REPRODUCTIVE BEHAVIORS OF TWO CONGENERIC, SYMPATRIC JUMPING SPIDERS: GENE FLOW BARRIERS AND POLYANDRY

by

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ABSTRACT

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This two part study compares aspects of jumping spider reproductive behavior and their implications 1) as gene flow barriers during the process of speciation and 2) in the occurrence of polyandry. Courtship behaviors of *Phidippus audax* and *P. carolinensis* were observed. *P. carolinensis* displays simple courtship behavior when compared to the visually complex behavior of *P. audax*. *P. carolinensis* males appear to be incorporating a vibratory element into their courtship display, and a possible stridulatory organ, located on the male pedipalp, is described. Large differences in courtship display, complexity of courtship badges, and phenologies between the species most likely prevent gene flow between them and may play a role in speciation within the genus.

Three prominent hypotheses on the forces driving the occurrence of polyandry (renewal of sperm supply, prolonged female choice, and fitness benefits) were tested in *P. audax*. Monogamously mated females were compared to polyandrously mated females in degree of multiple mating, clutch viability, and offspring size. Female spiders become choosier as their number of male encounters increase, and polyandrous females were more likely to mate multiply. There was a trend towards higher hatching success in polyandrous clutches, whereas degree of polyandry did not affect offspring size. Prolonged female choice is likely to be a strong driving force for polyandry in this species, and its possible mechanisms are discussed.

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PHIDIPPUS REPRODUCTIVE BEHAVIORS AND THEIR ROLES AS GENE FLOW BARRIERS

1.1 Introduction

1.1.1 Species Definition

Many biologists find it difficult to define something as plastic as a species; something that is continually morphing into new shapes, displaying new behaviors, and adapting to new environments through time. But, as biology is strongly reliant on the classification and organization of forms of life in order to more clearly see their relationships to each other, biologists continue to try and define organisms as species at given points in time. For the purpose of this thesis, I will define a species as a group of organisms, alike in structure and reproductively compatible, that share a common evolutionary history and a distinct evolutionary future. This definition is a compilation of the 3 major species concepts (Typological, Biological, and Evolutionary) (Claridge, et al. 1997). With this definition in hand, I will present an overview of hypotheses on *how* a species becomes a species.

1.1.2 Speciation

Though there can be a multitude of forces that drive a speciation event, most biologists agree that it must start with the formation of a barrier to gene exchange between parts of a population. Once that barrier is in place, each new population may contain different assortments of alleles which may undergo different selection pressures and lead to divergent evolution between the two separated populations. If the barrier is in place for a long enough period of time, the populations could diverge to a point where they would no longer fit our species definition when combined and would thus be defined as separate species from each other. This is a fairly simple idea on its own, but it becomes rather complicated when we start looking at the variety of potential barriers to gene flow, and the variety of forces that may act upon populations post-barrier so as to drive the populations to speciation.

The easiest type of genetic barrier to envision is an actual physical barrier that prevents individuals from coming into contact with each other. In animals, geographic barriers are usually considered the first step in speciation. These barriers can take eons to form, such as a mountain range or an ocean that divides a population of organisms, or they can occur rather suddenly, such as a tract of deforested land dividing a patch of jungle or a dam built along a stream. In essence, divergent evolution occurring after the original barrier forms can lead to speciation, and reinforcement barriers that encourage assortative mating keep the newly formed species separate entities if they become sympatric again in the future.

A second mode of speciation can occur via sexual selection, where males that differ in secondary sex characters or behaviors are preferred differentially by females. When female preference is highest at the two ends of the males' phenotypic spectrum, different looking/behaving animals will mate assortatively. Assuming that these sex characters/behaviors and the preferences for them are heritable, sexual selection may be as effective a barrier to gene flow as any given physical barrier. I will hereafter refer to the first, population separating barrier, be it physical separation, sexual selection, or some other mechanism, as the “original” barrier, and to any other barriers to gene exchange that follow as “additional isolation” barriers.

Gene flow barriers can be divided into two major categories: those that occur after fertilization takes place (post-zygotic), and those that occur to prior to fertilization (pre-zygotic). If individuals from two newly formed sister species are able to produce a hybrid zygote, several gene exchange barriers may still prevent that offspring from passing its hybrid genes into the next generation. Genetic incompatibility is likely to be a major reinforcement barrier. In cases of post-zygotic genetic incompatibility, genotypes of sister species or ecotypes are close enough for successful zygote formation, but errors may occur in later development and the hybrid offspring may be less viable than purebred offspring. This appears to be the case with lake whitefish, where hybrids occur but suffer at least two times the mortality in controlled conditions as purebred offspring (Lu & Bernatchez, 1998). If a hybrid offspring from genetically incompatible parent species reaches adulthood, it is often sterile due to errors in the segregation of sex chromosomes during gamete formation (Dobzhansky 1936) or incompatibility of other genes that govern the reproductive process (Wu & Hollocher 1998). In cases where hybrids are fertile, they may be less fit than purebreds due to reduced adaptations to the environment if both parent species are adapted to an environmental extreme (Hendry et al. 2002). They may also experience reduced mating success when parent species prefer to mate with conspecifics and hybrids are rare and less available for mating, as in certain *Heliconius* butterflies (Naisbit *et al.* 2001).

If post-zygotic barriers exist, then pre-zygotic barriers should be selected for since parental investments in hybrid offspring would come at an increased cost to their inclusive fitness. Pre-zygotic barriers are those that prevent individuals from locating each other for mating, prevent them from recognizing one another as potential mates, prevent copulation, or prevent union of sperm and egg. Each of these areas is receiving study, and there are cases in support of their wide occurrence in nature and importance in speciation. For instance, many animals rely on specific signals to locate potential mates, and female preference for conspecific male signals as opposed to those of other species (or populations) has been shown for the flashes of fireflies (Tamura *et al.*2005), the chirping of crickets (Mendelson & Shaw, 2002) and the bowers of Vogelkop bowerbirds (Albert, *et al.*, 2000). Many animals perform elaborate courtship displays prior to copulation. In species like these, preferences for conspecific courtship displays or behaviors over those of heterospecifics would reduce the likelihood of producing hybrid offspring. This preference has been implicated in driving speciation (original barrier) in African lake cichlids (Knight &Turner, in press) and Arizona jumping spiders (Masta & Maddison, 2002), or in supporting preservation of already separate species (additional isolation barrier), as in field crickets (Gray, 2005) and wolf spiders (Stratton & Uetz, 1981).

If courtship displays are brief or non-existent, or if they fail to discourage heterospecific matings, incompatible genitalia or reproductive physiology are other mechanisms thatreduce the risk of wasting energy on production of hybrids with reduced viability or fertility. The lock and key hypothesis thatwas proposed by Dufour in 1844 has been reexamined by various groups since (e.g.: Shapiro and Porter,1989; Schilthuizen, 2003). The lock and key hypothesis takes into account that insects (in the case of Dufour) have unique and perfectly paired genitalia within a species, and that this uniqueness probably prevents successful mating with other closely related species. Gametic incompatibility is certainly important for organisms that never meet but send their gametes out on wind or wave, where they likely encounter a variety of other species' gametes as well (e.g., plants, many marine invertebrates).   The evolution of this type of incompatibility is currently being documented in Panamanian sea urchin species (McCartney & Lessios, 2002), but may be occurring in animals that pair for mating as well (Alipaz *et al*, 2001).

1.1.3 TheRole of Courtship Behaviors as Gene Flow Barriers

In many cases, specific *courtship* behaviors or courtship-related morphologies may act as gene flow barriers. I shall define any behavior prior to or during pair formation that is intended to influence further reproductive behaviors or processes as courtship behavior. Under this definition, release of attractant chemicals, vocalizations or other noise used for mate attraction, physical displays directed towards a potential mate and, in some cases, the act of copulation itself can be considered courtship behaviors. Courtship can vary greatly in complexity (the types and numbers of signals used) between species (McKinney 1978), and also in its purpose. It is likely that the role of courtship varies from species to species. The following summary is not meant to be an extensive review of these roles, but will focus instead on courtship behaviors that may affect species formation and isolation, or are hypothesized to be important signals in jumping spiders.

For many species, especially those where the sexes do not cohabitate, locating or attracting a mate is an important courtship behavior. For many arthropods, pheromone trails released into the environment by one sex can be followed by the other to bring a potential pair together. This behavior has been well documented in moths and butterflies, where it is usually the female who sends out the come hither chemical signal (Thornhill & Alcock 1983), though there are cases where males are the signalers (Byers, 1983) Chemical courtship does not have to be limited to the function of mate location, however. In many cases, as with salamanders (Houck & Reagan, 1990) and lobsters (Bushmann & Atema, 1997), chemicals emitted by one sex or both appear to play an important role in sexual stimulation once the sexes have located each other. The sex pheromones of even closely related populations may vary in their chemical content (McElfresh & Millar, 2001), and there is strong evidence that individuals of the receiving sex frequently prefer signals of their conspecifics over those of other species or varieties (Leal 1996, McLennan and Ryan 1997, Kodric-Brown & Strecker 2001). There are many other behaviors besides chemical release that can serve a mate location function. Animals like frogs, crickets, and many birds attract mates with specific mating calls, while other animals use signals like bioluminescence or gaudy displays of brightly colored or oddly shaped body parts to advertise their location to potential mates.

Once a mate is located, the signaler often has to attract their attention and keep it in order to successfully mate. Many courtship behaviors, like elaborate displays of ornamentation in birds and dancing in jumping spiders may serve this purpose. Bright or contrasting colors help distinguish a potential mate from the surrounding environment (McNaught & Owens 2002), or may serve as signal amplifiers when they are coupled with other behaviors in multimodal displays (Uetz & Roberts 2002). But displays like these may serve several other purposes, as well. Not only does a showy display help focus attention on its displayer, but it may help the receiver to identify him as one of her own kind (Mayr, 1942), or to allow her to make decisions on his potential quality as a mate (Andersson, 1994).

For animals with estrous females or females who are polyandrous, courtship allows a male to assess the reproductive status of a female. Males with estrous females can detect if their mate is receptive through the chemical or behavioral cues she sends him. Males with polyandrous females may be able to detect whether or not she has been previously mated and make mating decisions based on such information. This may be the case with several spiny orbweaving spiders (Bukowski & Christenson, 1997; Bukowski *et al*, 2001), where males release more sperm to virgin females and are more likely to undergo more copulation bouts with virgins than with mated females.

In these studies, male spiny orbweavers spend equal time courting either treatment of female prior to copulation, suggesting that is during copulation that they receive mating status information.

Courtship may often serve as a trigger for further physiological responses that lead to successful reproduction. An early study using ring doves indicated that females were much more likely to display reproductive behaviors (correlated with increased reproductive hormone levels) such as nest building if they were exposed to courting males (Erickson & Lehrman, 1964). This is certainly the case when we consider copulation as a form of courtship because in many species females require copulation to induce ovulation (Marler & Hamilton, 1966). Other reproductive processes can be affected by copulation as well. In fruit flies, substances in male ejaculate actually speed up the timing of oviposition and make females less likely to remate (Wolfner 1997).

Before we can implicate original or additional isolation barriers and their roles in speciation, we need to accumulate adequate information on the morphologies, behaviors, and other traits that may be creating such barriers to gene flow between related species. In the following study, I compare reproductive behaviors and morphological characters of two sympatric species of jumping spider with the goal of identifying potential gene flow barriers within the genus *Phidippus*.

