

ENVIRONMENTAL HETEROGENEITY AND PHENOTYPIC VARIATION: THE  
EVOLUTION OF MALE BODY SIZE IN A GOLDEN ORB-WEB SPIDER

By

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To my family

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Phenotypic variation is a guiding principle that underlies most major processes in evolutionary biology, including speciation, extinction, and adaptation. Here I address how environmental heterogeneity in selection pressure can act on a small spatial scale to maintain male body size variation in the golden orb-web spider *Nephila clavipes*. Spider webs partition the environment into discrete patches, and for males who travel among female webs during adulthood in order to mate, female webs form a heterogeneous social environment. In the genus *Nephila*, females build unusually strong and long-standing webs, and males have a remarkable degree of variation in body size. Because large males have an advantage during male-male contests, the maintenance of this broad distribution of sizes over evolutionary time is a puzzle. I address how web-to-web variation in social environment could affect selection on male body size. To do this, I use field studies and mating experiments to investigate how female webs differ in two main characteristics that could affect the strength and direction of selection on male size: 1) the number of male competitors that cohabit on the web with the female, and 2) the quality of the female mate herself. In addition, using a modeling approach, I address how males could employ size-dependent strategies in

order to optimize their reproductive success in a heterogeneous environment. In this model I incorporate a variety of factors that could constrain male reproductive behavior, including male sperm limitation.

Female webs vary greatly in the number of male competitors present on the web (i.e. male density). In addition, the strength of selection on male size differs with density. Large males have a competitive advantage only in intermediate to large-sized male groups. When the number of competitors is low, small males are as likely to achieve copulations as large males. Secondly, the quality of the female web owner varies from web to web. From a male's perspective, female quality is a trade-off between sperm competition risk and female mortality risk. Males invest the most sperm when mating with a virgin female or a non-virgin female who is close to oviposition. As a result of this increased sperm investment, males sire more offspring when mating with either of these female types compared to a non-virgin female who is one month from oviposition. Furthermore, field studies show that the level of male competition at the web is a function of the quality of the female, which suggests that male density and female quality interact to affect male reproductive success in a size-dependent way.

Behavior and behavioral plasticity could play an important role in enabling small males to achieve similar fitness to large males if males can navigate the reproductive environment in an optimal way. However, for males of all sizes, male reproductive potential and male mating strategies could be constrained by male sperm limitation in this species. Using a histological and comparative phylogenetic approach, I assess the degree to which male mating rate could be limited by sperm depletion in this species. Findings show that spermatogenesis is synchronous, and testes are non-functional in

adult males. As a result, males have a finite quantity of available sperm, and once this sperm is depleted during one or a few copulations, males lose their ability to fertilize eggs.

I incorporate male sperm limitations and other constraints to male reproductive success as well as environmental heterogeneity into a dynamic state model in order to determine how male strategies and reproductive outcomes change as a function of body size. The model shows that male strategies differ particularly in terms of post-copulatory behavior, and large males are more likely than smaller males to stay behind and guard a female after copulation. Reproductive outcomes also differed between large and small males. Large males were slightly more likely to be monogynous compared to small males because they mate with virgin females at a higher rate and as a result deplete their sperm stores in a single mating. Thus, large male reproductive success is partially constrained by sperm limitation. Sperm limitation, however, did not promote choosiness in males unless it was very extreme (i.e. males were limited to a single mate). In this system, a variety of factors contribute to male reproductive success and male mating strategies, and as a result fitness differs more within a size class than between size classes. Males of all sizes achieve similar levels of reproductive success on average, which suggests that environmental heterogeneity may weaken selection on male body size by adding substantial within size class variation in fitness. A second interpretation is that males are able to use environmental heterogeneity to their advantage, navigating the reproductive environment in an optimal way for their size. Both of these scenarios could result in the maintenance of a broad distribution of male sizes in this species.

## CHAPTER 1 INTRODUCTION

Orb web spiders show some of the most extreme examples of sexual size dimorphism in animals. Many studies have addressed the evolution of this intersexual variation in size. Females can develop to large sizes in part because they are the sedentary sex (Fairbairn 2007), and large size corresponds to increased fecundity (Prenter et al. 1999). Males are smaller, and maximum size may be constrained by the demands of moving among female webs as adults in order to mate (Fairbairn 2007). However, in addition to extreme sexual size dimorphism, members of the genus *Nephila*, the largest web-building spiders in the world, show very broad within-sex size variation, but only in males (Vollrath 1980). In some species adult male size varies by an order of magnitude (Schneider & Elgar 2005). Because large males have a competitive advantage in almost every species in this genus, the maintenance of a broad distribution of male sizes remains a puzzle.

For arthropods in particular, body size is a complex trait that has a strong environmental component (Mousseau & Roff 1989). While there may be many proximate causes for size variation in *Nephila*, size and size variation must have a heritable component in order for the trait to be subject to evolutionary change. Some studies in spiders have addressed the degree to which environmental and genetic components contribute to size variation. For example, in the Mediterranean tarantulas (Lycosidae), low variance in female size relative to variance in male size has been suggested as evidence for stabilizing selection on female size and weak directional selection on male size (Fernandez-Montraveta & Moya-Larano 2007). The most comprehensive treatment of heritability in growth and size parameters in a spider is a

study of a cellar spider (Pholcidae) by Uhl et al. (2004). They found a narrow-sense heritability of 0.44 for body size (father-son regression). Furthermore, this study found significant family effects on the response to diet supplementation in growth and adult size. In spiders generally, size as well as gene by environment effects on size appear to have significant heritable components, and thus size and size variation may be subject to selection in these species (Uhl et al. 2004; Fernandez-Montravet & Moya Larano 2007).

*Nephila* species also show evidence of heritable variation in size. Environmental variation in food availability affects molt timing, but not necessarily adult male size, at least during the last three instars (Higgins 2000). Maximum size appears to be constrained by genetics and maternal effects (i.e. starting size; Higgins 1992; Higgins 1993, 2000). Furthermore, Higgins (1992, 1993, 2000) reports differences among populations within *N. clavipes* in average male size, which are presumably a function of genetic and environmental variation, or possibly an interaction between these two forces. Michalik and Rittschof (in press) found that a small sample of *N. clavipes* males collected in the wild and reared in a common garden lab setting had unusually long developmental times, and still showed a 1.5 fold range in size (cephalothorax width; Min = 1.43 mm, Max = 2.08 mm, S.D. = 0.14 mm), which suggests that maturation timing is plastic and broad, and at least some portion of the substantial variation in male size common to *N. clavipes* is due to genetic or gene by environment effects. In *Nephila* males, some component of size, and potentially the degree of plasticity in size, are heritable characteristics, with the potential to change over time in response to selection.

