). If this function applies to our study species, it is possible that the reason we found no effect of LWs during our experiments was because our trials were run in simple mating arenas with no other stimuli and thus contrast against the background was not required.

Although reliance on achromatic contrast may explain the apparent lack of importance of long-wave chromatic signals in our study, other explanations are possible. First, neural mechanisms may compensate for the absence of LWs such that females still perceive red coloration in the absence of the associated wavelengths (color constancy: Balkenius and Kelber, 2004; Chittka *et al.* 2014; Neumeyer, 1998). In this case, the perception of LWs remains important although females are unlikely to select males based on variation in chromatic properties of LW reflections. Accordingly, the decreased mating rates observed during **MR** treatments without enhanced illumination may have resulted from poor signal contrast in low light levels. Second, females may be capable of plasticity with regard to the types of visual signals used under different conditions. For example, blue and red ornaments may be redundant, with females using one or the other depending on the available lighting. Finally, while our results suggest that chromatic properties of LWs are not used in mate choice, they may be used in other contexts such as foraging, learning and navigation (de Ibarra *et al.* 2001; Hoefler and Jakob,

2006; Jakob *et al.* 2007; Taylor *et al.* 2016; VanderSal and Hebets, 2007); future work is needed test explicitly these hypotheses.

4.5.2 Role of vibratory signals in mating success

Similar to our findings for the chromatic features of long wavelength visual signals, our data suggest that vibrations do not play a crucial role in female willingness to mate. This finding is consistent with previous research (Girard *et al*. 2015) indicating that even though vibratory signaling predicts copulation, this effect is much smaller than for visual signals. Males may alter their use of vibrational signals in response to the substrate, vibrating more only when signals can be effectively produced (Gordon and Uetz, 2011; Gray *et al*. 2014; Heuschele *et al*. 2009; Patricelli *et al*. 2016; Partan, 2017). This shift in signal use may allow an animal to compensate for reduced efficacy in signal transmission in one sensory modality, for example by increasing the amount of time spent vibrating under low light condition. Similar results are reported for other types of spiders (Gordon and Uetz, 2011; Sullivan-Beckers and Hebets, 2014). In peacock spiders, males tend to produce more substrate-borne vibrations when females are not attentive to visual displays, which results in females reorienting themselves toward males and thus presumably paying more attention to visual displays (Girard *et al*. 2015). Thus we suggest that the interaction between vibratory and visual signals in our study species is not static but varies in response to the environmental conditions in which courtship occurs.

4.5.3 Multi-modal courtship signals

Animal courtship displays are complex and often involve multiple signals that employ more than one sensory modality. Presumably females evaluate more than one signal when making mate choice decisions. Recent models suggest that multiple signals evolve when different signals convey distinct types of information and thus serve distinct functions (Ay *et al*. 2007; Bro-Jørgensen 2010; Wilson *et al*. 2013). With regard to peacock spider signals, which employ multiple sensory modalities (i.e. vibratory versus visual signals), each modality may have unique functions and represent different axes of overall variation in courtship behavior. Vibratory signals likely function to draw a female's attention across long distances, when the female is not oriented toward a male (Girard *et al*. 2015; Girard *et al*. 2011), or when lighting conditions are less than ideal (this study). Once a female is attentive to a male, achromatic contrast patterns on the fan may become more important, possibly to enhance contrast with the background environment or as a signal of species identity (this study). Finally, it is possible that motion (dancing) is assessed as a signal of mate quality given that males that dance at higher rates are preferred as mates (Girard *et al*. 2015). Thus, although dancing is also a visual signal, it may function quite differently from color-based signals. More generally, we suggest that the evolution of complex signals in peacock spiders is driven by the need for different types of information (species identity, mate quality, multiple messages) and for maximal signal transmission in less than optimal environments (use of long wavelengths, behavioral compensation, redundant backups). Future studies are needed to investigate this hypothesis directly. As many have stated, trying to understand an organism that is unlike ourselves is the biggest challenge to animal behavior research (Nagel, 1974) but also one of the most meaningful as we seek to understand the natural world.

4.6 Acknowledgements

Chapter 5: Molecular phylogeny and courtship evolution of peacock spiders (genus: *Maratus*) and their relatives [5]

5.1 Abstract

Peacock spiders of Australia comprise at least sixty described species that are currently classified into two genera, *Maratus* (Karsch, 1878) and *Saratus* (Otto and Hill, 2017b) and several undescribed species. Whereas some peacock spiders are seemingly easily sorted into species groups based on morphological or behavioral characters, the evolutionary relationships among groups, and among species within groups, remain unclear. To help determine the phylogeny of this group of spiders, we use restriction site-associated DNA sequencing (RAD-seq) and maximum likelihood (ML) methods. To better interpret the patterns of complex signal evolution in this group, we also present data that we collected on male ornamentation and courtship displays. For the first time, our molecular phylogeny sheds light on relationships between peacock spiders and their relatives, including several major clades that emerge with strong support. Implications for the taxonomy and diversification of courtship displays in peacock spiders are also discussed.

5.2 Introduction

Peacock spiders of the *Maratus* genus are an extremely diverse clade of jumping spiders distributed across Australia. Males of this group are generally characterized by their conspicuously colorful abdomens and elongated, brush-adorned, third pair of legs, which are used in visual and vibrational courtship displays. The extensive radiation of peacock spider courtship ornaments and displays is comparable to that of the better-known birds of paradise (Irestedt *et al.* 2009; Scholes, 2008), but these animals have largely remained obscure until recently because of their small size. Previous research on jumping spiders provides evidence that complex signals have the potential to diverge rapidly (Masta and Maddison, 2002), and thus may be driving the pattern of extreme signal diversity we see in *Maratus* today. Thus far, peacock spiders have been divided into groups based on morphological and display features (Otto and Hill, 2017b). While the putative species groups are a helpful starting point for studying species diversity, a more comprehensive understanding of evolution in this genus has been hampered by the lack of a robust phylogeny.

Previous work has placed *Maratus* within a well-supported monophyletic subfamily, but distinguishing between *Maratus* and other closely related groups (*Hypoblemum, Jotus, Lycidas, Maileus, Maratus, Saitis, Prostheclina*) has been difficult (Zhang and Maddison, 2013; Zhang and Maddison, 2015). A few challenges to resolving these relationships include: many of the type species of closely related genera are not well known; large numbers of transition species in all groups remain undescribed; and genetalic structures, the primary morphological feature used to distinguish species, vary little across species (Otto and Hill, 2012). Originally, Żabka (1987) had separated the genera *Lycidas* (Karsch, 1878) and *Maratus* on the basis of the presence of opisthosomal flaps in the latter. However, many more recently described species, such as *M. linnaei* (Waldock, 2008), have minimal flaps or sometimes none at all, has rendered this distinction useless (Otto and Hill, 2012). Besides lateral flaps on the opisthosoma, Waldock (2002) proposed several other characters to separate *Lycidas* and *Maratus*, however, *Lycidas* was recently synonymized with *Maratus* (Otto and Hill, 2012), therefore the species previously associated with *Lycidas* have either been subsumed by *Maratus* or are awaiting assignment to another genus. One feature that still seems to encompass the vast majority of peacock spiders is their ability to raise and display colorful patterns associated with the abdomen (Girard and Endler, 2014; Hill, 2009). Based on this criterion alone, several new species have been placed within the genus over the last five years, but a robust molecular phylogeny is needed to uncover if these additions are valid as well as to reveal patterns of trait evolution among peacock spiders.