1.1.4 Courtship and Reproduction in Jumping Spiders

When asking questions about speciation, sexual selection, or courtship behavior, species in the spider family Salticidae are incredibly interesting research subjects. Commonly referred to as jumping spiders, salticids are widely distributed and highly diverse in their morphologies and life histories. They are visually oriented animals with well developed color vision that rivals our own (Land, 1985), but they also show responses to chemical and vibratory stimuli (Tietjen & Rovner 1982, Barth 1982). All three stimulus types probably play a role in their highly complex courtship behavior (Jackson, 1982), though visually based behaviors have received most of the attention in the literature. Like all spiders, adult male and female salticids are dimorphic, with males possessing several modified secondary sex characteristics, including paired sperm transferring organs located on the distal end of each pedipalp. Unlike many other spider families, where males are much smaller than females, salticid males often rival the female in body size. Male salticids are also more strikingly colored and ornamented than their typically drab females, and perform a complex courtship that contains a variety of behavioral elements and visual badges directed towards a female. Badges and behavioral elements differ between species, and, as mentioned previously, have been implicated as reinforcement barriers in a group of Arizona salticids in the *Habronattus pugillis* species group (Masta &Maddison, 2002). As is the case with many animals, it is the male salticid that does the displaying, while the female is considered to be the choosy sex. Jackson (1982) has outlined the major courtship behaviors of salticids in general, and I will review them here by describing a typical courtship bout followed by copulation:

A male salticid is likely to encounter a female conspecific’s dragline or sleeping nest before he locates the position of the female. When he encounters a female’s silk, he will tap it with his pedipalps, presumably processing chemical signals left on the silk by the female (Pollard, et al., 1987). He may then follow a dragline to the location of the female or increase searching behaviors in the immediate area of the encountered silk. Once a salticid male sees a female, he will typically begin courting her with one of two types of “dance” (in some species, both types are employed in succession). “Zigzag dancing” involves a side to side movement and “strait-line dancing” employs a forward and backward movement in relation to the female’s position (Jackson, 1982). Both types are usually coupled with various leg and palp waves and specific body postures. As a male closes in on a female during his dance, he will reach out and touch her face with his front legs and then attempt to crawl onto her back over her cephalothorax. During and after this mount he frequently taps the female with his legs, palps, and abdomen (Jackson, 1982). Occasionally, females may respond to a male’s approach by charging towards or striking at the male before becoming submissive, or they may reject their suitor by walking or running away from him.

Copulation occurs when a successfully mounted male will reaches under one side of the female’s abdomen with his front legs and rolls it to the side. This orientation is the correct position for him to contact one of the female’s external genital openings with his pedipalp. When palp/ epigynum contact is achieved, the male expands the hematodochae (inflatable sclerites within the palp), thereby inserting the tip of the sperm conduction portion of the palp (embolus) into the epigynum of the female. Immobile, capsulated sperm are transferred into the female's spermathecae shortly thereafter. As the female has two genital openings connected to paired spermathecae and the male has paired pedipalps, he will often disengage the first palp and roll the female's abdomen in the opposite direction in order to expose and inseminate this side as well. Copulation is usually terminated by the female, often when the male is attempting to switch sides (Jackson, 1982).

Members of the salticid genus *Phidippus* are good candidates for comparative reproductive studies. They are the largest of the North American salticids and are therefore easier to observe than smaller heterospecifics. Their large size also makes them easier to spot and collect in the field, and easier to rear to adulthood because they can take large, easy to rear prey. In North Texas, there are several sympatric *Phidippus* species that occur in high numbers. *Phidippus audax* and *Phidippus carolinensis* were chosen for this study because they were the most commonly encountered *Phidippus* during the time subject collections were made.

Several studies dealing with the reproductive behaviors displayed by various species of *Phidippus* have been conducted and provide background information for comparison. Reproductive behaviors of *P. audax* have been examined by several researchers (Taylor & Peck 1975, Roach 1988), but little is known about the courtship of *P. carolinensis*. This study will serve to document the courtship behaviors of *P. carolinensis* as well as compare reproductive behaviors between both species and determine whether any of the aforementioned modes of speciation or species reinforcement are applicable to these species.

1.2 Methods

1.2.1 Spider Collection and Housing

Immature *P. carolinensis* were collected in April and May, 2004, from the Ft. Worth Nature Center, a remnant prairie preserve in northern Texas. These animals were of intermediate age (2-3 molts from adulthood) and were frequently found upon metal structures, including fence supports and farm machinery. One spider was collected from a blooming *Asclepias* plant, and three were found on a wooden fence. Spiders could usually be found in close proximity to one another, with 3 or 4 animals sharing the same piece of farm equipment, etc. Though multiple searches were made throughout the nature center, populations of *P. carolinensis* seemed to be localized near human disturbance (i.e., where metal structures were present).

P. audax, a much more commonly encountered spider than *P. carolinensis*, was collected from a variety of locations throughout the length of the experiment. Several immature subjects were taken from the Ft. Worth Nature Center from the same locale as *P. carolinensis*, but the majority of animals were collected in and around human dwellings throughout the Arlington, TX, area. Whereas *P. carolinensis* individuals were closely synchronized in age at the time they were collected, *P. audax* was found in varying instars throughout the year.

Once in the lab, the spiders were housed in individual clear plastic containers with removable, rimmed lids. These lids allowed the salticids to build a retreat web in the upper corners without excessive disturbance when the lid was opened for food addition, etc. Several dried oak leaves were added to each container to allow the spiders a hiding place and simulate a natural environment. Immature spiders were fed a mixture of *Drosophila* adults, *Dermestes* larvae and *Tenebrio* larvae twice weekly until they reached adulthood. At this point they were feed once weekly. Water was continuously available in a shell vial plugged with cotton.

1.2.2 Mating Trials

To observe courting and copulatory behaviors, same-species pairs were introduced and observed in a clean container of the same type as their housing container. Because both male and female salticids are occasionally cannibalistic, mating trials were conducted on days immediately following feedings to insure that the spiders were satiated. Males were introduced to the courtship arenas first and allowed to sample a portion of fresh webbing taken from their assigned female's retreat web. This was done to alert the male of a female's presence prior to her introduction, as male salticids have been shown to pick up chemical cues in female silk (Taylor, 1998) and are more likely to court in its presence (pers. obs.). After a male showed an interest in the silk (usually by waving front legs, tapping palps, or displaying) a female was introduced into the container and the lid was placed on it to prevent the excited spiders from escaping. Once a male began courtship, all behaviors of both the male and the female were recorded, as well as latency times from beginning of courtship behavior to palpal insertion (Courtship Latency) and from insertion to termination of copulation (Copulation Latency). Matings were considered successful if a male was able to mount a female, and unsuccessful if he was not. Pairs were immediately separated after copulation or if either spider attempted to prey upon the other. A video recording was made of two pairings (one for each species) in 2005.

Behaviors displayed in successful matings and unsuccessful pairings were compared within a species in order to infer which behaviors are most important for a successful mating to occur, and also to determine the extent to which each sex controls the outcome of a given pairing. Behaviors were compared between species to determine if there is a difference in the courtship behavior repertoires between them. Courtship and copulation latencies were also compared between species.

1.2.3 Badges

Jackson (1982) defines badges as static properties of a spider's morphology that may serve a communicatory function. Coloration, pattern, and physical structures like tufts of bristles or apophyses could all be forms of jumping spider badges, as long as they convey information to conspecifics. Badges that are likely to convey information during courtship were identified in both males and females of *P. audax* and *P. carolinensis*, and compared between species.

1.2.4 Palp Imaging

Left pedipalps were removed from *P. audax*, *P. carolinensis*, and from preserved specimens of a third species, *P. clarus*, for comparison. Palp specimens were allowed to air dry and were then gold-palladium sputter coated. Three specimens from each species were mounted in three orientations (left side view, right side view, underside view) for a total of 9 mounted specimens per species. Specimens were examined and photographed using a JEOL 35CF scanning electron microscope and VitalSCAN imaging software. Length and width of the palpal bulb, along with the dimensions of the palpal apophysis and distal end of cymbium were compared using ImageJ software.

1.2.5 Statistical Analyses

Differences in courtship latencies and copulation latencies between *P. carolinensis* and *P. audax* were each analyzed using a Mann-Whitney test. Courtship behaviors that were displayed by both species were compared *between* them using Fischer's Exact Test. Binomial probabilities were calculated in order to compare behaviors between successful and unsuccessful matings *within* a species.

1.3 Results

1.3.1 Courtship Behaviors

For *P. audax*, 55 pairings were made, with an outcome of 24 successful matings and 31 unsuccessful encounters. For *P. carolinensis*, 25 pairings were made, with an outcome of 15 successful matings and 10 unsuccessful encounters.

As noted previously, the courtship behavior of *P. audax* has been documented on several occasions prior to this study, and their behavior here does not deviate greatly from these observations with the exception that female behavior is also described. Male *P. audax* typically spot a female while they are still some distance away from her and usually begin a courtship approach immediately. Courtship consists of rhythmic waves of the front legs as they are held up in a 90° angle from the body, coupled with simple up and down movements of the pedipalps and a zigzag approach. Females typically responded to the male's display by turning towards him. They would frequently wave one front leg and tap their pedipalps while watching a displaying male. As a courting male approached within a few centimeters of her, a female would often reach her front legs out and quickly strike the male with them, or she would prolong this striking action by meeting the male's outstretched front legs and pushing her chelicerae against his (wrestle). At this point, the female would either tuck in her legs and allow the male to mount (submissive), or she would turn from the male and walk or run away from him (reject). Male *P. audax* would frequently continue to court and follow females that had rejected them, and this extended courtship sometimes led to a successful mating minutes later. Successful courtship bouts averaged 2.8 ± 1.8 minutes in length. Forty four percent of the pairings in this study resulted in successful matings.

In *P.* audax, copulation typically began seconds after the male had mounted a submissive female and rolled her abdomen to access the epigynum. In many cases, males would dismount a restless female and re-court her. If she was again submissive, the male would resume copulation. Copulation times varied greatly in this species, with an average length of 30.3 ± 35.3 minutes. The shortest recorded *P.* audax copulation during this study lasted for 2 minutes, and the longest lasted 150 minutes.

In comparison to the zigzag courtship dance of *P. audax*, male *P. carolinensis* courtship is rather simple. Males frequently failed to engage females with courtship displays from a distance and would run or hop towards them instead. Once in close proximity to the female (<2 cm.), these hopping males would take up a rigid pose with the front legs held out in front of them at a 45° angle. This pose was frequently accompanied with a rapid flutter of the abdomen (buzz) and a one-at-a-time rotation of the pedipalps (palp grinding). If a male began courtship from a distance (>5cm), he would hold his front legs at a steeper angle to his body (about 90°) with the accompanied buzz and palp grinding. Courting males never moved rapidly toward their females; instead, they would slowly creep towards them while holding their rigid courtship posture. As a long-distance courting male approached his female, he would drop his front legs down to the 45° angle as he neared her. Female *P. carolinensis* would often watch a courting male with apparent interest, alternately waving their front legs and rapidly taping their pedipalps up and down. In two pairings, females moved towards their courting males, touched them with their front legs, and then become submissive. Female *P. carolinensis* were only rarely observed to strike a male, and were never observed to wrestle with one. Unlike *P. audax* males, *P. carolinensis* males were unlikely to follow a female that turned away from them during courtship. Even though males frequently failed to actively court females, sixty percent of the pairings in this study resulted in successful matings. Successful courtship bouts were not significantly different in length than those of *P. audax* (Mann-Whitney U = 112.5, P_2=0.9522), with an average courtship latency of 2.6±1.4 minutes.