Previous studies that have investigated how natural selection could maintain body size variation in *Nephila* have attempted to sum and compare the costs and benefits for males that are large versus small (Vollrath 1980; Cohn 1990; Uhl & Vollrath 1998; Vollrath 1998; Schneider et al. 2000; Uhl & Vollrath 2000; Schneider & Elgar 2001; Foellmer & Fairbairn 2005a; Schneider & Elgar 2005; Kasumovic et al. 2007). Here however, I take a broader approach by asking how, generally, natural selection acts to maintain phenotypic variation in any trait. Understanding the selection processes that maintain or constrain phenotypic variation is a central goal for many fields within evolutionary biology (Charlesworth & Charlesworth 1987; Badyaev & Foresman 2000; Bijlsma et al. 2000; Robertson & Rosenblum 2009). In this study I focus on one feature that affects phenotypic variation, environmental heterogeneity. Environmental heterogeneity is an important force that can lead to speciation on large geographic scales. However here I specifically address whether heterogeneity on a smaller scale, web to web variation in selection on male size, may facilitate male body size variation in *Nephila*.

Environmental heterogeneity promotes adaptive phenotypic variation because it can shift the optimal level of a phenotype spatially or temporally, and in some cases completely reverses the optimal level of a phenotype (Candolin et al. 2007; Engstrom-Ost & Candolin 2007; Wong et al. 2007; Gosden & Svensson 2008). Environmental heterogeneity can occur at a variety of scales in space and time, and relevant environmental differences can be both abiotic (Grant & Grant 2002; Juenger & Bergelson 2002) and biotic (Svensson & Sinervo 2004). Variation in social environment in particular is expected to affect the strength and direction of selection on a fine-

grained spatial scale (Levins 1968; Gosden & Svensson 2008). Web-building spiders are a special case of spatial environmental heterogeneity because spider webs partition the environment into discrete patches (Agnarsson 2003; Kasumovic et al. 2008; Rittschof & Ruggles 2010) with different social characteristics. Female webs can vary in the number and size of male competitors, which in turn is a function of the female herself (i.e. the female's quality, sexual receptivity, or reproductive state). Furthermore, males move from web to web searching for a female mate (Christenson & Goist 1979), and have the ability to modify their behavioral strategy in response to environmental variation.

Here I use field and lab experiments as well as a modeling approach to investigate how spatial variation in female quality affects male mating strategies, and may ultimately affect male body size evolution, in *Nephila clavipes*. First, I demonstrate that *N. clavipes* males show an unusual degree of sperm limitation, a reproductive constraint that affects male mating strategies. Second, I demonstrate that male competitive advantage is density-dependent in this species, and that female webs are highly variable in terms of male density, creating spatial heterogeneity in the strength of sexual selection on male body size. Third, I show that male reproductive investment changes as a function of female age, resulting in variation in female quality associated with age and mated status. Last, I integrate the factors relevant to male mating decisions, including male sperm limitation, variation in male-male competition, and variation in female value, into a dynamic state model, and using this model, I assess the strength of selection on male body size, and describe the strategies and tactics males use to negotiate a spatially variable reproductive environment.

## CHAPTER 2 A COMPARATIVE ANALYSIS OF THE MORPHOLOGY AND EVOLUTION OF PERMANENT SPERM DEPLETION IN SPIDERS

### **Background**

Sperm are small and numerous compared to eggs, which has led to the assumption that sperm are cheap to produce. However, recent studies have shown that sperm production is costly, and males can become sperm depleted (i.e. functionally sterile) at least for some period of time after mating (Nakatsuru & Kramer 1982; Dewsbury 1983; Wedell et al. 2002). The amount of sperm transferred during copulation affects a male's fitness in terms of sperm competition for the current mate (Parker 1970) and a male's ability to mate with subsequent females (Smith et al. 2009). As a result, sperm cost and male sperm depletion have broad implications for the evolution of male mating strategies in sexually reproductive animals (Møller 1991; Simmons et al. 1993; Bateman et al. 2001; Engqvist & Reinhold 2006).

Sperm depletion, the decrease in sperm number over successive ejaculates, is widespread in animals (Preston et al. 2001), but there is variation across species in the degree to which sperm depletion limits male mating opportunities (Radhakrishnan et al. 2009). Sperm depletion can be temporary, where males must undergo a reproductive latency period after mating in order to replenish their ejaculate (e.g. Lemaitre et al. 2009). In other cases, sperm depletion is permanent, where males are unable to replenish their sperm once it is used (Boivin et al. 2005). Because permanent sperm depletion (hereafter PSD) strongly constrains male mating ability, the mechanistic, ecological, and evolutionary bases of this phenomenon are of special importance to a variety of research areas, including the evolution of mating systems, male mate choice, sperm competition, and female sperm limitation.

Web-building spiders are popular for male mating strategy studies because in some species males show a variety of behavioral and morphological features that limit mating rate, including male sacrifice behavior (i.e. sexual cannibalism, e.g. Elgar 1991; Foellmer & Fairbairn 2003, 2004) and complete or partial genital breakage during copulation (genital mutilation, e.g. Schneider et al. 2001; Nessler et al. 2007b; Kuntner et al. 2009c). Mating behaviors that eliminate a male's ability to re-mate are collectively called terminal investment strategies (Andrade & Kasumovic 2005). Because these behaviors occur across a variety of distantly related species (Miller 2007; Uhl et al. 2010b), many studies have examined the selection pressures that maintain these extreme behaviors, and whether these selection pressures can be generalized across spider taxa (Schneider & Elgar 2001; Herberstein et al. 2002; Andrade 2003; Fromhage et al. 2003; Fromhage & Schneider 2006; Snow et al. 2006).

However, some species of spiders exhibiting terminal investment strategies also show evidence of PSD. In order to interpret the evolutionary history, causes, and consequences of terminal investment strategies in spiders, it is necessary to verify the amount of sperm available to a male across successive copulations (Herberstein et al. 2005; Michalik et al. 2010). Despite these broad implications, and although PSD was first suggested in spiders over 20 years ago (Christenson 1989), little is known about this phenomenon. Verifying whether PSD occurs, understanding its mechanistic basis, and assessing its evolutionary history are essential to interpreting the selection pressures driving terminal investment behaviors in this arthropod group.