In this study, we collected behavioral and molecular data from an extensive sampling of peacock spider taxa to resolve the phylogenetic relationships within the genus *Maratus* and to reveal signaling evolution patterns across the group. To do this, we characterized visual and vibrational signaling diversity for living individuals that were then collected and prepared as specimens. Using the DNA extracted from the same specimens, we generated restriction-site associated DNA (RAD) libraries for sequencing. After filtering and assembling our RAD sequences, we had data for a total of fifty-four peacock spider species spanning the major axes of the group's diversity. Here, we present the first strongly resolved molecular phylogeny for peacock spiders as well as preliminary findings regarding the evolution of elaborate male courtship traits. Given current taxonomy, the genus *Maratus* is a paraphyletic group containing several major, strongly supported monophyletic sub-clades that correlate with patterns of courtship behaviors. Importantly, some of the morphological/behavioral groupings we expected

to see were not supported by the molecular data, suggesting that these characteristics are not sufficient for robust taxonomic classification. Accordingly, the phylogeny and associated courtship characterizations provided here establish a critical comparative framework for future studies of *Maratus* morphology, behavior, and evolution.

5.3 Methods

5.3.1 Specimen Collection

Specimens representing a large proportion of the known *Maratus* diversity were collected across Australia (from ACT, NSW, QLD, SA, TAS, VIC, and WA) between: August 20th-Decemeber 16th, 2013; September 30th- December 8th, 2014; September 24th-December 24th, 2015. At least three individuals from each location were sampled when possible. For species thought to occur across a large geographic area, we sampled several populations located throughout their putative range.

5.3.2 Behavioral data

Visual and vibrational courtship signals were characterized using videos and laser vibrometer recordings that were obtained from each putative species (see Girard *et al.* 2011 for a detailed description of these methods) prior to their preservation in 100% ethanol.

5.3.3 Molecular Work

We used a Qnap DNA micro kit (Qiagen, Valencia, CA, USA) to extract and isolate genomic DNA from tissues of spider legs (N= 192 individuals, for a few very small-bodied taxa, whole spiders were used). We then generated two restriction-site associated DNA (RAD) libraries using the protocol provided in Ali et al. (2016). The only deviations from this protocol were that we did not complete the targeted bait capture step and we used Pippin Prep (Sage Science, Beverely, MA USA) instead of beads to select fragments between 250-600bp. Lastly, we sequenced our libraries on two lanes of Illumina HiSeq 4000 at the U.C. Davis Genome Center with 150bp paired end reads.

5.3.4 RAD Sequence Filtering/ Phylogenetic Reconstruction

We used the pipelines implemented in a custom script calling various external programs for processing ddRAD-seq data (pipelines are available at https://github.com/CGRL-QB3-UCBerkeley/RAD). Raw fastq reads were first de-multiplexed based on the sequences of internal barcodes with a tolerance of one mismatch. De-multiplexed reads were removed if the expected cut site (also one mismatch allowed) was not found at the beginning of the 5'-end of sequences. Exact duplicates were removed by using Super Deduper (https://github.com/dstreett/Super-Deduper). The reads were then filtered using Cutadapt (Martin, 2011) and Trimmomatic (Bolger *et al.* 2014) to trim adapter contaminations and low quality reads. The resulting cleaned forward reads for each individual were clustered using Cd-hit (Fu et al 2012; Li and Godzik, 2006); only clusters with at least two supported reads were kept. We used Blastn (Altschul *et al.* 1990) to compare clustered loci against themselves, as well as to remove any locus that matched a locus other than itself. We used this stringent filtering to remove any potential paralogs or loci containing repeats within an individual. The resulting RAD

loci from each individual were then combined for all individuals and the resulting marker sets were renamed and served as a master reference. We then used Blastn (Altschul *et al.* 1990) to compare markers from each individual to the master reference and we kept only those with unique hits. The unique markers from each individual served as a reference for that individual. We made the name of each marker in individual references consistent with that in the master reference.

Cleaned sequence reads from each individual were then aligned to their own reference using Novoalign (http://www.novocraft.com) and reads that mapped uniquely to the reference were kept. We used Picard (http://picard.sourceforge.net) to add read groups and GATK (McKenna et al. 2010) to perform realignment around indels. We finally used SAMtools/BCFtools and "vcfutils.pl vcf2fq" implemented in SAMTools (Li *et al.* 2009) to generate individual consensus sequences by calling genotypes and incorporating ambiguous sites in the markers. We kept a consensus base only when its depth was at least 3X or above and every locus that was retained contained no more than 80% missing data (Ns). We also masked sites within 5 bp window around an indel. We converted the resulting consensus fastq sequence file to fasta format using Seqtk (https://github.com/lh3/seqtk). Using Mafft (Katoh and Standley, 2013), the final filtered markers from each individual were aligned according to their name determined by comparing them to the master reference. Ambiguously aligned regions in alignments were then trimmed using Trimal (Capella-Gutierrez *et al.* 2009). To avoid too much missing data, we also removed alignments if more than 90% missing data (Ns) were present in 30% (or above) of the samples. We combined all filtered individual alignments in phylip format for phylogenetic analysis. Maximum-likelihood method was performed in RAxML-HPC v8.1.11 (Stamatakis, 2014) using the GTRGAMMA general time reversible model of nucleotide substitution with gamma distributed rate heterogeneity. Branch support was assessed with 1000 bootstrap replicates using the rapid bootstrapping algorithm.

5.4 Results

5.4.1 Molecular Data & Phylogeny

In total, molecular and behavioral data were collected for 54 unique species of *Maratus* (N=192 individuals) plus 5 additional species belonging to 4 different genera (*Jotus, Hypoblemum, Lycidas,* and *Saitis*) that served as outgroups. A complete list of species included in this study, with general collection localities (Figure 5.1) are provided in Table 5.1.

The number of reads (de-multiplexed reads that contain expected restriction cutting sites) we obtained from our RAD data set ranged from 101,077 to 3,623,248 among samples. We had an average of 16,637.6 (9,155-27,683) RAD markers per individual, but the number of shared orthoglous loci among all samples was low, likely due to the deep phylogenetic distances among many samples. After filtering our RAD data (no more than 30% of taxa were allowed to be missing), we were left with 161 individuals with an average per-individual coverage of RAD markers of 12.1X (ranging from 3.1X to 61.2X). The resulting concatenated alignments for RaxML analysis were derived from 524 markers with a total length of 60,904 kb (including 19788 SNPs, 11,962 of which are informative).

Figure 5.1. Map of collection locales. Numbers in circles (colored by clade) correspond to those in Figs. 5.2 & 5.19, and Table 5.1.

Table 5.1. Information on collection location and display traits for all species sampled. Cells with "-" are unknown values. See Figure 5.19 for examples of the various lateral fan flap categories (column 4) and overall fan shapes (column 5).