Figure 1.1: *P. carolinensis* in copula

Copulation in *P. carolinensis* was similar in appearance to that of *P. audax*, with males mounting females and immediately rolling their abdomen in order to insert their embolus tip into the epigynum (Fig 1). However, female *P. carolinensis* were not as restless as female *P. audax*; males very rarely dismounted and re-courted them. Copulation latencies were significantly longer in *P. carolinensis*than in *P. audax* (Mann-Whitney U=51, P=0.0012) with an average length of 86±50.4 minutes.

A list of observed behaviors (courtship and otherwise), their abbreviations and definitions is presented in Table 1. Many of these behaviors were displayed by both species. However, two behaviors, M.Fl and F.Wr, were only observed to occur in *P. audax* mating trials. Of these behaviors, male courtship displays were by far the most common behavior displayed by both species (Fig 2). Male *P. audax* courted their females in 90.9 percent of encounters (n=55), and male *P. carolinensis*males courted in 76 percent of encounters (n=25). Though *P. carolinensis* males exhibit a lower tendency to court than *P. audax* males, the difference is not significant (Fischer's Exact Test P_2=0.0889).

The remainder of observed behaviors occurred much less frequently than male courtship. All were observed in fewer than 30 percent of mating trials with the exception of two behaviors: female wrestle and female aggressiveness. Female wrestle was displayed only, and fairly frequently (49 % of encounters, n=55), by *P. audax* females, leading to a highly significant difference from *P. carolinensis*female s (Fischer's Exact Test P=<0.0001). There were aggressive females in both species, though aggressive behaviors were rarely displayed by *P. carolinensis*(observed in 8% of trials, compared to 34.6% of *P. audax* trials). Female *P. audax* were significantly more aggressive towards males than female *P. carolinensis* (Fischer's Exact Test P_2=0.0137).

Table 1.1: Behaviors Exhibited in Mating Trials.

Sex	Abbreviation	Description	Notes
males	M.Ct	Courtship Display	As described in text
	M.Ap	Approaches ♀ without courting	Includes hopping and running towards.
	M.Ag	Aggressive	Fangs bared, front legs rigid without wave
	M.Wch	Watches ♀ without display	Frequently w/pedipalp taps and single front leg wave
	M.Fl	Flees from ♀	
	M.Pr+	Pursues retreating ♀	
	M.Pr-	Does not pursue retreating ♀	
Females	F.Wch	Watches ♂ without approach	Frequently w/pedipalp taps and single front leg wave
	F.Ap	Approaches ♂	Includes hopping and stalking
	F.SS	Startled by unseen ♂, then submissive	Unseen male usually not courting
	F.Fl	Flees from ♂	
	F.Rj	Rejects ♂ by turning away from him	Occurs after ♀ observes courtship
	F.St	Strikes at ♂	As described in text
	F.Wr	Wrestles with ♂	As described in text
	F.Ag	Aggressive	Fangs bared, front legs rigid without wave

As mentioned previously, only male *P. audax* fled from their females. This behavior was observed in 23.6 percent of trials with *P. audax* for a significant difference between the two species (Fischer's Exact Test P_2=0.0071). The occurrence of one other behavior was also significantly different between the species: *P. carolinensis* males failed to pursue a rejecting female more frequently than *P. audax* males (Fischer's Exact Test P_2=0.0234). Though not strongly supported by statistical analyses, female *P. carolinensis* appear to watch males more frequently than *P. audax* (Fischer's Exact Test P_2=0.0889). They also appear to be startled by males and then become submissive more frequently than *P. audax* females (Fischer's Exact Test

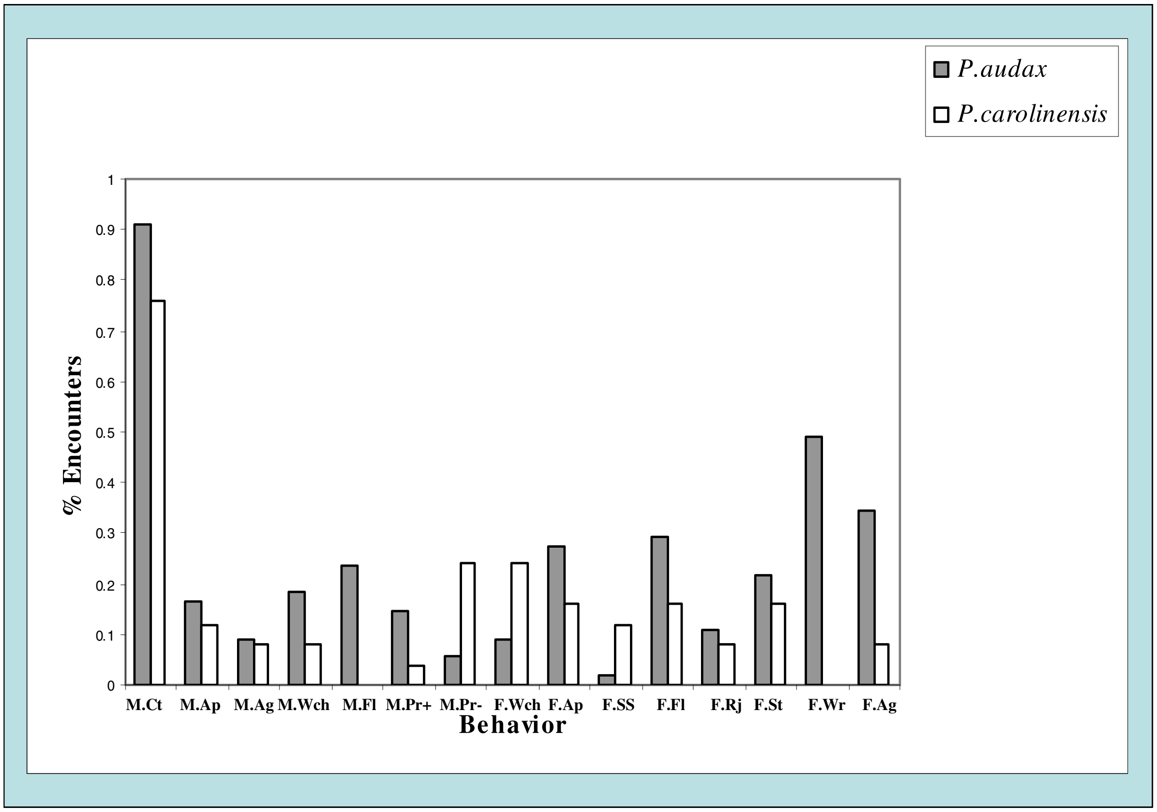

Figure 1.2: Behaviors exhibited during mating trials compared between species.

P_2=0.0850). The following behaviors were not statistically significant between species according to Fischer's Exact Test, but occurred more frequently with *P. audax*: M.Ap (P_2=0.7443), M.Wch(P_2=0.3226), M.Pr+(P_2=0.2602), F.Ap(P_2=0.3968), F.Fl(P_2=0.2715), F.St(P_2=0.7642). There appears to be no difference in the frequency in which either species' males are aggressive towards females (P_2=1) or either species' females reject courting males (P_2=1).

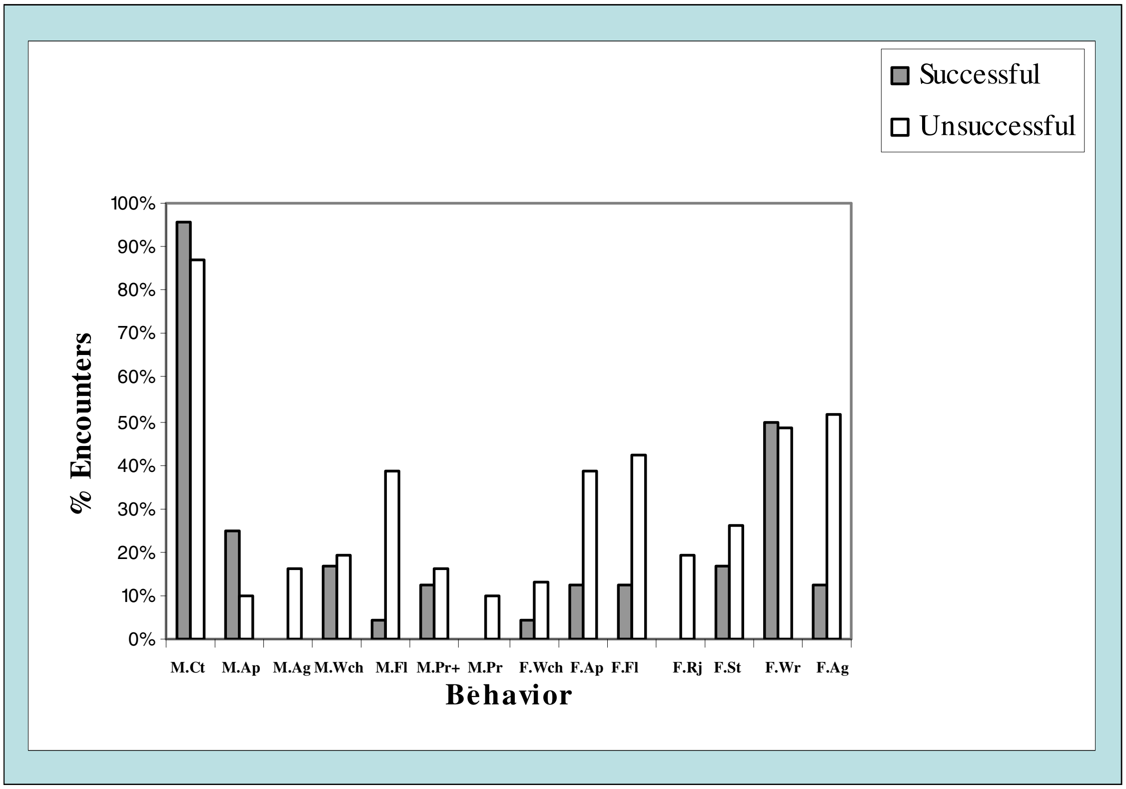

Figure1.3: A comparison of *P. audax* behaviors between successful matings and unsuccessful encounters.

Behaviors were compared between successful matings and unsuccessful encounters within a species by calculating binomial probabilities. In *P. audax,* all previously documented behaviors (Table 1) were compared except for F.SS, as it occurred so infrequently in this species (Fig 1.3).