The male reproductive system in spiders is unusual because males transfer sperm to the female using paired prosomal appendages called pedipalps that are

separate from the male genital opening. After the maturation molt, males ejaculate sperm through their genital pore onto a sperm web and draw the sperm into their pedipalps, a process called sperm induction (Foelix 1996). The sperm remains in the pedipalps until copulation. Studies that propose sperm depletion in spiders have examined the pedipalps for the presence or absence of sperm (Christenson 1989), which would indicate temporary sperm depletion because males can re-induct sperm before, during, or after copulation (Table 2-1). However, examination of the testes is required in order to demonstrate that sperm depletion is permanent. The male testes are simple, paired cylindrical organs that are connected to the genital pore by paired deferent ducts (Michalik 2009). Sperm is produced in the testes and then temporarily stored in the deferent ducts until ejaculation. cursory examination of the adult male testes suggests that sperm depletion may result because male testes atrophy or do not produce sperm during adulthood (Table 2-1; Herberstein et al. 2005; Michalik et al. 2010).

In the current study, I verify that sperm depletion in spiders is permanent using the golden orb-web spider *Nephila clavipes*. Spiders in the genus *Nephila* are known for genital mutilation and male sacrifice behavior, which suggests permanent sperm depletion may be present in this group. More importantly, extensive mating experiments in *N. clavipes* provide the best evidence of any species studied that sperm depletion may be permanent (Christenson 1989). Using morphological measurements from specimens sacrificed at different life stages, I examine how the size of the testes changes as males mature, induct sperm, and age. I use light and transmission electron microscopy to confirm that decreased testes size corresponds to a decrease in the

amount of tissue devoted to spermatogenesis. In addition, using a comparative phylogenetic framework and phylogeny-based statistics, we test the evolutionary relationships between PSD and other characters that limit male mating rate, including genital mutilation, male sacrifice behavior, and sexual size dimorphism.

## **Material and Methods**

### **Collection and Rearing**

Third and fourth instar juvenile *Nephila clavipes* were collected from mixed oak habitats within the Ordway-Swisher Biological Station in Melrose, Florida (Putnam County). Juveniles were reared at the Lab of General and Systematic Zoology of the University of Greifswald in cylindrical plastic containers (5x10 cm). The tops of the containers were covered with cheesecloth, and the bottoms were open. The open bottoms sat on top of moistened towels to provide humidity, and males were housed together on a shelf under natural light cycle at room temperature. In the containers males constructed prey-capture webs and were fed daily with two *Drosophila* flies.

Because I was interested in comparing changes in the testes as males approach and pass their maturation molt, I sacrificed sub-adult males across a range of times that spans the period between the sub-adult and adult molt (see Table A-1: Real age and standardized age for all males in the study). Furthermore, I sacrificed adult males across a range of times that spans the period from the maturation molt to death ( $N_{\text{sub-adult}} = 18$ ,  $N_{\text{adult}} = 19$ ; see additional file 1). My experimental set-up simplifies the true reproductive experience for adult males because we did not allow males to mate, nor did we provide females as cues for sperm production. However, in a series of behavioral experiments in this species, Christenson (1989) gave males the opportunity to copulate with multiple receptive females in succession, and males failed to transfer

any sperm in this scenario. This suggests that any processes occurring in the male genital system that limit re-mating ability are independent of male mating history and female cues.

### **Sample Dissection and Preparation**

Virgin male specimens were dissected in phosphate buffer (0.1M, pH 7.2) with 1.8% sucrose added (PB). The isolated genital systems were fixed in 2.5% glutaraldehyde (Merck Chemicals Ltd., Nottingham, UK) in PB and pictures for the analyses of the gross morphology were taken using an Olympus DP10 digital camera mounted on an Olympus ZX 7 stereomicroscope. For the histological and transmission electron microscope (TEM) analyses samples were post-fixed in PB buffered 2% OsO<sub>4</sub> (SERVA Electrophoresis GmbH, Heidelberg, Germany). After being washed in PB, the genital system was dehydrated in graded ethanol and embedded in Spurr's resin (Spurr 1969). For the light microscope (LM) analyses semi-thin sections (700 nm) were made with a Diatome HistoJumbo diamond knife at a Leica ultramicrotome UCT and stained according to Richardson et al. (Richardson et al. 1960). Sections were documented using a Zeiss MCr digital camera mounted on an Olympus BX60 compound microscope. For the TEM analyses ultra-thin sections (50 nm) were made with a Diatome Ultra 35° diamond knife at a Leica ultramicrotome UCT and stained with uranyl acetate and lead citrate according to Reynolds (Reynolds 1963). Examination was performed with a JEOL JEM-1011 electron microscope at 80 KV. Images were taken with a side-mounted Olympus MegaView III digital camera using the iTEM software (Olympus Soft Imaging Solutions GmbH, Münster, Germany).

## Measurements and Calculations

In order to compare changes in the genital system before and after the maturation molt, it was necessary to evaluate both male age groups along a single continuum of age. For this reason, adult males were assigned a “standardized age” that is the age at which they were sacrificed relative to the sub-adult males in the study (Table A-1: Real age and standardized age for all males in the study). For example, the oldest sub-adult male was sacrificed on day 72 after his sub-adult molt, so the adult sacrificed one day after his own maturation molt received an age of 73.

In order to determine how the male reproductive system changes with age, measurements of body size, testis and deferent duct width and length, and the ratio of generative tissue to total testis tissue were taken for all males in the study (N = 37). All body measurements were taken from digital photographs (Zeiss Discovery V20 with Zeiss MCr camera) using the IntMess module in the program Zeiss AxioVision 4.8 (Carl Zeiss MicroImaging GmbH, Göttingen, Germany). A linear measurement of male body size was taken as the width of the prosoma at its widest point. In addition, after dissection, the length and width of one testis and one deferent duct were measured per individual. For each individual, the most intact of each organ was selected for measurement. Because of their irregular shapes, total testis and deferent duct lengths were measured using the curve tool of the IntMess module, which traces along the length of non-linear objects. Widths of the testis and deferent duct were taken as linear measurements. Because testis and deferent duct widths vary along the length of both organs, the width of each organ was measured at three locations per individual, the 25%, 50%, and 75% points along the length. These widths were averaged to estimate the true organ width. Using the assumption that the testis and deferent duct are

approximately cylindrical, the length and average width measurements were used to calculate testis and deferent duct volumes.

To assess changes in generative tissue over male lifetime, I calculated the ratio of generative tissue to total testis area from a single stained testis cross-section per individual. Cross-sections were magnified using an Olympus BX60 light microscope and photographed with a Zeiss MCr camera. In spiders, spermatogenesis occurs in cysts, which are bordered by thin extensions of the somatic cells located at the periphery of the testis (e.g. Michalik et al. 2006). At the end of spermatogenesis, sperm cells accumulate in the lumen of the testis. Thus, I defined generative tissue as the total area of the testis filled with either spermatogenic cysts or lumen. The borders of the testis, the testis lumen, and the spermatogenic cysts were traced using the IntMess module to approximate area. The resulting testis cysts area and lumen area were summed, and this value, divided by the total testis area, gives the ratio of generative tissue to total testis tissue.