Species	Collection Locale/(# of samples)	Raises Fan	Lateral Fan Flap	Overall Fan Shape	Leg III Use	White Brush Leg III Tarsi	Elongated Spinnerets Display	Vigorus Tapping	Vibrations Produced (Before Pre-Mount)	Pre-mount Display
1) J. auripies	Sydney, NSW (2)	No	No	N/A	No	No	No	No	Yes	Partial
2) S. virgatus	Cowan, NSW (2)	No	No	N/A	Yes	No	No	Yes	Yes	No
3) S. mutans	Newcastle, NSW (2)	No	No	N/A	Yes	No	No	No	Yes	Yes
4) L. scutulatus	Sydney, NSW (2)	No	No	N/A	-	No	No	-	-	-
5) H. albovittatum	Brisbane, QLD (2)	Yes	No	N/A	Yes	No	No	No	-	Partial
6) M. speculifer	Freemantle, WA (1)	Yes	No	N/A	Rarely	No	No	No	No	Partial
7) M. sp. "flame"	Lake Gairdner, SA (1)	Yes	No	N/A	No	No	No	No	No	Partial
8) M. sp. "carmel"	Spring Hill Salt Flats, SA (2)	Yes	No	N/A	No	No	No	No	No	Partial
9) M. robinsoni	Newcastle, NSW (3)	Yes	No	N/A	Rarely	No	No	No	No	Partial
10) M. purcellae	Canberra, ACT (4)	Yes	No	N/A	No	No	No	No	No	Partial
11) M. sp. "meteor"	Lake Eyre, SA (3)	Yes	No	N/A	No	No	No	No	No	Partial
12) M. nigromaculatus	Boondall Wetlands Reserve, QLD (3)	Yes	Modified Hair	N/A	Yes	Yes	No	No	No	Yes
13) M. chrysomelas	a: Flinders Ranges National Park, SA (2) b: Tregoie National Park, QLD (2)	Yes	No	N/A	Yes	Yes	No	No	No	Yes
14) M. spicatus	Koonidoola Regional Bushland, WA (3)	Yes	No	N/A	No	No	No	No	No	Partial
15) M. watagansi	Watagans National Park, NSW (1)	No	No	N/A	Yes	Yes	No	Yes	Yes	Yes
16) M. spelendens	Normanhurst, NSW (2)	Yes	Yes	Round	Yes	Yes	No	Yes	Yes	Yes
17) M. martimus	Esperance, WA (2)	No	No	N/A	Yes	Yes	No	Yes	Yes	Yes
18) M. pavonis	a: Heardsman Lake, WA (4) b: Namadgi National Park, ACT (2) c: Melbourne, VIC (2) d: Orford, TAS (2)	Yes	Minimal	Round	Yes	Yes	No	Yes	Yes	Yes
19) M. literatus	a: Nhil Swamp Wetland Reserve, VIC (1) b: Booroopki Swamp, VIC (2)	Yes	Minimal	Round	Yes	Yes	No	Yes	Yes	Yes
20) M. cf. leo	Waterfall Gully, SA (3)	Partially	No	N/A	Yes	Yes	No	Yes	Yes	Yes
21) M. leo	Darlington, SA (3)	Partially	No	N/A	Yes	Yes	No	Yes	Yes	Yes
22) M. vultus	Strathdownie, VIC (2)	Yes	Minimal	Round	Yes	Yes	No	No	Yes	Yes
23) M. albus	a: Port Adelaide Beach, SA (1) b: Flinders Chase National Park, SA (3)	No	No	N/A	Yes	Yes	No	Yes	Yes	Yes
24) M. anomalus	a: Newcastle, NSW (1) b: Dunmore State Forest, QLD (1)	Yes	No	N/A	Yes	Yes	No	Yes	Yes	Yes
25) M. sceletus	Wondul Range National Park, QLD (3)	Yes	No	N/A	Yes	Reduced	Yes	No	Yes	Yes
26) M. cinereus	Stanthrope, QLD (2)	Yes	No	N/A	Yes	Reduced	Reduced	No	No	Yes
27) M. michaelorum	Carnavon Gorge, QLD (1)	Yes	No	N/A	Yes	Yes	No	No	Minimally	Yes
28) M. neptunus	Butterwick, NSW (3)	Yes	No	N/A	Yes	Yes	Reduced	No	Yes	Yes
29) M. cf. neptunus "red-rogers"	Cassilis, NSW (1)	Yes	No	N/A	Yes	Yes	Reduced	No	Yes	Yes
30) M. aurantius	Orange, NSW (3)	Yes	No	N/A	Yes	Reduced	Reduced	No	Minimally	Yes

Table 5.1. Continued from previous page.

Species	Collection Locale/(# samples)	Raises Fan	Lateral Fan Flap	Overall Fan Shape	Leg III Use	White Brush Leg III Tarsi	Elongated Spinnerets Display	Vigorus Tapping	Vibrations Produced (Before Pre-Mount)	Pre-mount Display
31) S. herperus	Canberra, ACT (3)	Yes	No	N/A	No	No	No	Yes	Yes	Partial
32) M. amabilis	a: Mt. Barney, QLD (2) b: Mount Kaputar National Park, NSW (1) c: Cowan, NSW (2)	Yes	Yes	Elliptical	Yes	Yes	No	Yes	Yes	Yes
33) M. speciosus	Trig Beach, WA (2)	Yes	Modified Hair	N/A	Yes	Yes	No	No	Yes	Yes
34) M. vespertilio	a: Bluff's Knoll, WA (2) b: Mt. Majura Nature Reserve, ACT (3)	Yes	Yes	Lobbed	Yes	No	No	No	Yes	Yes
35) M. velutinus	Ku-ring-gai Chase National Park, NSW (2)	Yes	No	N/A	Yes	No	No	No	Yes	Yes
36) M. proszynskii	Canberra, ACT (3)	Yes	No	N/A	Yes	No	No	No	Yes	Yes
37) M. harrisi	a: Kanangra-Boyd National Park, NSW (1) b: Booroomba Rocks, ACT (1) c: Wadabilliga National Park, NSW (2)	Yes	Yes	Lobbed	Yes	Yes	No	Yes	Yes	Yes
38) M. calcitrans	Black Mountain, ACT (1)	Yes	No	N/A	Yes	Yes	Yes	No	Yes	Yes
39) M. jactatus	Wondul Range National Park, QLD (3)	Yes	Minimal	Round	Yes	Yes	Yes	No	Minimally	Yes
40) M. ottoi	Venman Bushland National Park, QLD (3)	Yes	No	N/A	Yes	Yes	Yes	No	Minimally	Yes
41) M. digitatus	Mount Kaputar, NSW (2)	Yes	Yes	Lobbed	Yes	Yes	Yes	No	Yes	Yes
42) M. eliasi	Nuga Nuga National Park, QLD (3)	Yes	Yes	Diamond	Yes	Yes	Yes	No	Minimally	Yes
43) M. elephans	Tamworth, NSW (3)	Yes	Yes	Elliptical	Yes	Yes	No	No	Yes	Yes
44) M. volans	a: Mt. Barney, QLD (2) b: Cowan, NSW (3)	Yes	Yes	Elliptical	Yes	Yes	No	No	Yes	Yes
45) M. plumosus	Ku-ring-gai Chase National Park, NSW (3)	Yes	Minimal	Lobbed (Posterior)	Yes	Reduced	No	No	Yes	Yes
46) M. cf. plumosus "plumosassy"	Lower Glenelg National Park, VIC (3)	Yes	Minimal	Lobbed (Posterior)	Yes	Reduced	No	No	Yes	Yes
47) M. tasmanicus	a: Piccaninnie Ponds Conservation Park, SA (1) b: Melbourne, VIC (2) c: Mount William, TAS (2)	Yes	Yes	Elliptical	Yes	Yes	No	No	Yes	Yes
48) M. australis	Esperance, WA (1)	Yes	Yes	Lobbed	Yes	Yes	No	No	Yes	Yes
49) M. personatus	Cape Riche, WA (1)	No	No	N/A	Yes	Yes	No	No	Yes	Yes
50) M. pardus	Cape Le Grande, WA (1)	Yes	Yes	Elliptical	Yes	Yes	No	No	Yes	Yes
51) M. avibus	Cape Arid, WA (1)	Yes	Yes	Elliptical	Yes	Yes	No	No	Yes	Yes
52) M. caeruleus	Middle Island, WA (2)	Yes	Yes	Elliptical	Yes	Yes	No	No	Yes	Yes
53) M. sp. "col. mustard"	Yalgorup, WA (1)	Yes	No	N/A	Yes	Yes	No	No	Yes	Yes
54) M. clupeatus	Gnangara, WA (2)	Yes	Yes	Elliptical	Yes	Yes	No	No	Yes	Yes
55) M. madelineae	Dardanup, WA (3)	Yes	Yes	Elliptical	Yes	Yes	No	No	Yes	Yes
56) M. linnaei	Two People's Bay, WA (3)	Yes	No	N/A	Yes	Reduced	No	No	Yes	Yes
57) M. vespa	Lake Jasper, WA (1)	Yes	Minimal	Lobbed (Posterior)	Yes	Yes	No	No	Yes	Yes
58) M. sarahae	Bluff's Knoll, WA (2)	Yes	Yes	Elliptical	Yes	Yes	No	No	Yes	Yes
59) M. mungaich	Mount Dale, WA (3)	Yes	Yes	Elliptical	Yes	Yes	No	No	Yes	Yes

The best tree resulting from our ML analysis is shown in Figure 5.2; all nodes with less than 70% bootstrap support were collapsed. All putative species sampled were supported as monophyletic with one exception; our two outgroup samples of *L. scutulans*. At this time, it is unclear what the true placement of this species should be relative to the other outgroup members. Although the phylogenetic relationships among some species remain incompletely resolved, seven major monophyletic *Maratus* clades were revealed and strongly supported by our bootstrap analysis: speculifer clade (bs=100%), chrysomelas clade (bs=100%), anomalus clade (bs=100%), pavonis clade (bs=100%), digitatus clade (bs=100%), volans clade (bs=100%), and the mungiach clade (bs=70%). Several of these clades are mostly congruent with species group divisions previously proposed by Otto and Hill (2017b) on the basis of several morphological and behavioral similarities. Here we outline, by clade (when applicable), specific aspects of the morphological and behavioral characteristics we examined in our study (these data are also summarized in Table 5.1).