Only two *P. audax* behaviors occurred more frequently in successful matings than unsuccessful encounters: M.Ap and F.Wr, but the differences in occurrences between types of encounter were not significant for either behavior (M.Ap P_2=0.5078, F.Wr P_2=0.7011). Several behaviors occurred significantly more often in unsuccessful encounters than in successful matings (Table 2). Males fled from females (P_2=0.0034) and females were aggressive towards males (P_2=0.0044) much more often in unsuccessful encounters. Females also approached males (P_2=0.0352) or fled from

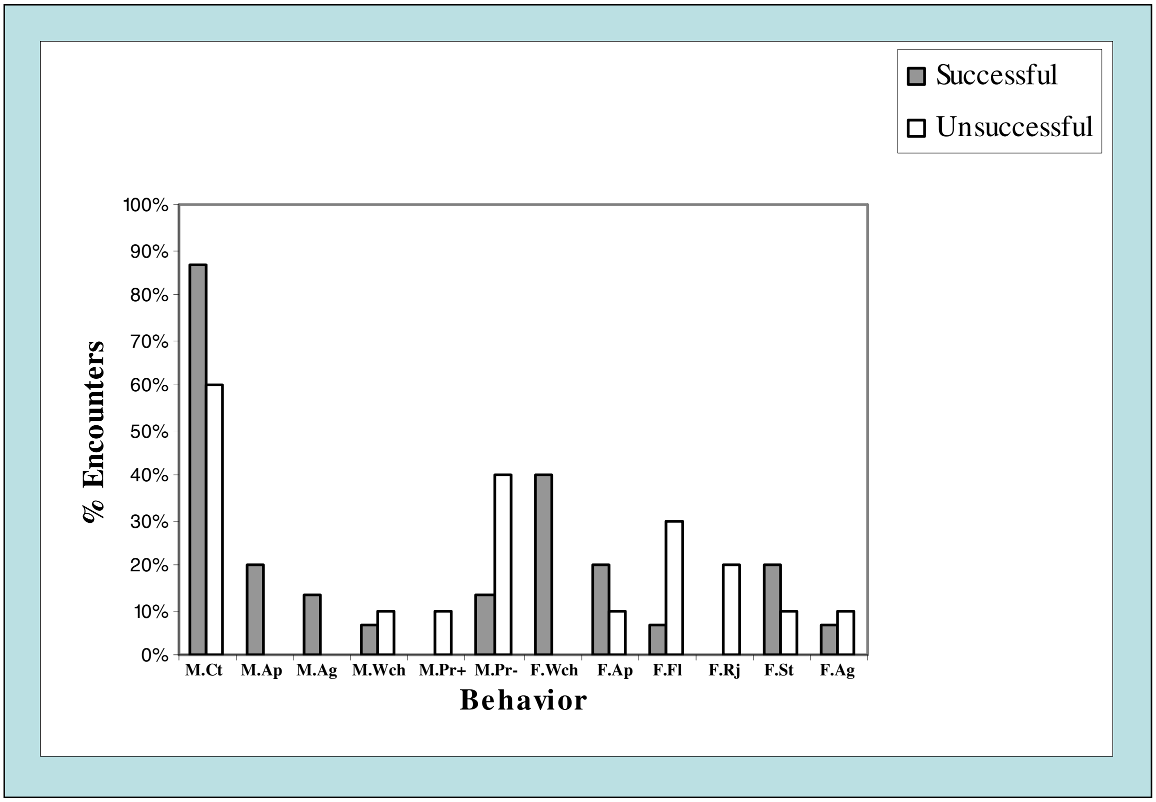

Figure 1.4: A comparison of *P. carolinensis* behaviors between successful matings and unsuccessful encounters

them (P_2=0.0213) more often in these encounters. F.Rj was only observed in unsuccessful encounters and the difference in number of observations was significant (P_2=0.0313). Both M.Ag and M.Pr- were also only observed in unsuccessful encounters, but since they were both observed at low frequencies, the difference between successful and unsuccessful encounters was not significant for either (M.Ag P_2=0.0625; M.Pr- P_2=0.25). There were no significant differences in occurrence of M.Ct (P_2=0.6718), M.Pr+ (P_2= 0.7266), F.Wch (P_2=0.375), or F.St (P_2=0.3877).

Unlike *P. audax*, several *P. carolinensis* behaviors (Fig 4) were only observed in successful matings. F.Wch was one of these behaviors, and occurred frequently enough during successful matings for there to be a statistically significant difference from unsuccessful encounters (P_2=0.0313, Table 2). M.Ap and M.Ag also only occurred during successful matings, but observations were infrequent and not significantly different from unsuccessful encounters (M.Ap P_2= 0.25; M.Ag P_2=0.5). Alternatively,

Table 1.2: Statistical Summary Table for Between and Within Species Behavior Comparisons. (*) indicates statistical significance at α=0.05.

Behavior	Between Species (Fischer's Exact Test P)	*P. audax* within species (Binomial Probability P)	*P. carolinensis* within species (Binomial Probability P)
M.Ct	0.0899	0.6718	0.1671
M.Ap	0.7443	0.5078	0.25
M.Ag	1.0	0.0625	0.5
M.Wch	0.3226	0.7539	1.0
M.Fl	** 0.0071	** 0.0034	N/A
M.Pr+	0.2602	0.7266	1.0
M.Pr-	* 0.0234	0.25	0.6875
F.Wch	0.0889	0.375	* 0.0313
F.Ap	0.3968	* 0.0352	0.625
F.SS	0.085	N/A	N/A
F.Fl	0.2715	* 0.0213	0.625
F.Rj	1.0	* 0.0313	0.5
F.St	0.7642	0.3877	0.625
F.Wr	*** <0.0001	0.7011	N/A
F.Ag	* 0.0137	** 0.0044	1.0

two behaviors were only observed in unsuccessful encounters: M.Pr+ and F.Rj. Neither of these behaviors occurred frequently enough to be significantly different from successful matings (M.Pr+ P_2=1, F.Rj P_2=0.5). M.Ct, F.Ap, and F.St. occurred more frequently in successful matings, but not significantly so (M.Ct P_2=0.1671; F.Ap P_2=0.625; F.St P_2=0.625), and M.Pr-, M.Wch, F.Fl, and F.Ag occurred more frequently in unsuccessful encounters but, again, not significantly so (M.Pr- P_2=0.6875; M.Wch P_2=1; F.Fl P_2=0.625; F.Ag P_2=1).

1.3.2 Badges

Possible courtship badges for each species and sex were identified (Fig 5) based on the parts of the body displayed towards potential mates during the courtship. Since females typically observe males face-on in courtship, male badges were likely to be displayed on the first and second pairs of legs and the anterior portion of the cephalothorax. Because females face males during courtship and males climb over them during copulation, female badges were likely to be found on the same body parts as males, as well as the dorsal cephalothorax and upper side of the abdomen.

Badges in adult male *P. audax* include white setae tufts found on the palpal elbow and around the joints of the foreleg. During courtship, both the forelegs and the pedipalps are moved in a rhythmic fashion, and the contrasting tufts may help call attention to this movement. Male *P. audax* chelicerae are usually larger and differently shaped than those of females and juveniles; they may function as a badge by aiding in sex recognition or by indicating male quality. Female *P. audax* also have tufts of setae on their forelegs, but, in contrast to males, these tufts are less pronounced and usually do not contain as many white setae. Since female *P. audax* often wave at or strike at males with their forelegs, it is possible that these tufts serve a communicatory purpose. Like males, females have metallic green chelicerae, but they are similar in shape to

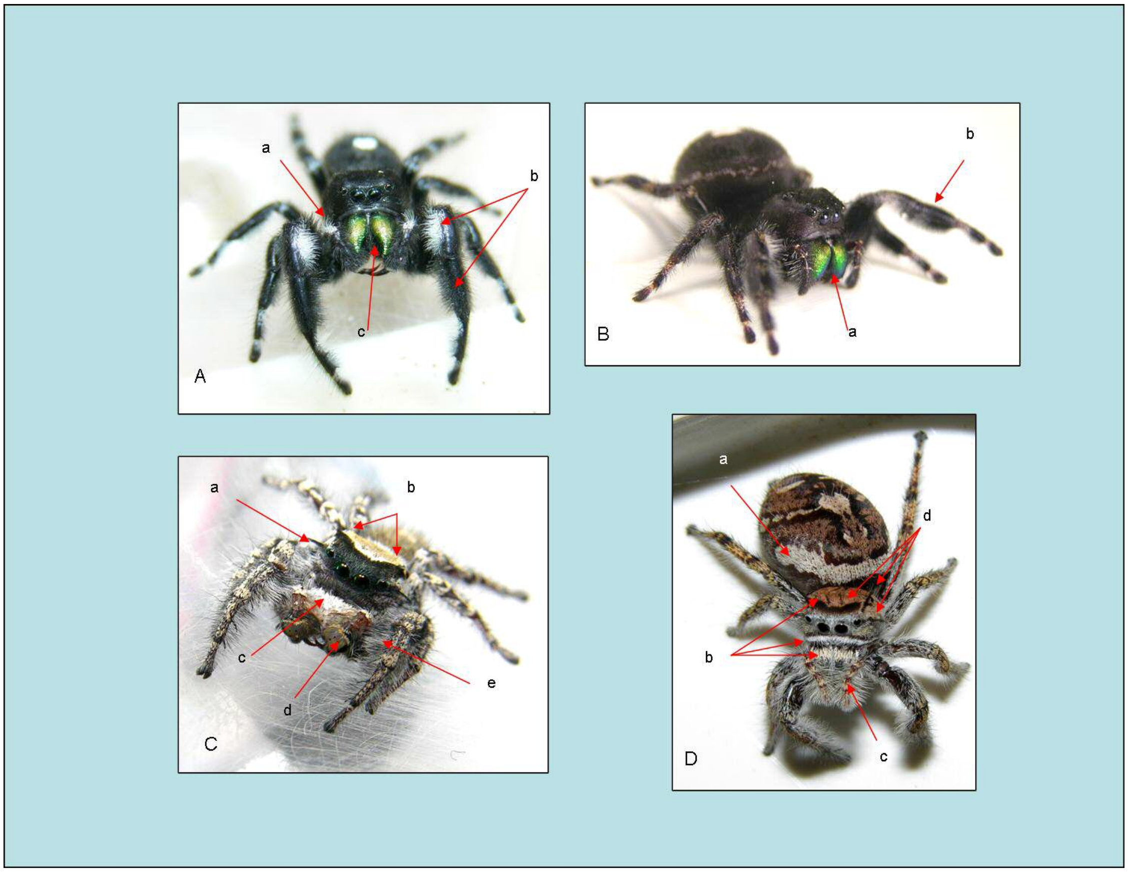

Figure 1.5: Potential courtship badges on both male and female *P. audax* and *P. carolinensis*. A) *P. audax* adult male a) White patch on elbow of pedipalp b) Alternating black and white setae tufts on foreleg c) Enlarged, metallic green chelicerae B) *P. audax* adult female a) Metallic green chelicerae b)Black setae tufts on foreleg C) *P. carolinensis* adult male a) Black mid-ocular tufts b)Thick, black anterior eye band bordered by black setae fringe c) White clypeus fringe d) Cymbium of pedipalp rounded and glossy with dark spots, elbow with white patch e) Gray fringes along forelegs D) *P. carolinensis* adult female a)Thick, white basal band on abdomen b) Alternating colors in ocular bands and clypeus band and fringe c) Gray fringe on pedipalps d) 3 distinct sets of ocular tufts

those of juveniles. It is possible that they serve a species recognition function in courtship.

Adult male and female *P. carolinensis* have a higher degree of sexual dimorphism than *P. audax*, therefore leading to a larger variety of potential courtship badges. Size and position of ocular bristles, width, number and color of clypeal and ocular bands, and various fringes on the forelegs and pedipalps all differ between the sexes and are all potential badges. However, the form of ocular bristles and clypeal and ocular banding seen on adult females is often similar in older juvenile instars, suggesting that these structures may serve to camouflage the spiders from predators. Nonetheless, it is possible that they serve a secondary function as courtship badges in adults.

1.3.3. Palp Images

Of the six palpal measurements taken (Fig 1.6: A, B), all but two were significantly larger in *P. audax* (Table 1.3). *P. audax* males have longer cymbiums with wider and deeper distal ends, as well as longer palpal apophyses. This is not surprising, as *P. audax* males are generally larger than *P. carolinensis* males (Edwards 2004).

Table 1.3: Statistical Summary Table for Palpal Measurements. All measurements recorded in microns. (*) indicates statistical significance at α=0.05.