### **Male Sperm Induction**

In order to assess whether the process of sperm induction corresponds to changes in the testes, we assessed male pedipalps for the presence of sperm. To do this, for each adult male in the study (N = 19), I removed the left pedipalp and soaked it in clove oil (Sigma Aldrich Chemie GmbH, Munich, Germany) for 3-5 hrs. and then examined the pedipalp under a light microscope. Using this method, we could visualize sperm through the pedipalp cuticle in order to determine sperm presence or absence.

### **Statistics**

All statistical analyses were performed using the SAS program JMP 7.0 (SAS Institute Inc., Cary, NC, USA). For comparisons of testis and deferent duct volume for

sub-adult versus adult males, the data were natural-log transformed in order to normalize the data distributions, and analyzed using two-tailed t-tests. All other data were analyzed without transformation. Male generative tissue changes were analyzed using a Kruskal-Wallis Test.

### **Character Optimization and Phylogenetic Comparative Analyses**

In order to assess the evolutionary history of PSD and other male mate-limiting characteristics in spiders, I elaborated on Miller's (Miller 2007) phylogenetic hypotheses of araneoid spiders, which assessed the evolutionary history of genital mutilation, male sacrifice behavior, male accumulation on female webs (i.e. male-biased operational sex ratio), and monogamy in this group. All analyses were based on the trees and character matrix of Miller (2007; see for detailed description of the phylogenetic hypotheses and characters: [1] genitalia mutilated during copulation (G), [2] sacrifice behavior (S), [3] sexual size dimorphism (D), [4] male accumulation (A), [5] male monogamy (M)). Additionally, I coded the character "permanent sperm depletion" (P) based on the present data and published and unpublished results (Michalik 2006; Michalik et al. 2006; Michalik & Hormiga 2010; Michalik et al. 2010; Michalik unpublished observations) as follows: 0, *spermatogenesis ongoing in adulthood*; 1, *spermatogenesis only in subadult stage*. The Miller (2007) phylogeny is based on morphological data, but given the available genetic data, at the time of the study, this tree was the most robust phylogeny, particularly for resolving relationships among the Nephilids.

The character optimization was carried out with the Maximum Parsimony method (MP) implemented in the software Mesquite 2.7.2 (Maddison & Maddison 2010). To evaluate whether origins of one specific character state ("dependent character") is more concentrated on branches with another specific character state ("independent

character”) we performed the concentrated changes test (CCT; Maddison 1990). The CCT was run in MacClade 4.0 (Maddison & Maddison 2000) using 100,000 replicate simulations with the ancestral state unspecified and actual changes considered. We analyzed the correlation between PSD and genital mutilation, male sacrifice behavior, sexual size dimorphism, monogamy, and male accumulation based on the four phylogenies used by Miller (2007). Since the tests in all four phylogenies resulted in nearly identical p-values we restricted Tab. 2 to the results based on the phylogenetic hypothesis shown in Fig. 2-5 (topology Kuntner et al. ((TA)(EN)); Miller 2007).

## Results

In order to account for the effect of body size on testis size, I analyzed the variation in male body size and the relationship between body size and testis size. For males used in our analyses, body size showed limited variation; prosoma width ranged from 1.43 to 2.08 mm (Mean = 1.85 mm, SE  $\pm$  0.023 mm), and regression analyses showed that prosoma width was not significantly correlated with either testis or deferent duct volume ( $F_{1,35} = 0.022$ ,  $R^2 = 0.0006$ ,  $P = 0.88$ ;  $F_{1,35} = 2.55$ ,  $R^2 = 0.068$ ,  $P = 0.12$ ). For these reasons, I did not account for body size variation in any analyses of the genital system.

### Mechanistic Basis of Permanent Sperm Depletion

Testis volume was significantly greater for sub-adult males compared to adult males (two-tailed t-test,  $t_{35} = 5.89$ ;  $P < 0.0001$ ; Fig. 2-1A). In contrast to testis volume, male deferent duct volume did not differ between sub-adult and adult males (two-tailed t-test,  $t_{35} = 0.34$ ;  $P = 0.74$ , Fig. 2-1B). Thus, for my analyses, I focused on processes in the testis.

Male testis volume changed dramatically with male standardized age (Fig. 2-2A). Within the adult male group, testis volume showed a steep decline with male age that can be described as a negative logarithmic function (Fig. 2-2; Slope = -0.018,  $R^2 = 0.72$ ). The further decrease in volume for adult males is related to the induction of the seminal fluid into the male pedipalps (Fig. 2-2B).

The changes in testis volume corresponded to changes in the proportion of testis tissue devoted to sperm production. Combining all data, these changes over time can be described as three distinct phases (Fig. 2-3; Kruskal-Wallis Test,  $X^2 = 17.26$ ,  $P < 0.0002$ ). In phase one (Fig. 2-3, left), testis volume increases, and the majority of the testis tissue is devoted to sperm production. The peak testis volume corresponds to early spermatogenesis during which germ cells divide and spermatids start to differentiate nearly synchronously. The sperm cells in this phase are large and spherical (Fig. 2-4A). Over the course of spermatogenesis, the spermatids become more compact, indicated by the condensed chromatin in the nucleus and the relatively small amount of cytoplasm in the cell (Fig. 2-4B). At the end of spermatogenesis, the main cellular components coil within the sperm cell (Fig. 2-4C), a process that occurs in all other spiders studied (Alberti 1990). The loss of volume of the spermatids due to the condensing and coiling processes induces phase two (Fig. 2-3, middle), where the spermatogenic cysts around the sperm cells contract, resulting in shrinking of the testes and a decrease in generative tissue. By the end of phase two, sperm cells have moved from the contracted cysts into the lumen of the testes (Fig. 2-4D) and they subsequently move into the lumen of the deferent duct. Sperm induction initiates phase three (Fig. 2-3, right); seminal fluid is discharged out of the testes and deferent duct through the

genital opening. The absence of seminal fluid in the lumen results in further shrinking of the testes and near absence of generative tissue. The seminal fluid consists of spermatozoa and secretion (Fig. 2-4D), which is produced by the somatic cells of the testis.