5.4.2 Morphological and Behavioral Data

"speculifer clade"

Species in this group include (Figures 5.2 & 5.3): *M. speculifer, M. sp. "flame"* and *M. sp. "carmel."* It is likely that *M. fimbriatus* fits into this group as well, but molecular data are needed to confirm. Males of the speculifer clade lack lateral fan flaps yet still raise their abdomens to display to females. These spiders lack typical *Maratus* features, such as tufted legs and a traditional pre-mount display. The pre-mount display of these species (described in Girard *et al*. 2011) is different in that legs III are not extended at 90° angles from the body, but instead remain touching the substrate. In the three species examined, vibrations were not used prior to the modified pre-mount display. Lastly, the use of third legs in male displays is either severely reduced or nonexistent and instead legs I are primarily employed.

"chrysomelas clade"

This group is contains (Figures 5.2 & 5.4): *M. chrysomelas, M. niagromaculatus, M. sp. "meteor,"* as well as *M. robinsoni, M. purcellae,* and *M. spicatus,* which were previously placed in a "spicatus" group by Otto and Hill (2017b). According to our phylogeny, members of the *spicatus* do not comprise a monophyletic clade and thus this grouping is not utilized here. However, as Otto and Hill (2017b) predicted, these three species are closely related to *M. chrysomelas.* Males of the chrysomelas clade do not have lateral fan flaps although *M. niagromaculatus* has erectile bristles adorning the outside of their abdomen. All individuals raise their fans during courtship. None of these species use vibrations prior to mounting females. The only species with a true pre-mount display are *M. chrysomelas* and *M. niagromaculatus*. The pre-mount displays of all other members of the chrysomelas clade are similar to those exhibited by members of the speculifer clade. *M. chrysomelas* and *M. niagromaculatus* are also the only members of the chrysomelas group to have a white brush of setae on the tarsi of their third legs, with these legs being used extensively during courtship (*M. robinsoni* was observed using third legs on very rare occasions).
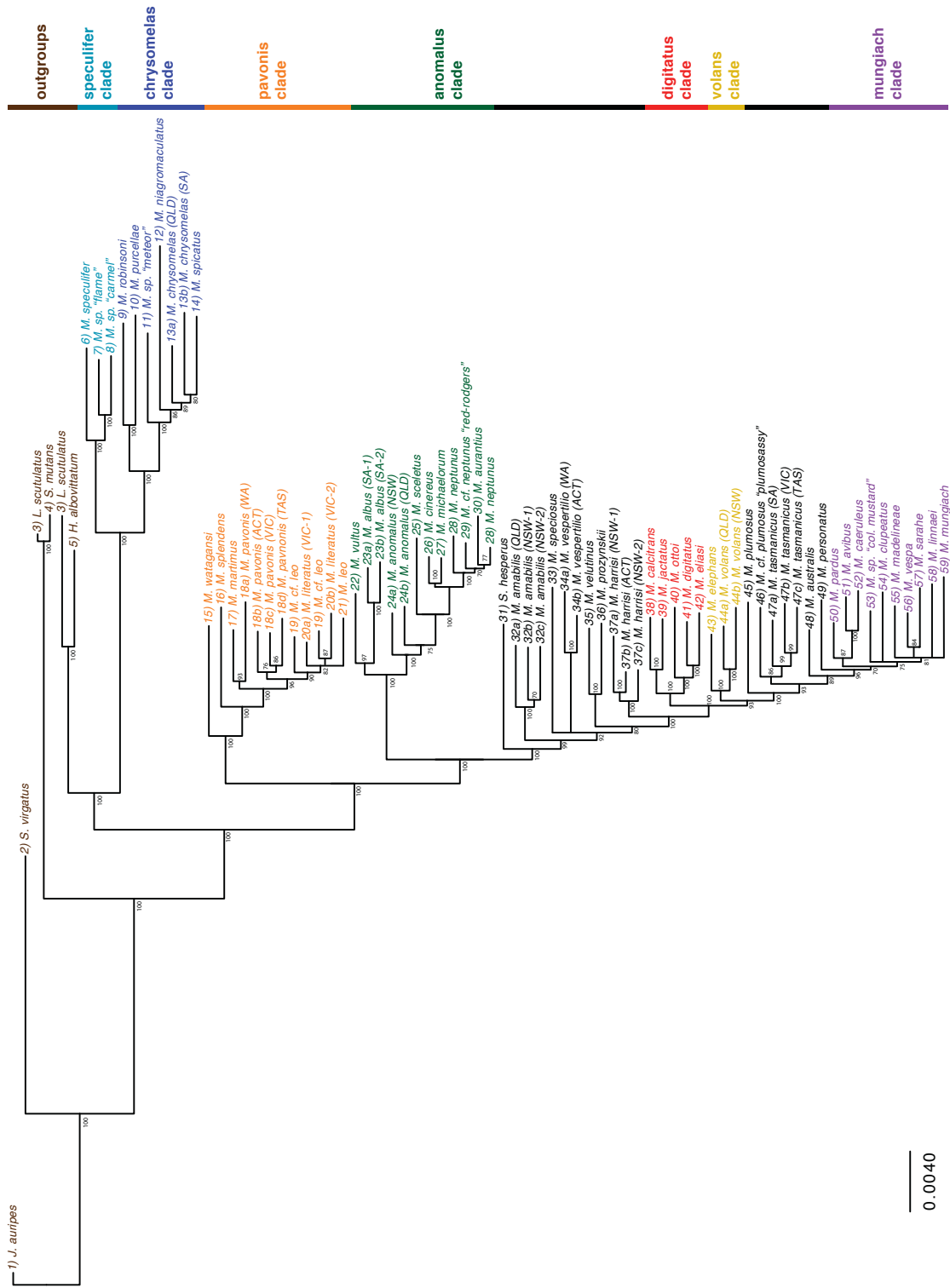
Figure 5.2. RaxML Maximum likelihood phylogeny for peacock spiders and their relatives. Bootstrap values are shown at each node; nodes with values below 70 were collapsed.

Figure 5.3. Members of the speculifer clade: *M. speculifer* and *M. sp. "flame"* (from left to right).

Figure 5.4. Members of the chrysomelas clade: *M. Chrysomelas,* and *M. niagromaculatus* (top, from left to right); *M. sp. "meteor," M. spicatus, M. robinsoni*, and *M. purcellae (*bottom, from left to right).

"pavonis clade"

The pavonis clade is comprised of the following species (Figures 5.2 & 5.5): *M. pavonis, M. splendens, M. leo, M. cf. leo, M. watagansi, M. martimus,* and *M. literatus*. This group also likely includes *M. montanus*. All males of this clade use ornamented third legs extensively during courtship. Additionally, males of this group produce some of the most powerful vibrations throughout courtship, including percussive taps on the substrate that are generated by the abdomen and third legs. *M. splendens* is the only member of this group to have sizable lateral fan flaps, whereas most other members of the group have minimal (*M. literatus* and some M. pavonis morphs) or no (*M. watagansi, M. leo, M. martimus*) flaps. *M. leo,* and *M. cf. leo* are known to only lift their abdomen slightly during displays while, *M. martimus,* and *M. watagansi* do not lift their abdomens at all. All the other members of this species group lift their abdomens completely when displaying to females.