Structure	*P. audax*	*P. carolinensis*	t-test P-value
Apophysis Length	251.2	149.5	* 0.0123
Apophysis Width	77.0	74.6	0.8381
Cymbium Depth at Tip	733.6	507.6	** 0.0031
Cymbium Length	2266.9	1844.1	* 0.0170
Cymbium Median Width	889.8	921.2	0.6153
Cymbium Width a t Tip	581.1	321.3	** 0.0013

However, the width of the apophysis in *P. carolinensis* males was relatively equal to that of *P. audax*, and the median portion of their cymbium is slightly wider than *P. audax* cymbiums. This is probably because the cymbium is rounded in *P. carolinensis* and rectangular in *P. audax*. The diameter of the *P. carolinensis* cymbium widest at the

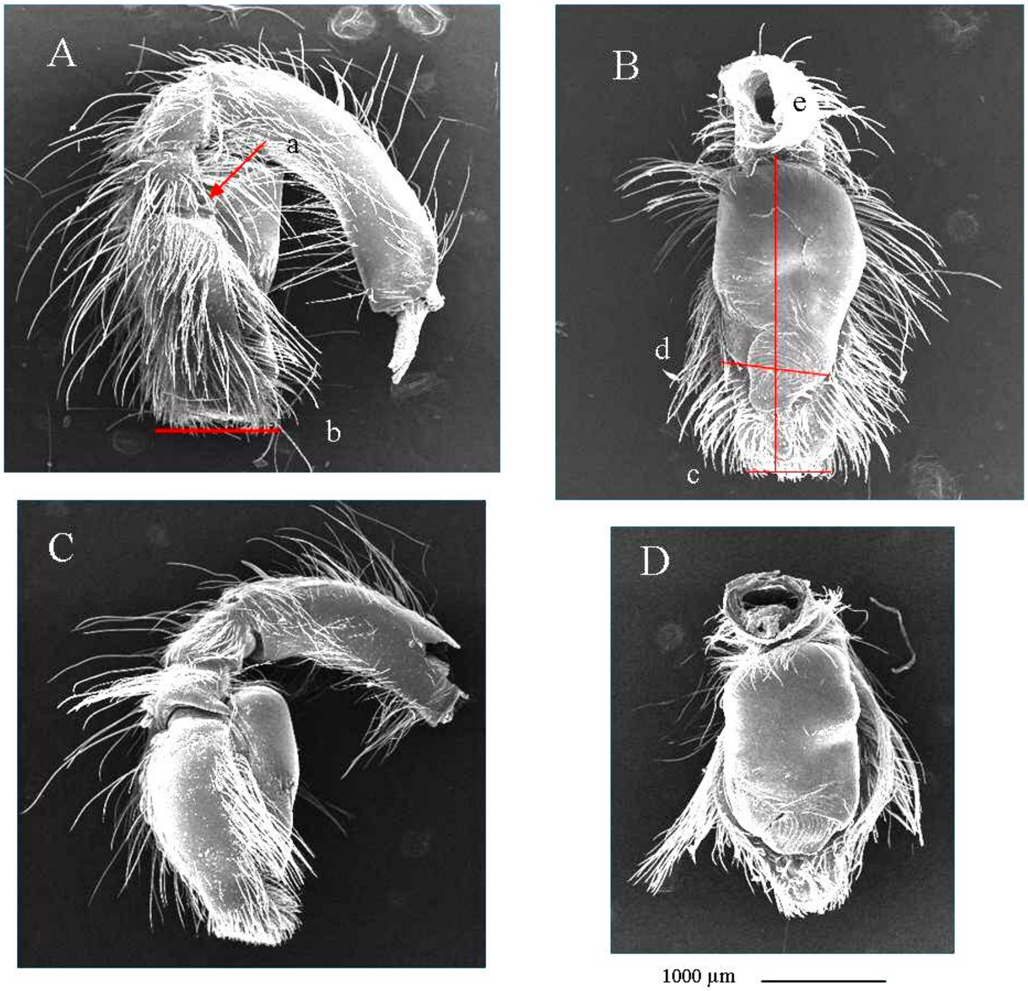

Figure 1.6: SEM micrographs of left pedipalps from *P. audax* (A, B) and *P. carolinensis* (C, D). A, C - a proximal, lateral view (the side of the pedipalp that contacts the female). B, D – Underside of the pedipalp. Measurements taken: a) length and width of apophysis, b) depth of distal end of cymbium, c) width of distal end of cymbium, d) width of middle portion of cymbium, e) length of palpal bulb.

median portion and narrows at the distal end, resulting in a significantly smaller cymbium tip width (t-test df=4, p=0.0013) and depth (t-test df=4, p=0.0031) than in *P. audax.*

The tibial apophysis is generally thought to aid males in locating the correct placement of the palp during copulation by connecting with a receptive groove on the female's venter (Coddington & Levi 1991). A closer look at the thickened apophysis of *P. carolinensis*, coupled with the observation of palp grinding during courtship, may reveal a second function of the apophysis in this species. There is a furrow on the

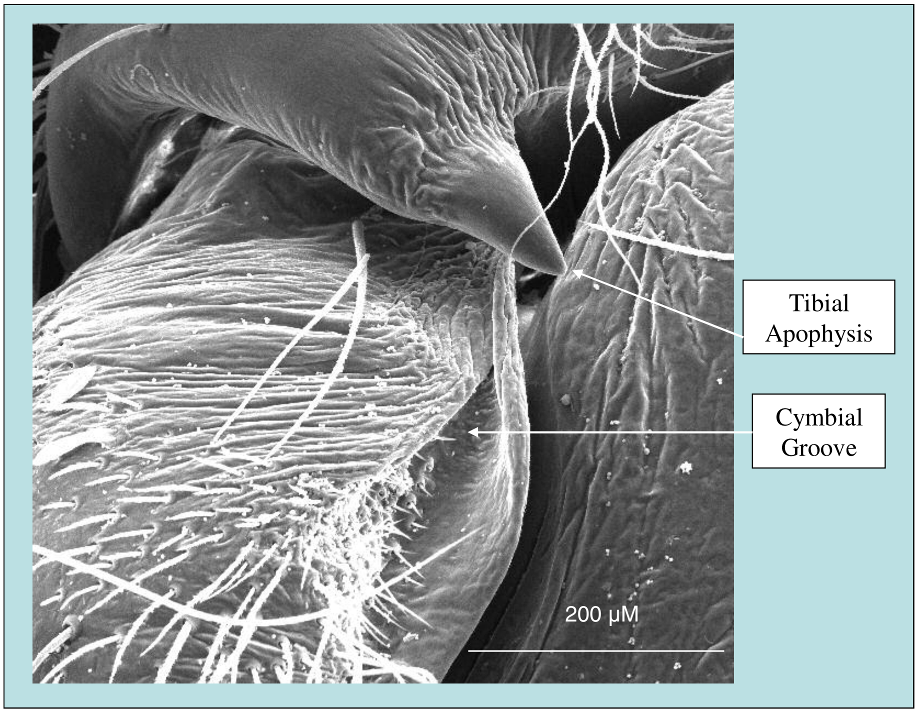

Figure 1.7: SEM image of possible stridulatory mechanism on *P. carolinensis* palp.

cymbium adjacent to the tibial apophysis (Fig 1.7), and it is likely that during palp grinding behavior, male *P. carolinensis* are rotating the cymbium so that the apophysis is rubbed through this furrow, creating vibrations that can be detected by a nearby female. If this is indeed the case, a short, thick apophysis is not surprising, as these dimensions would make it more robust to the mechanical forces placed on it during stridulation.

1.4 Discussion

A zigzag courtship dance is common in the genus *Phidippus* (Taylor & Peck 1975, Jackson 1982, Robertson & Stevens 2002). Male *P. audax* invest a great deal of energy into this active, visually stimulating dance, and it is likely that observant females are able to pick out elements of the dance that differ between males as indicators of overall male vigor and quality. Female *P. audax* also appear to "test" their courting males by striking at or wrestling with them as they approach. If a male is unable to overcome the forelegs of a wrestling female, he is unable to mount her and copulate successfully. In fact, most of the observed "rejection" behavior displayed by female *P. audax* occurred immediately after they had wrestled with a courting male, suggesting that the wrestle itself provided the female with information about the quality of the male. As larger, vigorously courting males are more likely to overcome the powerful courtship wrestle of a large female, large male size and vigor are likely to be selected for. This may explain, in part, why *P. audax* is one of the largest salticids in North America (Edwards, 2004).

Male *P. audax* fled from their females in over 20% of encounters and females fled from their males in close to 30% of encounters, suggesting that both sexes consider each other as potential threats, and with good reason. Both male and female *P. audax* frequently showed aggression towards each other during encounters. On several occasions, animals had to be forcefully separated with tweezers after one attacked the other, and often one animal, if not both, sustained serious injuries. On several occasions, males were observed to court and retreat from aggressive females at the same time. As they widened the distance between them, the retreating males would cease courting and flee. This suggests that the courtship dance displayed by male *P. audax* may serve the primary function of short circuiting the female's prey recognition and hunting behavior neural pathways long enough for a male to accomplish his purpose, whether it be approach or retreat. If this is the case, females may not be receiving adequate quality signals at this point in courtship and the need to test their males by engaging them in a wrestle following the courtship dance makes sense.

Both male and female *P. audax* appear restless during courtship, and males would frequently hop off and recourt females before mounting them again. Because of their large size and bright black and white coloring, *P. audax* may be more susceptible to visual predators than other, more camouflaged species like *P. carolinensis*. Typically, their excellent vision would cue them to an approaching predator, but during copulation, neither male nor female has their eyes positioned for a clear view of approaching danger. Restlessness, frequent breaks in copulation, and relatively short copulation times in many of the pairings observed may be side effects high predation risk during copulation.

The courtship display of *P. carolinensis* differs greatly from *P. audax* and other zigzag dance courtships within the genus. Instead of flashy approach movements, *P. carolinensis* males employ a static courtship posture along with possible vibratory elements. These elements include an abdomen buzz, which may be a common courtship component in jumping spiders (Maddison & Stratton 1988b), and palp grinding, which is, to my knowledge, an undescribed mode of stridulatory communication in the jumping spiders, though other forms of stridulation are known to occur (Maddison & Stratton 1988a, Elias et al 2003). It is possible that these vibratory components focus a female's attention towards the posturing male, as female *P. carolinensis* watch and wave at courting males significantly more than female *P. audax* do. Female interest in courting males seems to be extremely important for successful mating in this species because males rarely approach females rapidly when in courtship posture and interested females will oftentimes approach courting males.

It is possible that female *P. carolinensis* pick up fewer mate quality cues from the courtship of their males than *P. audax* females. Female *P. carolinensis* were never observed to engage courting males in a wrestle, and strikes by females usually occurred before they fled from males, suggesting a defensive rather than mate-testing behavior. Though male courtship was equivalent in length to that of *P. audax*, copulation latencies for *P. carolinensis* were, on average, close to 3 times as long as those for *P. audax*. The cryptic coloration of both males and females may make longer copulations more feasible from a predation standpoint, but since sperm is transferred early in copulation, we must examine the *need* for such lengthy encounters. This question and a possible explanation (cryptic female choice) will be revisited in Section II of this thesis. For now, let us assume that the act of copulation itself sends important male quality signals to the female. If this is the case, *P. carolinensis* females may rely on these signals more than female *P. audax* because they appear to be exposed to fewer quality indicators (i.e., vigor, strength) prior to copulation.

Because little is known about the species distributions and genetic relationships in this genus, it is difficult to implicate specific physical barriers to gene flow as original, species forming barriers, though this may certainly be a speciation mechanism in the group. Currently, there is more evidence suggesting that sexual selection is playing a major role as an original barrier to gene flow in *Phidippus*, as it has already been implicated in speciation in the jumping spider genus, *Habronattus* (Masta & Maddison 2002). The drastic difference between *P. audax* and *P.carolinensis* courtship behaviors and badges indicates that strong selective forces have been at work on these traits, a hallmark of sexual selection.

Because of the nature of this study, only potential pre-zygotic gene flow barriers were observed. As I only conducted same-species mating trials, I am unable to address issues of hybridization, hybrid inviability, etc, directly. However, a hybridization study conducted on allopatric populations of *P. audax* suggests a degree of genetic incompatibility between them (Taylor & Peck 1975) and it is probably safe to assume that genetic incompatibility between separate species would be even higher.