### **Evolutionary History of Permanent Sperm Depletion**

The optimization of PSD suggests at least three independent origins of this trait in araneoid spiders, i.e. within Nephilidae, Araneidae and Theridiidae (Fig. 2-5). Based on the lack of behavioral data (scored as missing data, see also Miller (2007)), the presence of PSD in *Clitaetra* and *Herennia* (Nephilidae) as well as in the closest related taxa *Argiope* (Araneidae) is ambiguously optimized and should be addressed in future studies. As indicated by the CCT, the evolution of PSD is significantly correlated to and dependent on genital mutilation, male sacrifice, male monogamy, male accumulation, and sexual size dimorphism (Table 2-2).

### **Discussion**

*Nephila clavipes* sperm maturation occurs only during the sub-adult instar, and spermatogenesis is completely absent in adult males. In addition, after the maturation molt, all sperm is inducted into the pedipalps at one time (Myers & Christenson 1988). As a consequence, the amount of sperm available to males for mating is limited to the sperm contained in the male pedipalps, and once it is used, males lose their ability to fertilize eggs. These data strongly suggest that when males deplete their sperm, it is permanent.

PSD, characterized by nearly synchronous spermatogenesis and termination of spermatogenesis in the sub-adult instar, is unusual in spiders. In most spiders, spermatogenesis is ongoing throughout adulthood (Michalik & Uhl 2005) and all stages

of spermatogenesis are observed in the testes at the same time (Michalik & Huber 2006). Moreover, in most species, males generally recharge their pedipalps before, after or even during courtship and copulation (Huber 1998; Knoflach 2004), indicating active sperm production in adult males.

PSD occurs in distantly related spider groups (Fig. 2-5). It has been documented in two spider families, Theridiidae (Michalik et al. 2010), and Nephilidae (*Nephila clavipes* and *N. senegalensis*; *Nephilengys malabarensis* and *N. borbonica*; this study and Michalik and Kuntner unpublished data). In addition, it has been suggested to occur in a third family, Araneidae (Herberstein et al. 2005). Because these three families of spiders are distantly related, and because PSD occurs in some but not all members of these families, (Fig. 2-5; Michalik, unpublished data), it is likely that PSD has evolved independently at least three times (Fig. 2-5). One plausible hypothesis for this distinct distribution of PSD is that the trait evolved in correlation with other traits that limit male reproductive rate (Miller 2007).

Terminal investment behaviors, which eliminate male re-mating ability (e.g. male sacrifice behavior and genital mutilation; Andrade & Kasumovic 2005) often co-occur (Miller 2007), and PSD appears to have evolved in lineages following the evolution of traits associated with male monogamy (Fig. 2-5, Table 2-2). Data suggest that in lineages with high levels of male competition (i.e. male accumulation; Fig. 2-5; Miller 2007), which is often a consequence of extreme sexual size dimorphism (Miller 2007), males are limited to monogamy, and genital mutilation and male sacrifice behaviors have evolved as mechanisms of paternity assurance (Fig. 2-5; Miller 2007; Uhl et al. 2010b). As a consequence, unlimited sperm production became unnecessary in these

groups because males typically mate once (Knoflach & van Harten 2001; Foellmer & Fairbairn 2003). The evolution of PSD may be an energy-saving mechanism that has evolved in some groups with terminal investment strategies and monogamy because it betters males' chances of locating and successfully achieving copulations with a female.

Sperm production and maintaining the function of the testes is energetically costly (Van Voorhies 1992) particularly if males do not feed as adults. In the moth *Plodia interpunctella*, starved males show decreased sperm numbers (Gage & Cook 1994). Similarly, in the adder *Vipera berus*, males lose as much mass during periods of sperm production (when males are immobile) as they do during periods of active mate search (Olsson et al. 1997). In spiders, extreme sexual size dimorphism, which is a consequence of fecundity selection on females (Coddington et al. 1997; Hormiga et al. 2000; but see also Corcobado et al. 2010), results in large sedentary females and mobile searching males. In species that require webs for prey-capture, males that search for females cannot build webs and so typically do not eat during adulthood (Christenson et al. 1985), which makes them vulnerable to starvation.

The hypothesis that PSD is an energy-saving adaptation has some support in the genus *Tidarren* (Theridiidae). Males in this genus have unusually large pedipalps for their body size and so they castrate one of their pedipalps prior to sperm induction (Knoflach & van Harten 2000, 2001). Pedipalp removal increases male locomotor performance, giving males more stamina during mate search, and allowing males to find females more quickly (Ramos et al. 2004). The high locomotor performance that is characteristic of *Tidarren* is unusual among spider species (Ramos et al. 2004). Thus in this genus one result of increased energetic demands could be that males divert oxygen

and other resources away from the testes and into the muscles leading to a loss of testes function (PSD; Michalik et al. 2010).

The need for physical endurance is one characteristic that *Tidarren* appears to share with another sperm-depleting group, Nephilidae. Most of the Nephilid spiders have high post-copulatory energy requirements. Male sacrifice behavior is rare but genital mutilation is common, limiting male re-mating ability (Fig. 2-5; Miller 2007; Kuntner et al. 2009b). However, males guard females after mating, which decreases the probability that the female will re-mate (Christenson & Goist 1979; Christenson et al. 1985; Linn et al. 2007). The termination of spermatogenesis might be an energy-saving measure that allows these males, who no longer have functional pedipalps, to spend extended periods of time fighting after copulation (up to two weeks in *N. clavipes*; (Christenson et al. 1985).

In contrast, in the genus *Latrodectus* (Theridiidae), the widows, male sacrifice behavior is common, but preliminary results suggest that PSD does not occur in this genus (Michalik et al. unpublished data for *L. hasselti*, *L. hesperus* and *L. geometricus*; Table 2-1). However, in *Latrodectus*, even when males are not cannibalized during copulation, they die soon after mating (Forster 1992). Thus, *Latrodectus* species do not appear to share the level of physical stamina and survivorship found in *Tidarren* and the Nephilids, possibly because *Latrodectus* species do not have the same pre and post-copulatory energetic demands. This preliminary hypothesis might explain the conspicuous absence of PSD in *Latrodectus*, even though these species are well known for high rates of genital mutilation, male sacrifice behavior, and monogamy (Andrade 2003; Stoltz & Andrade 2010). Future studies should address this problem.

In the third family of spiders that show PSD, the Araneids, PSD is only found in *Argiope* (Herberstein et al. 2005), one of the few genera in this family with high rates of genital mutilation (Nessler et al. 2007a, 2008) and male sacrifice behavior (Foellmer & Fairbairn 2003, 2004). Although extreme sexual size dimorphism is common in the Araneids, it does not typically lead to monogamy in this group (Miller 2007), which may explain the rarity of PSD in the family. Thus although sexual size dimorphism can lead to monogamy in some cases (Fromhage et al. 2005; Miller 2007), the patterns in the Araneids suggest that either genital mutilation or male sacrifice behavior, which impose the strongest constraints on male re-mating opportunity, must be present for PSD to evolve. Further analyses of the Araneids should test for PSD more broadly to determine whether it occurs only in groups with genital mutilation or sacrifice behavior, or if extreme sexual size dimorphism alone (which only somewhat constrains male mating rate) is sufficient to favor its evolution.