"anomalus clade"

The anomalus clade is comprised of the following species (Figures 5.2 & 5.6): *M. anomalus, M. albus, M. aurantius, M. cinereus, M. michaelorum, M. neputunus, M. cf. neptunus "red-rodgers," M. vultus,* and *M. sceletus,* which was originally hypothesized by Otto and Hill (2017b) to be part of the calcitrans group. According to genital as well as general morphology (Otto and Hill, 2017b), this group also likely includes *M. julianneae, M. kochi,* and *M. lentus.* This clade in is particularly phenotypically diverse with regard to courtship behavior. Vibrational signals were observed in the courtship displays of most species, although the extent to which these vibrations are used varies. *M. anomalus* and *M. albus* are the only members of this clade to utilize intense percussive tapping to punctuate their visual displays. In contrast, vibrations are severely dampened or are rarely used by *M. cinereus, M. cf. neptunus* and *M. aurantinus. M. vultus* is the only species in this group to have any form of lateral fan flaps, although the rounded flaps of *M. splendens* are very minimal relative to those in other *Maratus* species. *M. albus* males do not raise their fans during courtship. Ornamented tarsi are present in most species, although brushes are severely reduced in *M. sceletus, M. cinereus* and *M. aurantius*. Lastly, elongated spinnerets are used in the displays of *M. sceletus* males, and to a much lesser extent (in spinneret size and movement) in *M. cinereus, M. neptunus, M. cf. neptunus "red-rodgers,"* and *M. aurantius* males.

S. hesperus

S. hesperus (Figures 5.2 & 5.7) males have no fan and no ornamentation on the tarsi of legs III. This is the only peacock spider species outside of the speculifer and chrysomelas clades that does not use its third legs during courtship, including the final pre-mount display. *S. hesperus* males use powerful vibrations during courtship with very vigorous percussive tapping by legs III and the abdomen.

M. amablilis

M. amabilis (Figures 5.2 & 5.8) have large lateral fan flaps and ornamented third legs. They also make use of vigorous percussive tapping during their displays. Males of this species use a normal pre-mount display.
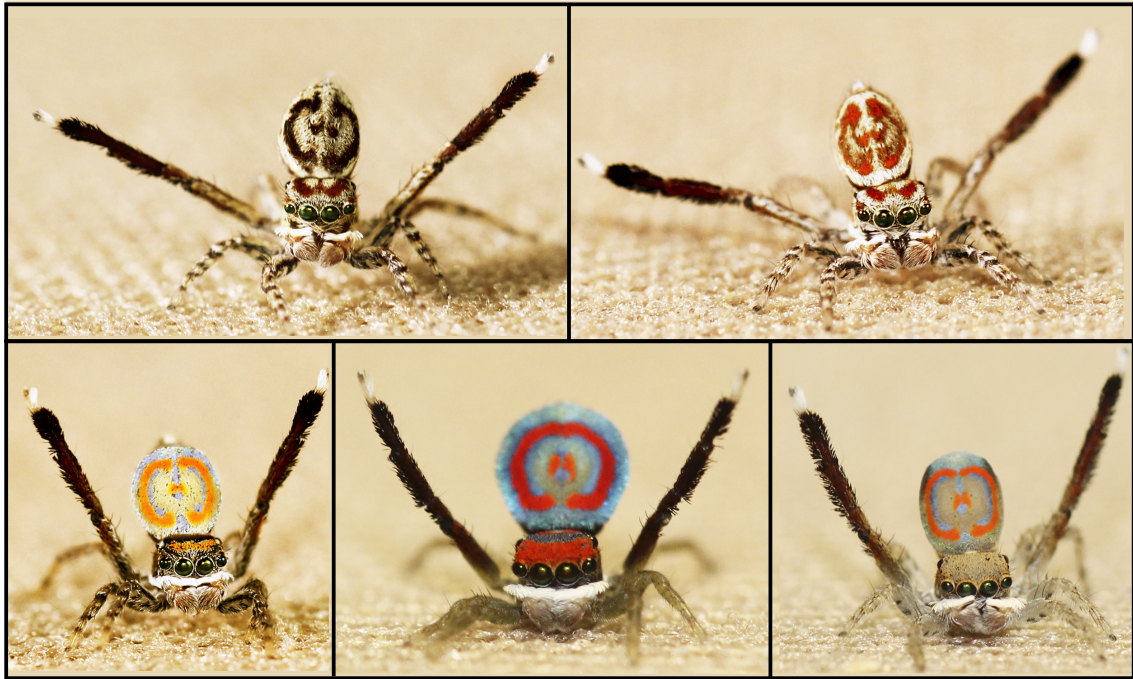
Figure 5.5. Members of the pavonis clade: *M. cf. leo, M. leo* (top, from left to right); *M. literatus, M. splendens,* and *M. pavonis* (bottom, from left to right).

Figure 5.6. Members of the anomalus clade: *M. neptunus, M. cf. neptunus "red-rogers," M. aurantinus,* and *M. cinereus* (top, from left to right); *M. sceletus, M. anomalus, M. michaelorum, M. vultus,* and *M. albus* (bottom, from left to right).

Figure 5.7. *S. hesperus.*

Figure 5.8. *M. amabilis.*

M. speciosus

M. speciosus (Figures 5.2 & 5.9) has no fan, but instead has erectile bristles along the outside of its abdomen that are inflated and splayed out when abdomen is raised. Legs III of this species are ornamented and used in male courtship displays, as are vibrations (no tapping though). This species has a normal pre-mount display.

M. vespertillio

M. vespertillio (Figures 5.2 & 5.10) has large fan flaps that are lobed. Although legs III are not ornamented with white tarsi they are still used extensively during male courtship. *M. vespertillio* has a normal pre-mount display.

M. velutinus

M. velutinus (Figures 5.2 & 5.11) does not have a fan nor does it have third leg ornamentation. This species, however, raises and uses both its abdomen and third legs during male courtship. This species also produces vibrations but without percussive elements. Males engage in a normal pre-mount display.

M. proszynskii

M. proszynskii (Figures 5.2 & 5.11) is very similar to *M. velutinus*, see description above.

M. harrisi

M. harrisi (Figures 5.2 & 5.12) is likely very closely related to *M. lobatus* (not included in this study). Similar to *M. vespertillio, M. harrisi* males have large lobed fan flaps that are raised with the abdomen and unfurled during courtship. *M. harrisi* produce percussive vibrations akin to those made by male *S. hesperus* and *M. amabalis*. The tarsi of legs III have white brushes and are used often during courtship. Males engage in a normal pre-mount display.