Hybridization studies in salticids are mostly limited to pairings considered to be subspecies (Masta & Maddison 2002) or distinct populations within a species (Taylor & Peck 1975). However, Edwards (1980) successfully mated and raised viable offspring from crosses between two closely related species of *Phidippus*: *P. otiosus* and *P. regius*. *P. regius* is thought to have diverged recently from *P. otiosus*, as they still share many morphological characters (Edwards, 2004) and are sister species out of seven North Carolinian *Phidippus* species analyzed for genetic divergence (Terranova & Roach 1987). To my knowledge, a complete genetic phylogeny including both *P. carolinensis* and *P. audax* has not been done, but Edwards (2004) has recently revised relationships in the genus by creating a phylogeny of all described *Phidippus* species based on 70 morphological characters. According to Edwards' intensive study, *P. audax* and *P. carolinensis* are quite divergent from each other, with *P. carolinensis* being more basal and *P. audax* more recently derived. Given this information, it is probably unlikely that hybridization between the two species could take place. However, without hybridization studies, this possibility cannot be completely ruled out.

Species specific courtship displays and badges are potential additional isolation barriers, no matter if they've arisen through sexual selection or as a mechanism to prevent hybrid matings. In the case of *P. audax* and *P. carolinensis,* it is highly unlikely that hybrid matings would ever take place in the wild. Even if the two very different-looking spiders recognized each other as potential mates, their drastically different courtship displays and badges would unlikely convey the correct information between species to lead to a successful mating.

Male embolic and female genitalic structures are quite conserved in salticids, and this trend holds true for *Phidippus* species as well (Edwards 2004). Though there are enough differences in the shape and orientation of these structures to tell species apart taxonomically, these differences may not be enough to prevent successful insemination between species. It is unlikely that the “lock and key” hypothesis of species reinforcement is at work within the genus *Phidippus* except in species that are strongly divergent in structure from each other. Differences in palpal structure between *P. audax* and *P. carolinensis* are mostly in structures of the cymbium and palpal apophysis, which do not play a role in sperm intromission. It is likely that the rounded, relatively bare cymbium of *P. carolinensis* accompanied by the long setae fringe on each side functions as a courtship badge, while the thickened apophysis and accompanying cymbial groove function as a stridulatory organ during palp grinding.

Phenology can also play a major role in whether gene flow can occur or not. Because adult *P. carolinensis* are only found in the early summer, and adult *P. audax* are most common in the spring months, it is unlikely that a mixed species pairing would ever occur in the wild; however, because low numbers of adult *P. audax* are found throughout the year in the study area, I cannot rule out the possibility of a mixed species adult encounter occurring.

In conclusion, several courtship-related mechanisms have the potential to serve as barriers to gene flow in the genus *Phidippus*. The most effective of these mechanisms is likely to be the large difference in the types of courtship behaviors performed by each species. *P. audax* has an active courtship with many visual components and a strong female participation component (i.e., wrestle) while *P. carolinensis* has a simple visual display coupled with several vibratory elements. Overall appearance differs greatly between the two species and may aid in species recognition prior to courtship. Genitalic morphologies are unlikely to prevent insemination between species, but because the two species are phylogenetically and phenologically divergent, hybridization is unlikely to occur with much success. Further studies are needed to determine whether hybridization is indeed a possibility between this sympatric pair of species.

CHAPTER 2

INVESTIGATION INTO FORCES DRIVING POLYANDRY IN *PHIDIPPUS AUDAX*

2.1 Introduction

Polyandry, or the mating of a female with more than one male, is a common occurrence across many diverse groups of animals. There are currently several theories as to what forces are responsible for the presence of polyandrous females across a large and varied group of species. Of course, each theory will not sufficiently explain polyandry for each of these species and it is likely that different combinations of these proposed forces are driving the evolution of polyandry in species that vary in their life histories.

Arthropods are a group of animals that are receiving much attention in the field of polyandry research. In this chapter, I will introduce some of the current theories on forces driving polyandry that are applicable to arthropods in general and to salticid spiders specifically.

2.1.1 Possible Forces Driving Polyandry in Arthropods

One of the most obvious potential reasons for polyandry is a female's need to renew a dwindling sperm supply. In the late 1940's, Bateman (1948) exemplified this by showing that female *Drosophila melanogaster* in a food stress treatment produced more offspring when they were allowed to copulate with multiple partners than when mated singly when compared to females in an *ad libitum* food treatment. Bateman reasoned that only the starved females needed to re-mate sooner, probably because starved males produced less sperm than those in the well-fed group. Other arthropod studies have also indicated the need for sperm as a motive for re-mating (Taylor 1967, Nakagawa et al 1971). When sperm quantity is a major factor for a female's fitness, females may mate with whichever male is present in order to obtain enough sperm, thus leading to the occurrence of polyandry.

An aspect of Darwin's sexual selection theory, the choosey female, may explain polyandry in species where females are fairly sedentary, and males roam to find their mates. In this case, females are not able to observe many males at once and choose the one they deem the highest quality (classic Darwinian sexual selection). Instead, sit-and-wait females may mate with the first male that comes along to insure a sperm supply. Successive males may then be "tested" for quality, or compared to the first male in some way so that the female chooses to mate with males of higher quality than her first mate, and rejects males she deems to be of lower quality (Halliday, 1983). In an extension of this theory, dubbed "Cryptic Female Choice" or "Selective Fertilization", the indicators of quality that a female uses to choose the sire(s) of her offspring may be tied in with the act of copulation itself. In cases like these, females may have internal mechanisms to pick and choose between the sperm from multiple males based on their copulatory performance.

Evolutionary biology stresses that increased fitness, or the increased ability of an individual to produce more viable offspring and send more of its genes into the next generation, is strongly selected for. Polyandry may have evolved in some species because it increases the fitness of the female and/or her offspring and is therefore selected for. Polyandry may help the female accrue direct or indirect fitness effects. For instance, in species where males contribute a nutritional or chemical "gift" to the female at the time of mating, it may greatly benefit a female to mate with more than one male and receive multiple gifts that she can put towards offspring production. This seems to be the case with gryllid crickets (Tregenza & Wedell, 1998), where males attach a nutrient rich jelly to their spermatophores which females remove and consume shortly after mating.

Large degrees of inbreeding or outbreeding may result in litters that are less viable because of genetic incompatibilities between their parents. In these cases, a female may reduce her chances of producing unviable offspring, thus increasing her fitness, by accepting sperm from several males. It is suggested that genetic incompatibility may be the driving force for polyandry in the beetle-riding pseudoscorpion, *Cordylochernes scorpioides* (Zeh, et al., 1998).

Finally, polyandry may indirectly affect the fitness of a female's offspring in several interesting ways. Many studies have compared the fitness of females that were multiply mated with those that remained monogamous. In several of these cases, offspring from polyandrous mothers do show an increased fitness over monogamous-mother treatments. For instance, in the bulb mite, *Rhizoglyphus robini*, daughters of polyandrous mothers were more fecund than those of monogamous females (Konior, et. al., 2001). Female *D. melanogaster* that were allowed a higher degree of mate choice (polyandry) produced larger adult offspring with higher survivorship under stressful conditions than the offspring of monogamous flies (Promislow, et al, 1998). Polyandrous decorated crickets, *Gryllodes sigillatus*, produced heavier sons that would live longer and produce more sperm than smaller offspring from monogamous matings (Sakaluk et al. 2002). Some explanations for these phenomena are that females may invest more in their offspring if they are allowed to choose the father (Konior, et. al., 2001), or that certain quality traits like adult offspring size or viability may be due to sperm competition within polyandrous mothers, where the highest quality sperm fertilize the eggs (Sakaluk, et. al. 2002). Since natural selection works upon traits that influence fitness in one way or another, females that accrue fitness benefits from polyandry would be selected for along with their polyandrous behavior.

2.1.2 Polyandry in Spiders

It is widely acknowledged that polyandry occurs in most, if not all, spider species (Austad 1984). Spiders are a fascinating group to use in polyandry studies because all of the aforementioned forces leading to the evolution of polyandrous mating systems may be at work within this group. Because males carry sperm in their pedipalps, their sperm supplies may be limiting as they may not have time to recharge the pedipalp before encountering another female. Female spiders are often sedentary, with males seeking them out to breed. This type of mating system frequently limits the number of males a female is exposed to at one time, creating the possibility of mate choice over time. The possibility of cryptic mate choice is also present in the spiders. Spider sperm is encapsulated and immobile until the female activates it prior to fertilization of her eggs. After copulation, sperm is usually stored in receptacle organs within the female's body called spermathecae. It may be possible for a female to keep ejaculate from different males separate and then selectively activate one male's sperm over another's based on quality information that she receives from her mates, though evidence of this is scarce. Another interesting aspect of spider reproductive behavior touted by cryptic choice supporters is that male spiders release sperm quite early during copulation, but copulation may continue for many more minutes (or even hours) beyond insemination. It has been suggested than this extended copulation may be sending signals of a male's quality to the female (Eberhard 2004) and facilitating cryptic female choice. However, this behavior may serve a sperm plug formation (Masumoto 1993) or mate guarding purpose instead (Thornhill & Alcock 1983, Harari et al 2003).

The following study compares aspects of reproductive behavior between monogamous and polyandrous females of *P. audax* in order to determine if there is a variation in the degree multiple matings between treatments and to point towards likely forces that are responsible the occurrence of polyandry in this species.

2.2 Methods

2.2.1 Procedure

Collection, housing, and care of spiders were the same as in the courtship behavior study reported in Section I. Because the spiders reached adulthood at different times, regular surveys of the animals were taken in order to determine their life stage and sex. Once animals were reproductively mature, they were separated between treatment groups. Females were randomly assigned as Monogamous (M) or

Polyandrous (P). M females were randomly assigned encounters with the same male 3 times in a row, and P females were randomly assigned 3 different males, one time each. Because males were limiting, the same male was often assigned to two females, but males were never mated twice in one day to ensure that they had time to recharge their pedipalps before their next encounter. Encounters were spaced at least 48 hours apart from each other to allow time for any physiological processes necessary for a female to register that she had been mated to occur.

Because salticid males are known to pick up on chemical cues in female silk draglines that enhance searching or courtship behavior (Taylor 1998), filter paper containing traces of silk from females assigned to a particular male was placed in his habitat 24 hours prior to an encounter. Males were also allowed to sample fresh silk from their assigned female's nest webs immediately prior to introduction of the female into the mating arena.

Mating trials were carried out using the same methods as in Section I, and copulation latencies were recorded. Mated females were then allowed to lay and guard eggsacks. Spiderlings were removed and counted once they had molted into the first instar and began to disperse from the nest web. Because initial size has been shown to be positively correlated with increased survival in spiders (***), it was chosen as a measure of offspring fitness. Carapace widths (measured as the distance between convex portions of the posterior lateral eyes) were measured on all first instar spiderlings and averaged to get an overall estimate of size across a given litter. To determine if polyandry or lack thereof had an effect on clutch viability, numbers of unhatched eggs and offspring that died prior to reaching the first instar were also recorded for each egg sack. Hatching success was measured as the ratio of first instar spiderlings to total clutch size (spiderlings + unhatched eggs). Though female *P. audax* have been recorded to lay up to 6 fertile batches in a row following a single mating in the lab (Roach 1988), it is extremely unlikely that they survive long enough to lay more than 3 in a natural setting, so only data from the first 3 eggsacks laid by mated females were taken into consideration.