It is important to note that there is variation in the occurrence of genital mutilation within the family Nephilidae, even though PSD may be present in all species (see Fig. 2-5). In *Nephila fenestrata*, males commonly break off the distal tip of the embolus during mating rendering the pedipalp useless (Fromhage & Schneider 2006), while in other species (e.g. *N. edulis*), embolus breakage is rare or absent (Kuntner et al. 2009b; Uhl et al. 2010b). In *N. clavipes* specifically, the evidence for embolus breakage is equivocal (Kuntner et al. 2009b; Uhl et al. 2010b), and recent work in *Nephilengys borbonica* has shown that genital mutilation is a labile trait that occurs only in certain mating contexts (Kuntner et al. 2009a).

In the Nephilids, character optimization and CCT suggests that genital mutilation preceded the evolution of PSD, and mutilation was secondarily lost in certain species (Kuntner et al. 2009b; Fig. 2-5, Table 2-2). The loss of genital mutilation could have occurred because changes in the species' mating systems allowed males to benefit from mating multiply, or because females escaped male plugging behavior over evolutionary time (i.e. antagonistic co-evolution of genitalia; Kuntner et al. 2009b). However, while males in these species may have regained the ability to maintain intact pedipalps during copulation, they may not be able to regain functional testes once PSD has evolved. Instead, in some species (e.g. *N. clavipes*) males may prudently allocate sperm, particularly when mating with non-virgin females (Christenson & Cohn 1988). Thus it remains unclear whether PSD results in monogamy in all cases. Future studies should confirm the occurrence of PSD in Nephilids, determine the relationship between PSD and male mating rate, and examine the evolutionary lability of PSD relative to other terminal investment behaviors. The Nephilid spiders, which show a broad range of mating systems and terminal investment behaviors, make an ideal group for this comparative study.

### **Final Remarks**

Here we verify that sperm depletion in *Nephila clavipes* is permanent, and describe its mechanistic basis. cursory studies in other species suggest that spiders exhibiting PSD share this common mechanism (Michalik et al. 2010). Although PSD is an unusual phenomenon, it appears to have evolved multiple times in association with genital mutilation, male sacrifice behavior, and other traits associated with monogamy (e.g. sexual size dimorphism). In general, PSD could be an energy-saving adaptation, although the factors favoring it (e.g. pre-copulatory mate search or post-copulatory mate

guarding) may be species-specific. Future work will explore the costs of sperm production, the energetic benefits of PSD, and employ a broad comparative phylogenetic approach to address the relationships between PSD, male terminal investment behaviors, and environmental factors that constrain male mating rate in spiders.

Table 2-1. Overview of sperm depletion in spiders as it pertains to sexual cannibalism and genital mutilation.

Family	Species	PSD	TSD	Sexual cannibalism	Genital damage	Source
Nephilidae	<i>Nephila clavipes</i>	yes	yes <sup>F</sup>	no	no	present study; (Christenson 1989)
	<i>Nephila plumipes</i>	?	no	yes	yes, partial genital breakage	(Schneider & Elgar 2001; Schneider et al. 2001; Schneider et al. 2008)
	<i>Nephilengys malabariensis</i>	yes	?	yes	yes, emasculation	(Danielson-François 2006)
Tetragnathidae	<i>Glenognatha emertoni</i>	?	no	no	no	(Danielson-Francois & Bukowski 2005)
	<i>Tetragnatha versicolor</i>	?	no	no	no	(Herberstein et al. 2005; Uhl et al. 2010b)
Araneidae	<i>Argiope keyserlingi</i>	yes	yes	yes	yes, partial genital breakage	(Schneider et al. 2005b; Schneider et al. 2006)
	<i>Argiope bruennichi</i>	?	yes <sup>T</sup>	yes	yes	(Bukowski & Christenson 1997b)
	<i>Micrathena gracilis</i>	?	yes <sup>T</sup>	no	no	(Bukowski et al. 2001)
	<i>Gasteracantha cancriformis</i>	?	yes <sup>T</sup>	14.7 %**	no	(Andrade & Banta 2002; Snow & Andrade 2004); Michalik and Andrade (unpublished)
Theridiidae	<i>Latrodectus hasselti</i>	no	yes	yes	yes	(Knoflach 2004; Molina & Christenson 2008)
	<i>Netiscodes rufipes</i>	?	yes <sup>T</sup>	no	no	(Knoflach & van Harten 2001; Michalik et al. 2010)
	<i>Tidarren argo</i>	yes	?	yes	yes, emasculation	(Costa 1998)
Lycosidae	<i>Schizocosa malitiosa</i>	no*	no	no	no	(Huber 1995; Eberhard 2004)
Anyphaenidae	<i>Anyphaena accentuata</i>	?	no	no	no	(Danielson-François 2006)

In order to summarize direct evidence for sperm depletion in spiders, only studies that explicitly addressed sperm usage by sperm counts were considered. PSD (permanent sperm depletion; yes/no): In cases where the testes were examined, was PSD confirmed? TSD (temporary sperm depletion; yes/no): In cases where the pedipalps were assessed for the presence of sperm after copulation, were the pedipalps absent of sperm? The superscript T denotes that the occurrence of TSD depends on the time spent copulating, and F denotes that the occurrence of TSD depends on the status of the female mate (i.e. virginity). Note that TSD does not necessarily suggest PSD since spider males usually re-induct sperm. \*males that were able to re-induct fathered significantly more offspring than those that were prevented from re-inducting, \*\*male is killed by female but not consumed.

Table 2-2. Hypotheses of the evolution of PSD. Hypotheses were tested using the concentrated change test for one of the phylogenies used by Miller (2007; topology “Kuntner et al. ((TA)(EN))”).

Independent	Dependent	p-value	g, l of dep. (g ind.)
PSD	genital mutilation	0.52	+10, -4 (+3)
PSD	male sacrifice	0.49	+6 (+2)
PSD	male monogamy	0.086	+7, -1 (+3)
	male		
PSD	accumulation	0.166	+6, -1 (+3)
PSD	size dimorphism	0.25	+7, -5 (+3)
genital mutilation	PSD	0.006	+3 (+3)
male sacrifice	PSD	0.0018	+3 (+2)
male monogamy	PSD	0.00069	+3 (+3)
	male		
accumulation	PSD	0.005	+3 (+3)
size dimorphism	PSD	0.03	+3 (+3)

When optimization was ambiguous, accelerated transformations were preferred to minimize the number of gains in the character of interest. The right column indicates the total number of gains (g) and Losses (l) of the dependent character (dep). The number of gains in the presence of the independent character (ind) is given in parentheses.