"calcitrans clade"

Species in this group include (Figures 5.2 & 5.13): *M. calcitrans, M. jactatus, M. ottoi, M. eliasi*, and *M. digitatus.* Note: despite their pronounced morphological and behavioral similarities, this clade does not contain *M. plumosus* or *M. sceletus.* Males of all species in this group inflate elongated spinnerets during courtship, which are waved at females. Members of this clade are additionally typified by an asymmetric display, in which the raised abdomen is alternatingly waved toward one side and then the other (legs III are also extended one at a time in alternating directions). Species in the calcitrans clade all have white setae on third leg tarsi. Presence of a lateral fan flap varies across species, with flaps ranging from large in *M. digitatus* and *M. eliasi* to non-existent in *M. ottoi* and *M. calcitrans.* Vibrations (excluding percussive tapping) are employed by all species during courtship, albeit minimally in *M. eliasi, M. ottoi* and *M. jactatus.*

"volans clade"

The volans clade is comprised of only two species (Figures 5.2 & 5.14): *M. volans* and *M. elephans,* but contrary to Otto and Hill (2017b) does not include *M. pardus.* Males in this group raise their abdomens during courtship and extend large lateral fan flaps that form an

Figure 5.9. *M. speciosus.*

Figure 5.10. *M. vespertillio.*

Figure 5.11. *M. velutinus* and *M. proszynskii* (from left to right).
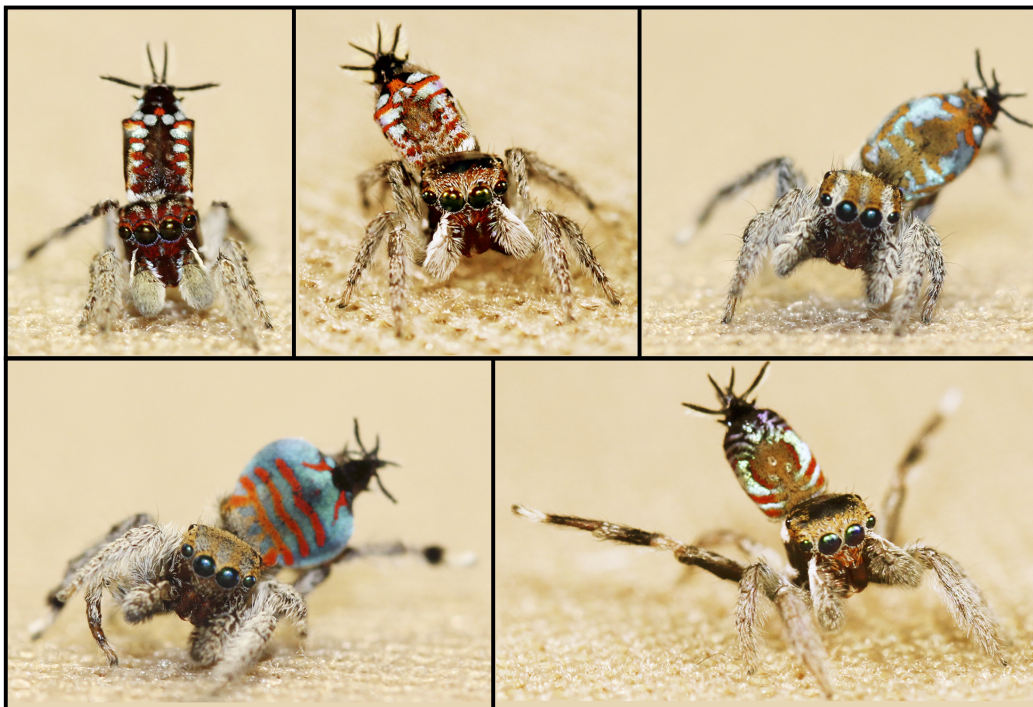
Figure 5.12. *M. harrisi.*

Figure 5.13. Members of the calcitrans clade: *M. digitatus, M. eliasi,* and *M. calcitrans* (top, from left to right); *M. jactatus,* and *M. ottoi* (bottom, from left to right).

Figure 5.14. Members of the volans clade: *M. volans* and *M. elephans* (from left to right).

elliptical shape when opened. *M. volans* and *M. elephans* make use of vibrations throughout their courtship displays, including the pre-mount display, although no percussive tapping is produced as part of these signals. Legs III of both species are ornamented with white brushes on the tarsi.

M. cf. plumosus "plumosassy"

M. cf. plumosus "plumosassy" (Figures 5.2 & 5.15) is very similar to *M. plumosus*, see description below. Despite this similarity, our molecular data did not support this species being more closely related *M. plumosus* than they were to *M. tasmanicus* (Figure 5.2, bs=86%).

M. plumosus

M. plumosus (Figures 5.2 & 5.15) males have minimal fan flaps, restricted to the posterior portion of the abdomen. Spiders of this species lack leg III tarsi ornamentation but use both their raised abdomen and third legs to perform an asymmetrical display similar to that of calcitrans group members. Inflated spinnerets, however, are not used in the display of *M. plumosus*. Light vibrations are produced intermittently throughout courtship. The pre-mount display proceeds normally in this species.

M. tasmanicus

M. tasmanicus (Figures 5.2 & 5.16) has large fan flaps that are lobed, similar to that of M. *harrisi*. Legs III are ornamented with white tarsi and are used extensively during male courtship. *M. vespertillio* produces light vibrations throughout courtship and has a normal pre-mount display.

M. australis

M. australis (Figures 5.2 & 5.16) is very similar to *M. tasmanicus*, see description above. Despite this similarity, our molecular data support *M. personatus* as more closely related to *M. australis* (Figure 2, bs= 89%).

M. personatus

M. personatus (Figures 5.2 & 5.17) does not have fan flaps and does not raise its abdomen during courtship. This species uses ornamented third legs and vibrations to court females. *M. personatus* has a normal pre-mount display.

"mungiach clade*"*

This group includes the following species (Figures 5.2 & 5.18)*: M. mungiach, M. avibus, M. caeruleus, M. madelineae, M. sarahe, M. vespa, M. pardus, M. linnaei, M. clupeatus, M. sp. "col. mustard."* Morphological, behavioral and geographical evidence suggests this group likely also includes *M. bubo, M. hortorum, M. karrie, M. melindae,* although we were not able to include specimens of these species in our phylogeny. The majority of males in this group have ornamented third legs (white brushes on tarsi, reduced in *M. linnaei*), as well as large lateral fan flaps. When opened, these flaps make an elliptical fan shape. As exceptions, *M. vespa, M. linnaei* and *M. sp. "col. mustard"* have either greatly reduced (*M. vespa*) or no flaps (*M. linnaei*). While males of this group use vibrations prior to and during the pre-mount display, they do not exhibit percussive tapping of the third legs or abdomen.

Figure 5.15. *M. cf. plumosus "plumosassy"* and *M. plumosus* (from left to right).

Figure 5.16. *M. australis* and *M. tasmanicus* (from left to right).

Figure 5.17. *M. personatus.*

Figure 5.18. Members of the mungiach clade: *M. avibus, M. caeruleus,* and *M. linnaei* (top, from left to right); *M. madelinae, M. mungiach,* and *M. sarahe* (middle, from left to right); *M. sp. "col. mustard," M. pardus, M. clupeatus,* and *M. vespa* (bottom, from left to right).

5.5 Discussion

This study provides a taxonomically broad phylogenetic analysis of peacock spiders using genome wide markers. In general, our goals were to a) characterize relationships between different species of *Maratus*, (b) investigate multi-modal courtship signal organization across the group and (c) make inferences about the evolution of species-level differences in complex courtship signaling. We sampled 59 species and used hundreds of molecular markers to generate a robust molecular phylogeny for this group, upon which our interpretations regarding signal evolution are based. First, our results challenge the current status of peacock spiders as monophyletic and, accordingly, our molecular phylogeny has important implications for the taxonomy of not only *Maratus*, but also that of closely related genera. However, our phylogenetic analyses largely corroborate several species groups proposed by Otto and Hill (2017b) and clarify instances where previous, morphologically based studies were unable to reveal the relationships between these taxa. Lastly, the topology of our tree lends support to the idea that *Maratus* displays have evolved from simple, primarily unimodal signals to more complex, multi-modal displays.

The recent transfer of several species of *Lycidas* to *Maratus* (Otto and Hill, 2017a; now *M. speculifer, M. chrysomelas,* and *M. niagromaculatus*) as well as the description of several new species of peacock spiders (Otto and Hill, 2017a; Otto and Hill, 2013; *M. robinsoni, M. purcellae and M. spicatus*) have made *Maratus* a paralyphyletic genus, as it no longer includes all decedents (e.g. *L. scutulans* and *H. albovittatum* are missing) of the most recent ancestor to all other *Maratus* species. Another problematic area of the phylogeny is the location of *S. hesperus*, which was placed in its own genus because its genitalia differ significantly from other *Maratus* species examined to date (Otto and Hill, 2017a). As there is typically limited interspecific variation in genital morphology of *Maratus* and closely related taxa (Otto and Hill, 2017a; Waldock, 2013), identification of distinct species of *Maratus* has relied largely on secondary sexual characteristics. Determining the limits of what constitutes a "true *Maratus*" is outside the scope of this paper, but the central placement of *Saratus hesperus* among other well established species of *Maratus* certainly advocates for a taxonomic revision of this species.