2.2.2 Statistical Analyses

A 3 x 2 contingency table, constructed from data pooled from both treatments, was used to determine whether females' tendencies to copulate changed as male encounters increased. Fischer's exact tests were used to compare the percentages of females mating between first and second encounters and between second and third encounters. If female choosiness is a factor, we would expect to see fewer females mating again after their first mating because not all second males would be of higher quality than the first. Alternatively, if genetic incompatibility is a strong driving force in this species, it is likely that the percentage of mating females between any given encounter will be relatively equal, as females are likely unable to know if a male is incompatible by appearance alone. Fischer's exact test was also used to compare the percentages of females mating multiply between M and P treatments. In this case, if female choosiness is a factor, we would expect to see fewer matings overall in the M treatment, because there would be no need to remate with the same male twice.

However, if sperm supply was an important driving force, females would be equally likely to mate with the same male as with different suitors.

There is evidence that in some spider species, males discriminate against previously mated females (Burkowski et al. 2001) while in others, they do not (Elgar et al. 2003). To determine whether male selectiveness is affecting the occurrence of polyandry in *P. audax*, Mann-Whitney U-tests were run to compare both courtship and copulation latencies of males paired with virgin vs. previously mated females.

In spiders, female size has been correlated with the number and size of offspring she produces (Marshall & Gittleman 1994, Simpson 1995, Skow & Jakob 2003). The total clutch size may also affect size of offspring (Lack 1947, Simpson 1995, Brown et al. 2003). In an effort to separate these maternal effects from those of polyandry, correlations were run on Female Carapace Width (CW)/Clutch Size, Female CW/Spiderling CW, and Clutch Size/Spiderling CW.

Previous studies have shown that in most spiders, clutch size tends to decrease as clutch number increases (Marshall & Gittleman 1994), and there is evidence that, in *Phidippus*, hatching success may decrease as well (Jackson 1978) To see if this is indeed the case for *P. audax*, hatching success was compared between clutches using a Kruskal-Wallace test on data pooled from both treatments.

Polyandry may have an effect on multiple aspects of spider fitness, both of a given female and of her offspring. In order to determine if P females or their offspring have a fitness advantage over those in the M treatment, hatching success and first instar offspring size were each compared between treatments using Mann-Whitney U-tests.

Hatching success was chosen to represent a female fitness trait, and offspring size was used as an offspring fitness trait, as larger offspring may have a competitive advantage over smaller ones.

2.3 Results

A total of 6 M and 12 P pairings were attempted. Of these, two females in the P group, though introduced to at least 3 different males, never mated and were thrown out of the analyses. Three other females in the P group mated only once because they either refused all but one suitor or they laid eggs immediately after their first mating. These females were included in their assigned treatment for behavioral analyses, but were moved to the M treatment for all clutch-related comparisons.

There was a significant difference in whether females accepted males based on the number of previous courtship experiences they had (3x2 contingency table, p=0.0047, first encounter n=16, second encounter n=15, third encounter n=11).

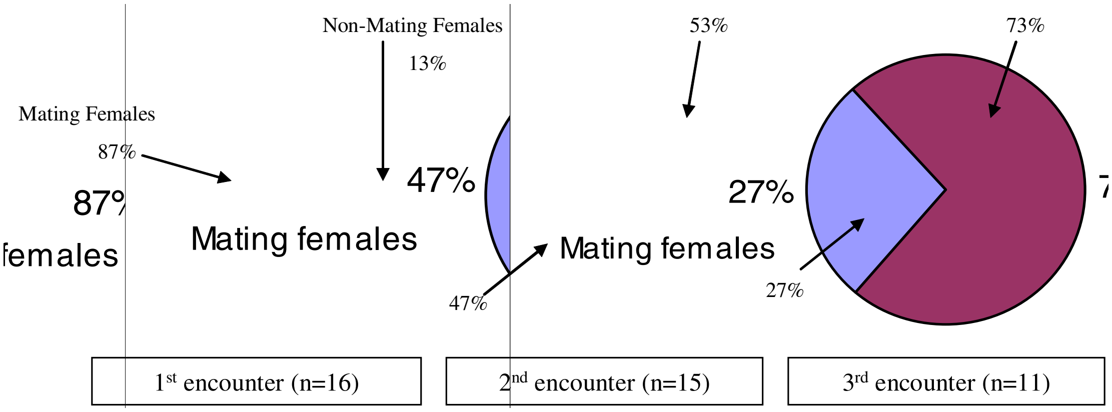

Figure 2.1 Percentage of females that mated in first, second, and third encounters.

Females became increasingly less receptive to males as encounters increased (Fischer's exact test first vs. second encounter p=0.0233, second vs. third encounter p=0.0034,

Fig.2.1). Though fewer females mated in third encounters than second encounters, this difference was not statistically significant (Fischer's exact test p=0.4379).

P group females (n=9) remated more frequently than M group females (n=6), though the difference between them was not significant (Fischer's exact test p=0.3147, Fig.2.2).

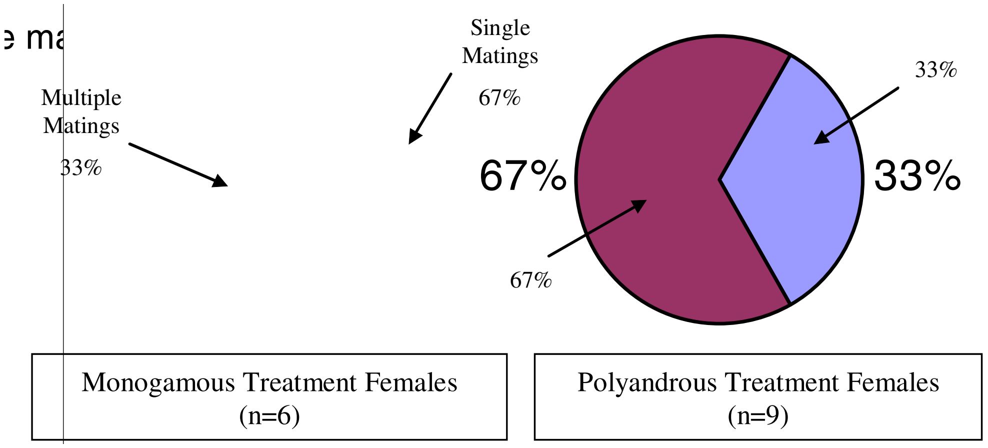

Figure 2.2 Percentage of females that mated multiply between M and P treatments.

There was no significant difference between male courtship latencies (Mann-Whitney U-test p=1.0) or copulation latencies (Mann-Whitney U-test p=1.0) between first (n=13) and second (n=8) matings. There was a large degree of variance in copulation latencies of both first and second matings. Males copulated with virgin females for an average of 27.6±26.7 minutes, and with previously mated females for 37.3±49.8 minutes.

Within the timeframe of this experiment, 10 female spiders laid at least one viable clutch. Five of these animals laid a second clutch of eggs, and four of these five laid a third clutch. Two M treatment females died prior to laying eggs. One female, mated to 3 males in the P treatment, died shortly after laying her first clutch of eggs. These eggs were unviable and had oozed out of the nest web in a congealed mass, suggesting a physiological problem prior to their deposition which is unlikely to be related to polyandry. Only one other female died after depositing her first clutch of eggs, presumably from starvation (females often refused food while guarding eggs). This female's clutch was viable and proceeded to hatch after her death. The remaining females that deposited fewer than three clutches were younger than three-clutch females and would presumably reach this number if the experimental timeframe were extended.

Across total clutches examined (n=19), clutch size does not appear to be correlated with female body size (r=-0.1158, p=0.6373, Fig 2.3A). Separation of data by clutch number did not lead to any significant correlations (First Clutch: n=10, r=-0.179, p=0.6238; second clutch: n=5, r=0.0148, p=0.9780; third clutch: n=4, r=0.698, p=0.3016).

When treatment and clutch number are pooled (n=18), there is no correlation between female size and first instar offspring size (r=0.107, p=0.6729, Fig 2.3B). Separation of clutch numbers provided no significant correlations, though in second clutches (n=5), larger females tended to hatch smaller spiderlings (second clutch: r=-0.5939, p=0.2906; third clutch: r=-0.8344, p=0.3724). Small sample sizes are likely responsible for this trend.

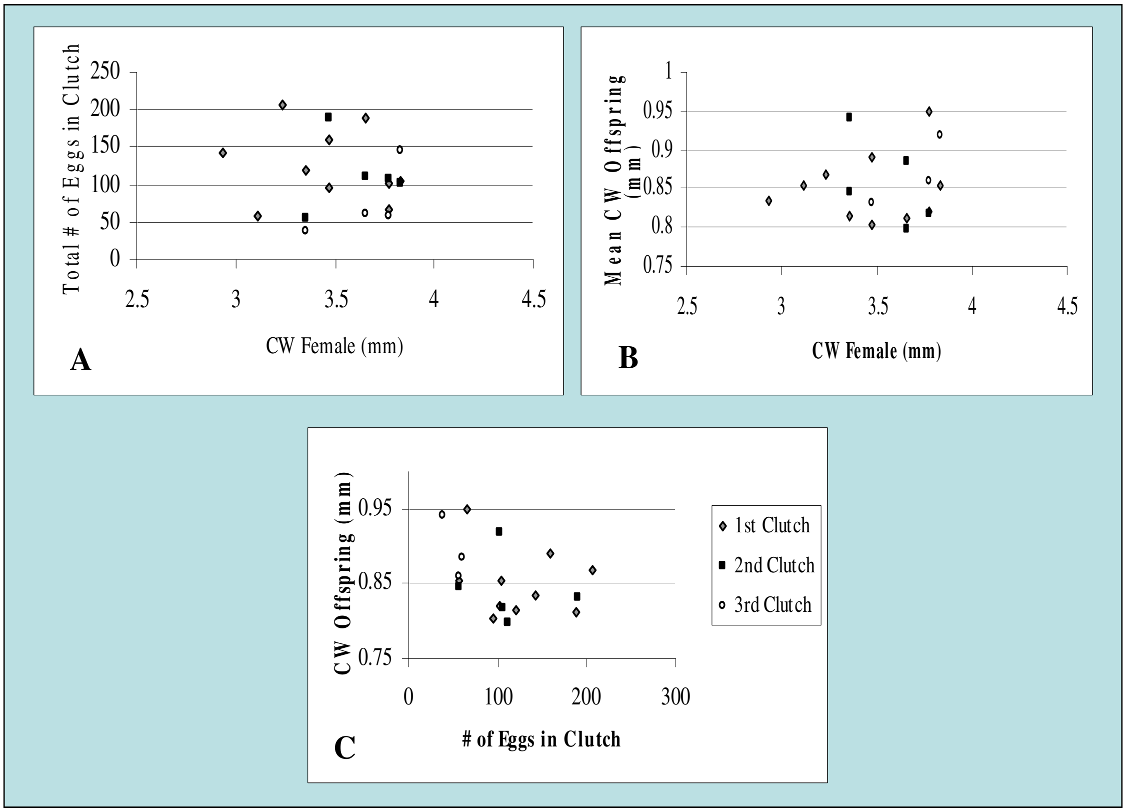

Figure 2.3: Scatter plots of A) Female size VS clutch size, B) Female size VS first instar offspring size, and C) Clutch size VS first instar offspring size.

Spiderlings tend to be smaller when clutch size is large, though the correlation is not significant (pooled data: n=18, r=-0.3612, p=0.1407, Fig 2.3C). Because mean female size is not significantly different between treatment groups (Mann-Whitney U-test, p=0.2627), this trend shouldn't affect outcomes due to polyandry.