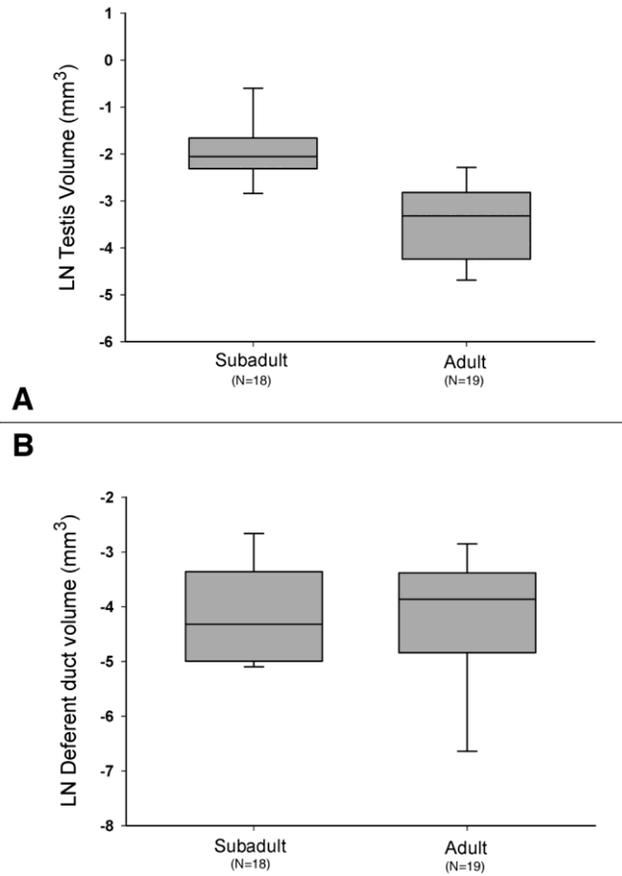


Figure 2-1. Testis volume vs. Deferent duct volume in *Nephila clavipes*. (A) Testis volume in sub-adult versus adult males ( $N_{\text{subadult}}=18$ ,  $N_{\text{adult}}=19$ ;  $F_{1,35}=34.7$ ;  $P<0.0001$ ). (B) Deferent duct volume in sub-adult versus adult males ( $N_{\text{subadult}}=18$ ,  $N_{\text{adult}}=19$ ;  $F_{1,35}=0.11$ ;  $P=0.73$ ).

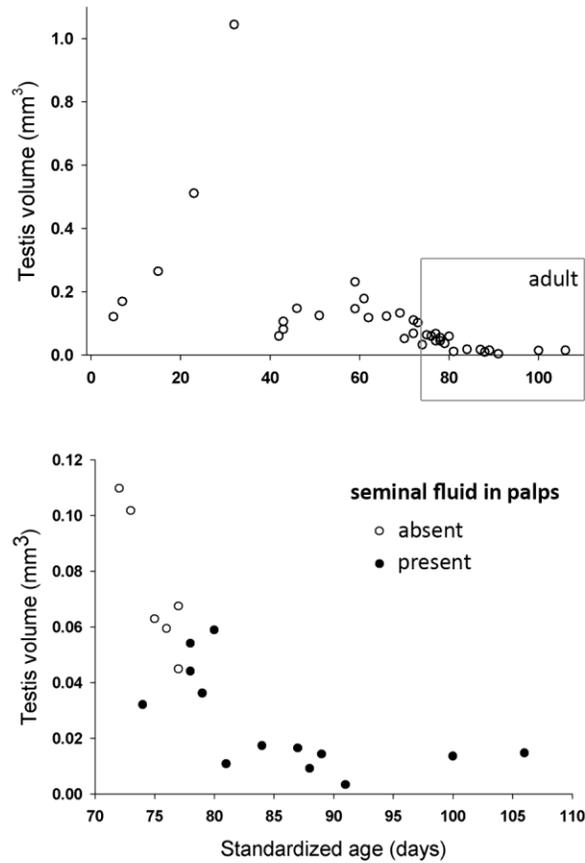


Figure 2-2. Trajectory of testis volume during male development in *Nephila clavipes*. The adult stage (box in upper graph) is enlarged to show the decrease in testis volume after sperm induction (Slope = -0.018,  $R^2 = 0.72$ ).