Taken together, our molecular and behavioral data provide strong support for seven distinct monophyletic sub-clades within the genus *Maratus*. The seven clades are as follows: speculifer, chrysomelas, pavonis, anomalus, digitatus, volans, and mungiach. While several species currently fall outside these sub-clades, the discovery of additional species may reveal more sub-clades within the genus. Interestingly, our phylogeny revealed that some of the morphological/behavioral groupings that we had expected were not supported with our tree. For example, based on fan shape and overall courtship behavior of male *M. plumosus* and *M. cf. plumosus "plumosassy"*, we anticipated that these species would be most closely related to members of the *calcitrans* group. Similarly, the inflated spinnerets exhibited by male *M. sceletus* during courtship lead us to hypothesize a phylogenetic position within the *calcitrans* clade for this species. Finally, *M. cf. plumosus "plumosassy"* and *M. tasmanicus* are sister taxa to each other rather than to the species with which they share an appearance, *M. plumosus* and *M. australis,* respectively. Similar apparent mismatches between phenotype and phylogenetic relationship occurred in other sub-clades in our tree. For example, some of the pavonis and mungiach group members with more similar phenotypes did not cluster together on the tree. This is illustrated with *M. madelineae,* which is more closely related to *M. linnaei* than either *M. mungiach* or *M. sarahe,* although these two species are much more similar to *M. madelineae* in

their displays, fan shape, size and coloration. For *M. pavonis*, we have a case in which individuals of the same species from geographically disparate populations are more distantly related to each other than they are to individuals classified as a different species; specifically, *M. pavonis* from WA are more closely related to *M. martimus* than to *M. pavonis* from the eastern states. Instead, the latter is more closely related to *M. literatus* and *M. leo*. It is possible that *M. pavonis* is currently made up of several geographically distinct cryptic species. However, disagreement between morphological/behavioral groupings and our molecular phylogeny could also stem from hybridization-mediated introgression, as well incomplete lineage sorting. Additional analyses are needed to explore these possibilities in greater detail.

Prior to this study, secondary sex characteristics were the only way to infer evolutionary relationships among different species of *Maratus*. For example, we had expected that the most ancient lineage of *Maratus* spiders are those now placed within the speculifer clade (*M. speculifer, M. sp. "flame,"* and *M. sp. "carmel"*) owing to: the relative simplicity of male morphological phenotypes, the differentiation of their displays as compared to other species, and the assumption that complex structure and morphology evolved later within *Maratus*. Several of these traits do mirror apparent phylogenetic relationships among species, which makes intuitive sense, as these are traits on which sexual selection acts. Moreover, paired with our phylogenetic data, the species characters examined in this study do indeed suggest that on the whole, evolution of male signaling has been from more simple displays, lacking vibrations and elaborate male ornamentation, to displays that incorporate more extensive vibrations and larger, more elaborately pattered fan/flaps (Figure 5.19). Features of closely related outgroups in our study (Table 5.1) provide evidence that the most recent common ancestor of all *Maratus* very likely had the following features: an opisothoma that was raised during courtship, no ornamentation on legs III, simple vibrations reserved for the pre-mount displays, and an incomplete pre-mount display that lacks the engagement of legs III. At present, inferences regarding the evolution of the use of legs III in any aspect of courtship is ambiguous based on comparisons with our outgroup and speculifer group members.

Our data suggest that the evolution of male courtship behavior in this group has moved toward greater complexity via a number of one-time evolutionary events (i.e. development of leg III ornamentation, development of courtship vibrations and development of fan flaps). Occasional reversions to simpler morphologies (reduced fan flaps, with less patterning, loss of leg III ornaments or use in male displays) are thus suspected to have occurred several times in the course of peacock spider evolution, perhaps most notably in species such as *M. personatus,* as well as in *M. velutinus* and *M. proszynski*. There are also specific aspects of complexity that have seemingly emerged independently in different lineages (elongated and inflated spinnerets, vigorous tapping). Additional phylogenetic analysis will be required to better understand trait evolution in this group and we suggest that these studies should focus on areas of the phylogeny where morphological and behavioral groupings fail to match relationships constructed using molecular data.
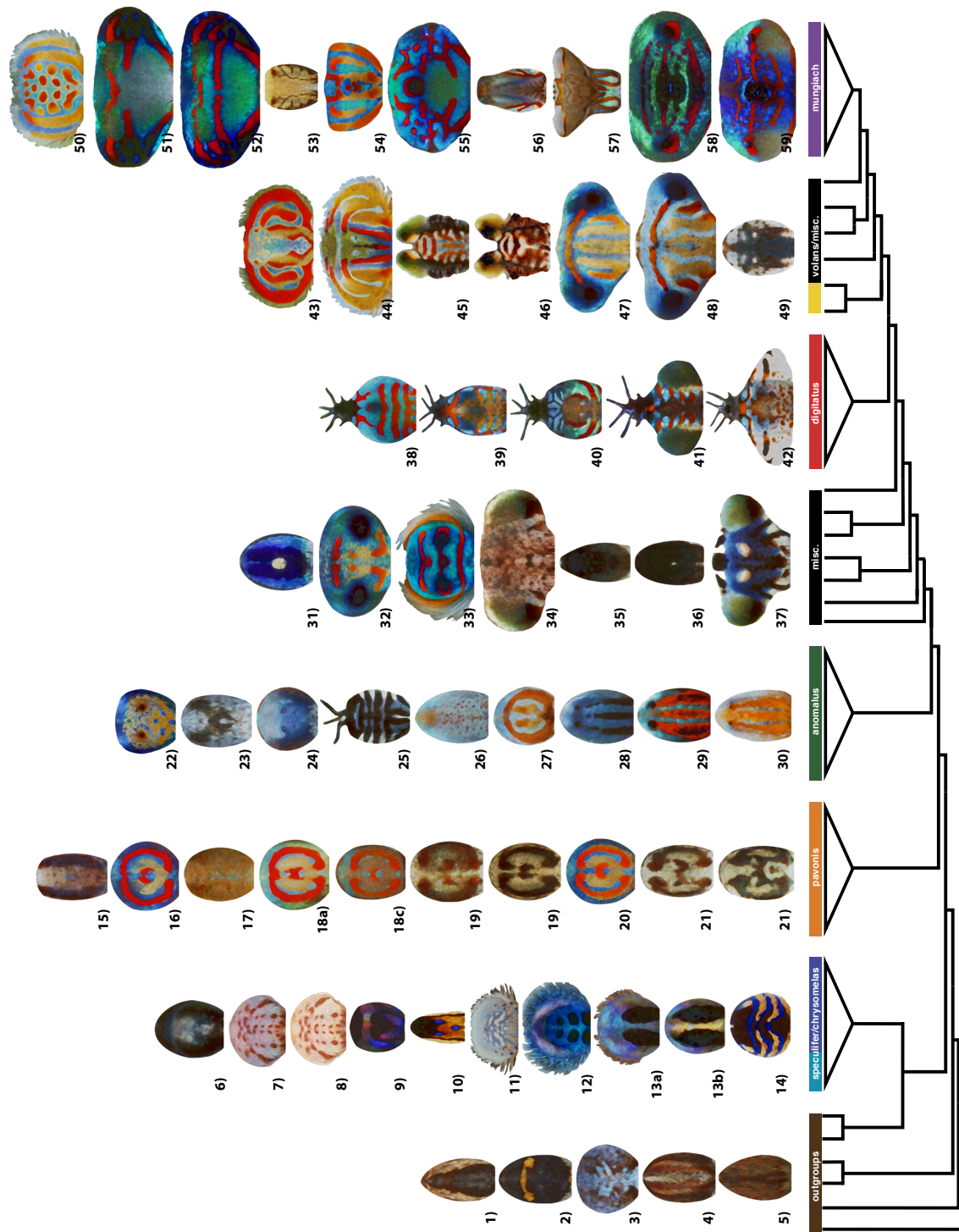
5.6 Acknowledgments

Figure 5.19. Fan diversity of *Maratus* clades: numbers to the left of each fan correspond to those in Figs. 5.1 & 5.2, and Table 5.1.