Hatching success appears to increase with successive clutch numbers (Fig 2.4), but differences across clutch number are not significant (Kruskal-Wallace test: H=2.61, χ^2_{crit}=5.99). The mean hatching success in first clutches (63.9%) is approximately 20% lower than second (83.8%) and third clutches (86.5%). Larger sample sizes may lead to a stronger trend towards higher hatching ratios in later clutches. Because there were approximately equal numbers of P and M clutches within each clutch number (1st clutch: 4P, 6M; 2nd clutch: 3P, 2M; 3rd clutch: 2P, 2M), potential clutch number effects should not confound those of polyandry.

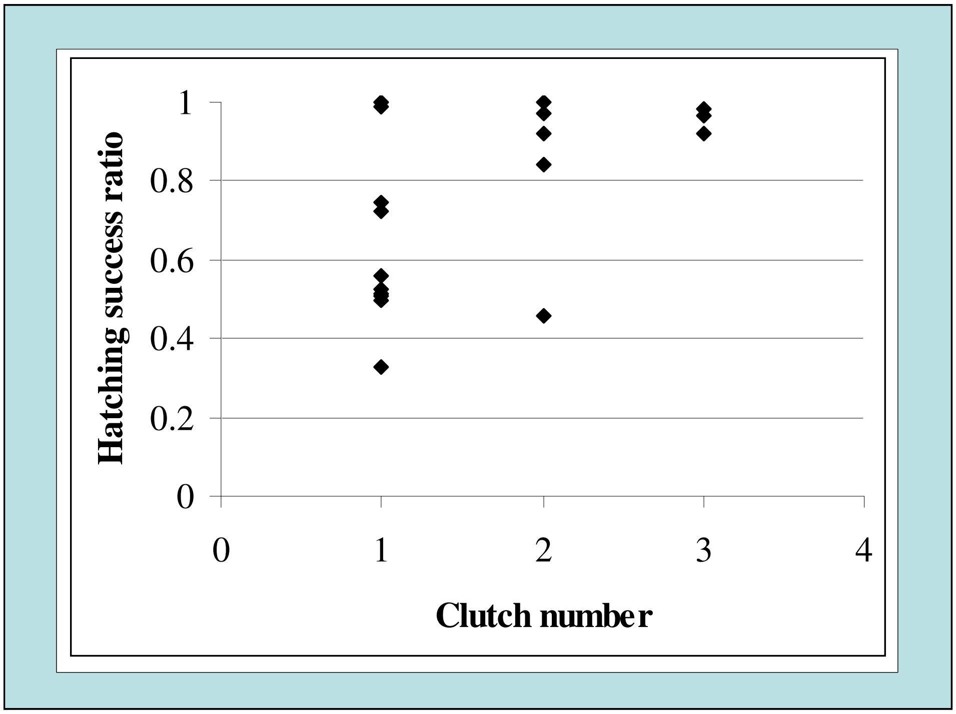

Figure 2.4 : Hatching success by clutch number

When all three clutches are pooled, hatching success is higher in P females, with an average of 80.5 % of eggs laid by polyandrous females (n=9) developing into live first instar spiderlings, compared to 68.9 % of those laid by monogamous females (n=9, Fig 2.5A); however, this difference is not statistically significant (Mann-Whitney U-test: p=0.2891). There is no difference in mean first instar sizes between treatments (n=9 for both) (Mann-Whitney U-test: p=1.0, Fig 2.5B).

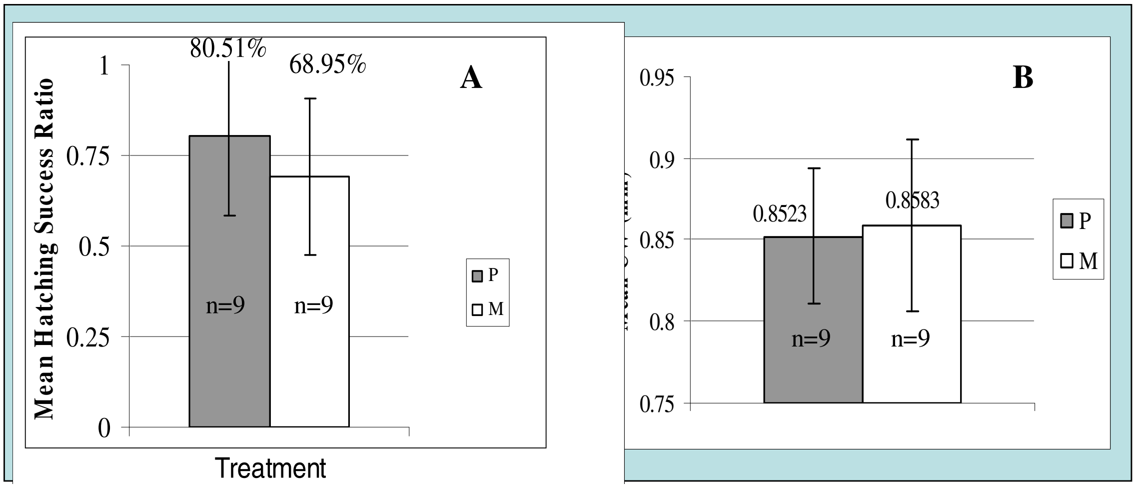

Fig 2.5: A) Mean hatching success between P and M treatments, B) Mean size of 1st instar spiderlings between P and M treatments. Error bars are ± one standard deviation.

2.4 Discussion

Most jumping spiders spin a sac-like silken retreat web that they use for night time shelter and molting; this is the case with *P. audax*. Though in-depth behavioral field studies have not been conducted on this species, it is assumed that juveniles and females remain in the vicinity of these webs for weeks at a time, while adult males wander in search of females (Givens, 1978). In many salticids, including *P. audax*, males usually mature earlier than females (Givens, 1978; Jackson 1978) and have been known to spin retreat webs on top of those of penultimate females (Jackson 1982, pers. obs.). This early-maturing strategy allows males to locate and perhaps guard virgin females until they are reproductively mature. Male territoriality after mating has not been observed in salticids; it is assumed that after mating with one female, a male moves on to locate another. In nature, a young female guarded by a male may not have a choice of whether to mate with him, as he can probably overpower her within her retreat web while she is still weakened from her final molt. However, if a virgin female is not guarded by a male during her final molt, it would be advantageous for her to mate with the first male that she encounters to ensure a future sperm supply.

In this study, virgin females were more receptive to males than previously mated females, essentially becoming choosier with experience. Obtaining an early sperm supply is likely the reason that these lab-reared females accepted more males when virgins. Once inseminated, females were free to be choosy in encounters with successive males. Only one P female mated with all three males presented to her, while several mated with two males and the remaining P females were moved to the M group for clutch related variables because they rejected all but one of the males they encountered. In all matings where a female rejected a courting male, the female allowed the male to approach her, engaged him in a wrestle, and then turned and walked away. Occasionally, females would stalk and attack courting males as they would any other prey item, but rejection after courtship was a more common behavior.

By allowing a male to court, and engaging him in a wrestle, a female may be comparing certain quality characters he possesses to those of her previous mate(s). Rejection likely occurs when a female deems her first mate to be superior to her current suitor. This may be why assigned P females were more likely to mate multiply than assigned M females. After 48 hours passes between encounters, an M female may not “remember” her assigned male, but she may now have a set of male quality “acceptance thresholds” in place from cues she received during her first mating. The same male is unlikely to exceed his own signals from the previous encounter and is more likely to be rejected on his second attempt to mate with the same female. However, two M females mated with the same male twice. In both cases, copulations were quite short in first encounters and the females were restless throughout second encounter copulations. It is possible that complete insemination was not achieved in first encounters and that these females accepted the same male to obtain more sperm. However, this is unlikely because spider insemination occurs very early in copulation (Schneider et al. 2004). A second explanation for multiply mating monogamous females is that aspects of their males' courtship displays "improved" between encounters (e.g.: vigor) or were equivalent to previous encounters (e.g.: size, strength), thus meeting the females' quality acceptance threshold. Female restlessness during copulation may indicate some degree of indecisiveness about the encounter; perhaps that a female's current suitor has only just met her acceptance threshold.

Because P females usually rejected at least one of their suitors, I believe it is unlikely that *P. audax* polyandry has arisen solely to combat genetic incompatibility, as seems to be the case with their fellow arachnids, the beetle-riding pseudoscorpions (Zeh et al. 1998). However, some degree of genetic incompatibility may occur in *P. audax*, especially in outbred populations (Taylor and Peck, 1975). Because subjects for this trial were collected from various populations throughout the Arlington, Texas area, the potential for genetic incompatibility may be higher than if they came from the same population. Indeed, there is a trend for M females to lay clutches of eggs with a reduced hatching success compared to P female clutches. However, I hesitate to assign polyandry as the direct cause of higher clutch viability because sample sizes are so small, and I have no information on sperm priority patterns in *P. audax* or the actual sires of a given clutch of offspring. A study on a much larger scale than this one may be able to clearly relate polyandry with hatching success in this species. Male sterilization studies would be necessary to determine sperm priority patterns, and paternity testing would also need to be incorporated in order to address genetic incompatibility questions (i.e.: Is one male more incompatible with one female vs. another?).

Polyandry does not appear to have an effect on initial offspring size in *P. audax*. This is not surprising, because studies that link the occurrence of polyandry to increased offspring fitness suggest that its effects show up later in life. For instance, offspring fecundity increases in polyandrous bulb mites (Konior et al. 2001), adult survival rates and perhaps body size increase in polyandrous fruit flies (Promislow et al. 1998), and adult male offspring of polyandrous decorated crickets are larger, potentially living longer and providing bigger nuptial gifts (Sakaluk et al. 2002). Unfortunately, salticid spiders are difficult to successfully rear in large numbers because of a high mortality rate in the early instars (Robertson and Stephens 2002, pers. obs.) and it may not be feasible to rear sample sizes large enough to test for effects of polyandry on older instars or adults. It is probably even more difficult to achieve a high degree of polyandrous matings, a hardship I share with other researchers who have attempted it (Elgar et al. 2003, for example).

Though this experiment specifically addresses polyandry in *P. audax*, different forces may be driving polyandry in related species. In Section I, I addressed behavioral differences in courtship and copulation between *P. audax* and *Phidippus carolinensis*. It was found that *P. carolinensis* has a relatively simple courtship with no female wrestling, but that copulation is, on average, three times longer than in *P. audax*. It is possible that female *P. carolinensis* are using specific cues associated with copulation itself as male quality indicators and "choosing" between various males' sperm for the fertilization of their clutches. In order to determine if this is the case, a similar polyandry study should be conducted, coupled with a detailed description of female genital morphology/physiology during several points of the reproductive process (i.e., 1st mating, 2nd mating, and fertilization/oviposition) and paternity testing of offspring.

In conclusion, prolonged female choice is likely the driving force for occurrence of polyandry in *P. audax.* Females tend to mate with the first male they encounter in order to insure adequate sperm supply, and then allow successive males to court them prior to accepting or rejecting them. By observing this courtship, females may be comparing quality-indicating signals from the male to an acceptance threshold of sorts based on previous encounters. Genetic incompatibility may also be a factor reinforcing the occurrence of polyandry in this species. Major forces driving polyandry may differ even in closely related species, as may be the case with *P. carolinensis*

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BIOGRAPHICAL INFORMATION

Stacey received her Bachelor of Science degree in zoology from Washington State University in 2002. Prior to and during this time, she had the opportunity to on various research projects, including a massive survey of parasitoid wasps and their aphid hosts, a study of grape arthropod pests and beneficials in conventional and organic vineyards, and studies concerning native and invasive species of lady beetles, to name a few. Entomology is Stacey's fondest endeavor, though she considers herself a naturalist by trade. After graduating from UTA, she plans on moving back to Eastern Washington State with her husband to pursue a collegiate teaching career and to get her hands back in the family farmer's market business.