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Appendices

A.1 UV Photography Protocol

A1.1 Equipment/Supplies
- Fujifilm Finepix IS Pro
- Hyper Utility HS-V3 Software
- Tripod
- Adapters/Filters:
  1. Fotodiox 55mm Filter Thread Lens, Macro Reverse Ring Camera Mount Adapter
  2. T-NI T-mount adaptor for F-mount
  3. Nikon BR-3 52mm Mount Adapter Ring
  4. Adorama Step-Up Adapter Ring 52mm Lens to 55mm Filter Size
  5. Hyperion DT-Ring SP54/M55 for DT54 and Hyperion Eyepieces
  6. 2" filter holder
  7. Baader UV-IR-Cut Filter 2"
  8. Unmounted Astrodon Sloan UV' filter (mounted on a 52-46 step-down adapter)
- Lens (UV5035B 50mm F/3.5 UV lens)
- Nikon Bellows
- UV-Vis spectrum light: i.e. Lamda LS Light (Type B Parabolic Cermax PE300BUV lamp in Damian's lab)

A1.2 Assembling the hardware

First, attach the bellows to the camera, then attach the battery pack, lastly attach the whole unit to the tripod. We highly suggest anchoring your camera set-up as it may be very heavy on one side. After the initial hardware set-up, refer to Figures A1.1 & A1.2 for Adapter/Filter placement- it is easiest to start with the UV/IR cut filter set-up.
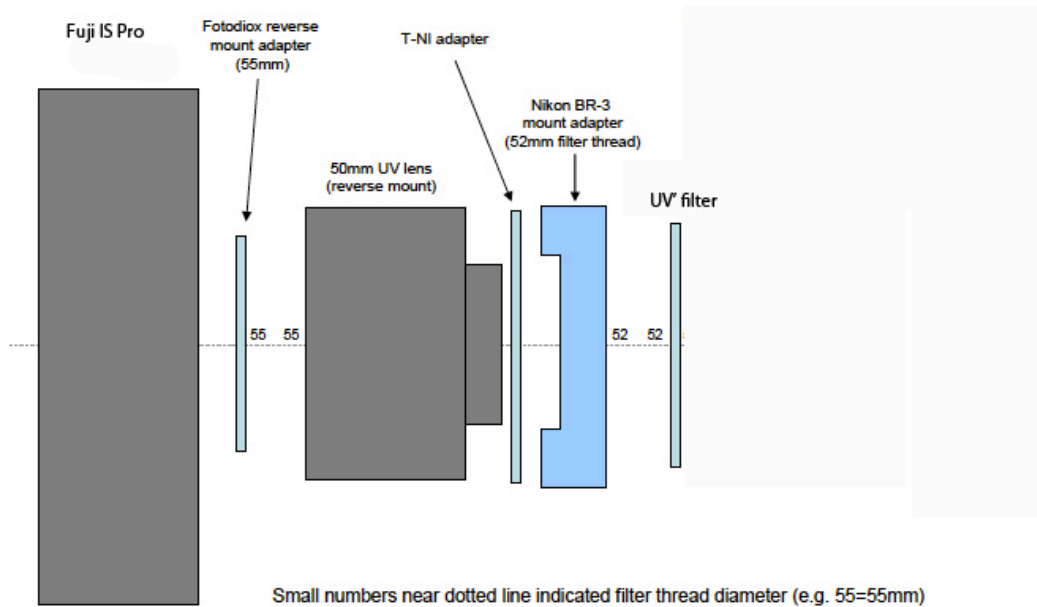
A1.3 Setting up the software

Make sure that your camera firmware is up-to-date and install HS-V3. Plug in USB cable linking camera to computer, then turn on camera.

Open "Studio Utility."
Under [shoot] select "control finepixS5 pro"
A control panel will open
Choose the camera select option
Wrench button: for file naming conventions
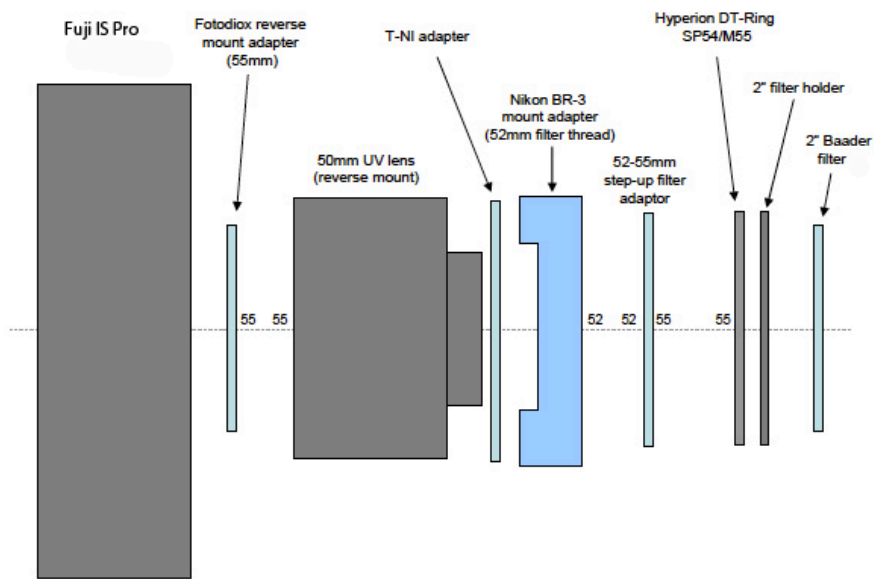
Figure A1.1. UV/IR cut filter set-up

Figure A1.2. UV' filter set-up

A1.4 Software Settings

- ISO- should probably be set at 100 (if pictures are not bright enough- may want to adjust this to be higher/more sensitive, but photo may become more fuzzy) but ISO should be set at the same value for all shots (i.e. not set on auto).
- Raw + Fine: this saves both jpgs and raw files
- Keep on M (for manual)
- Exposure time: We used 2.5" (seconds, don't get confused by 2.5 vs 2.5 ') for UV', and 50 for UV/IR cut (to let in less light) although this will be different if your light is less powerful.
- RGB- STD
- Image quality- select **Raw+F**
- Keep on **L**
- Leave everything else as is (on STD)
- White balance: use sun setting (or if possible, it's better to use the color temperature of your light source, in which case you select the "k" option and specify temperature of light source in kelvin)
- Memory mode: center weighted focus

Note: you can save your settings for later use.

A1.5 Focusing the camera system

Focus the camera set-up by adjusting the height of the camera from the table's surface as well as by adjusting the bellows (self-explanatory, but takes some playing around). Note: you can write down the Bellow's stop # that is used in each filter set-up to minimize refocusing effort between changes. Depending on how long it takes to run each specimen, you may be able to run multiple specimens using the UV/IR cut filter set-up before switching to the UV' set-up. This allowed us to minimize the amount of readjusting that needed to be done- we only re-focused between filters, rather than between individuals.

A1.6 Prepping Specimens

If you must kill your specimens, we highly recommend not freezing them because this process might alter the UV coloration. Killing with ethyl acetate is best.

A1.7 Camera Settings

- Put it in manual mode
- F-stop 16 (aperture, set manually on the lens): this allowed us to maximize focal plane, and our specimens were dead so a long exposure (small aperture) time was fine.
- UV lens fully in (doesn't really matter but best to be consistent)