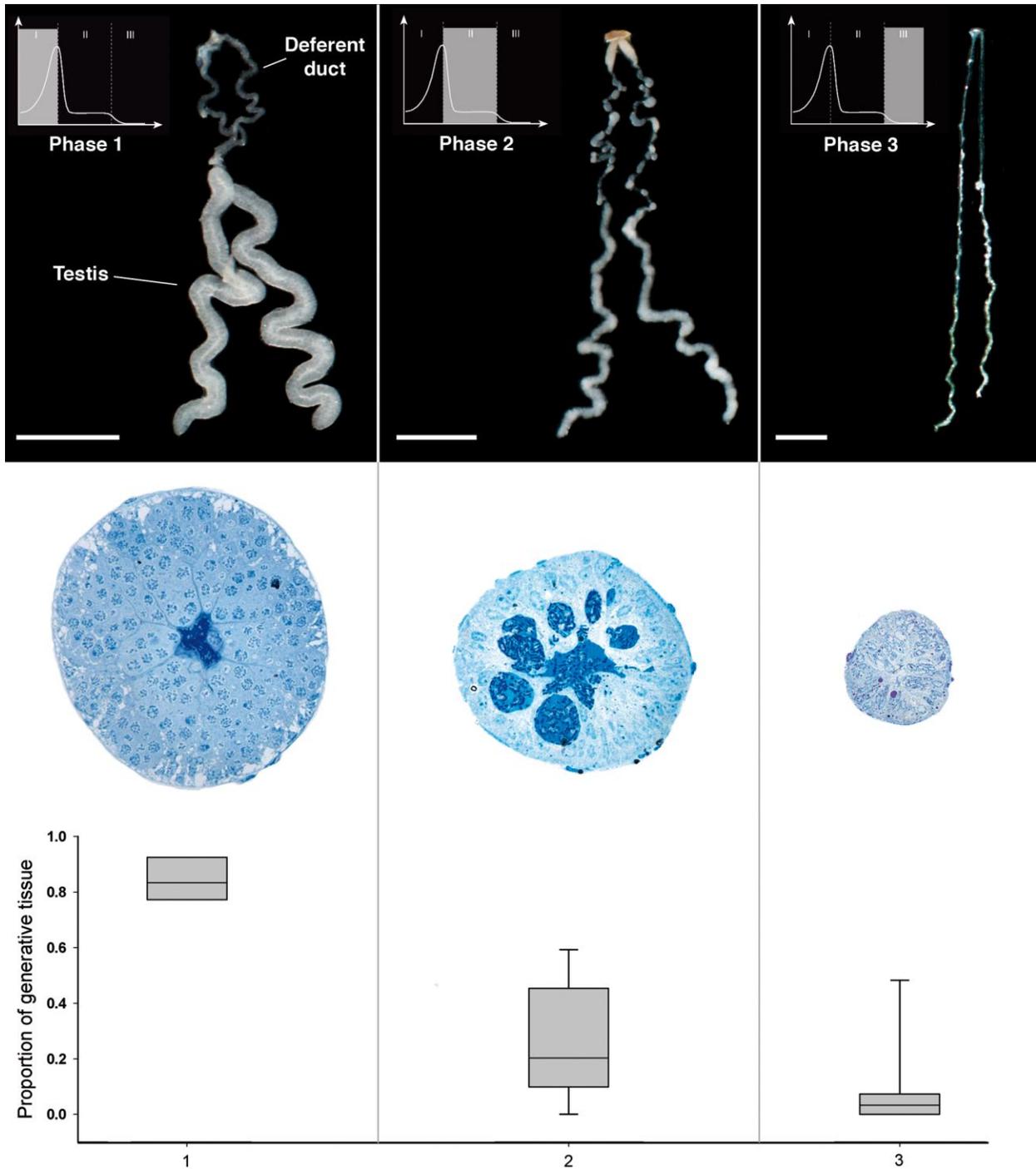


Figure 2-3. Development of the male genital system in *Nephila clavipes*. Three phases in the development of the male genital system (left to right) characterized by a dorsal view of the whole genital system (top, scale: 1mm), a stained cross-section of the testis (middle), and the analysis of the proportion of the generative tissue in testis (bottom; Kruskal-Wallis Test, one-way;  $X^2 = 17.26$ ,  $P < 0.0002$ ).

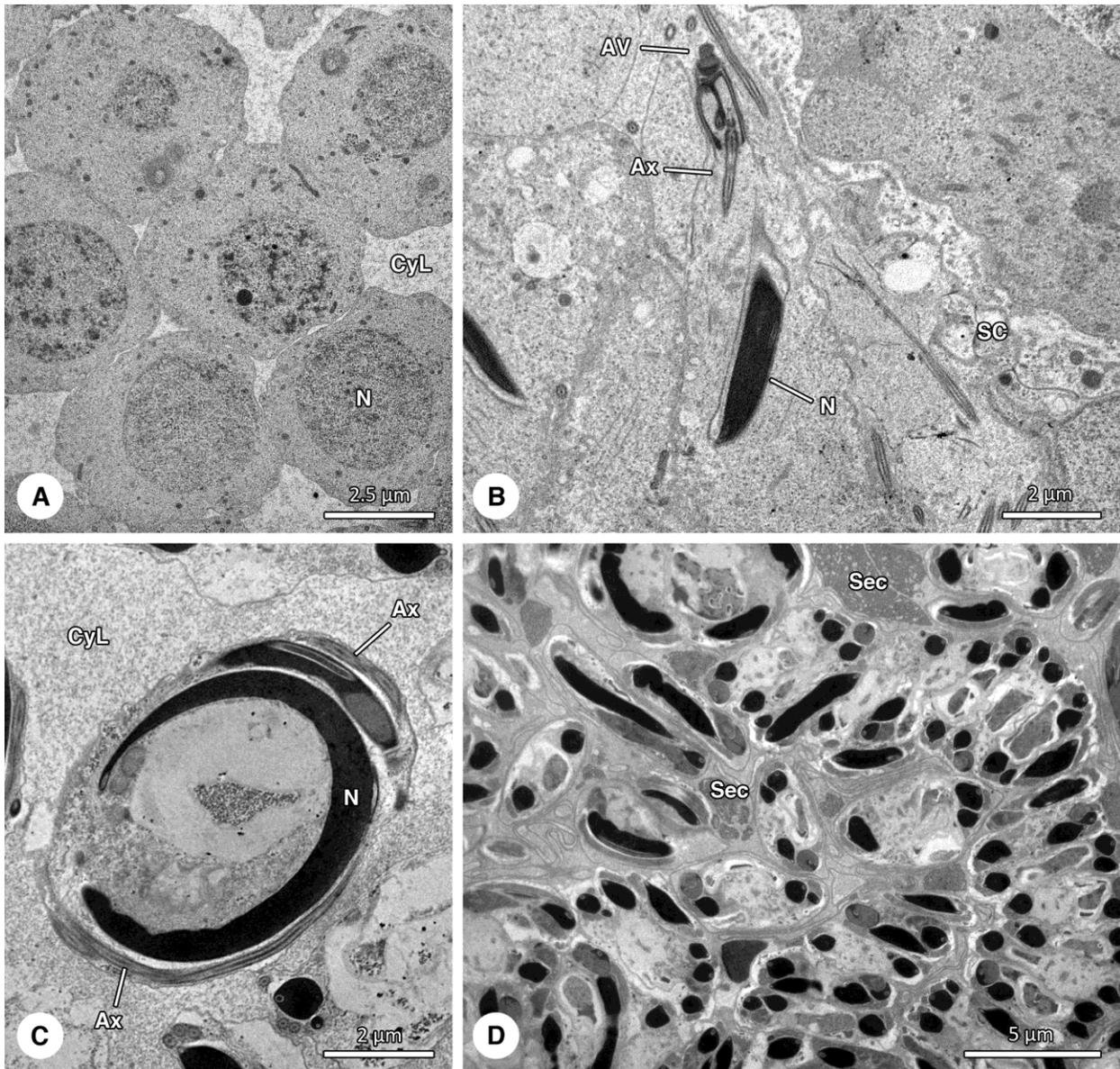


Figure 2-4. Ultrastructural aspects of spermatogenesis in *Nephila clavipes*. (A) Early spermatids. (B) Longitudinal section of mid-stage spermatids with condensed and elongated nuclei. (C) Coiled spermatids in a spermatogenic cyst. (D) Coiled sperm cells and secretion in the lumen of the testis. Abbreviations: AV, acrosomal vacuole; AX, axoneme; CyL, lumen of spermatid cyst; N, nucleus; SC, somatic cell; Sec, secretion.

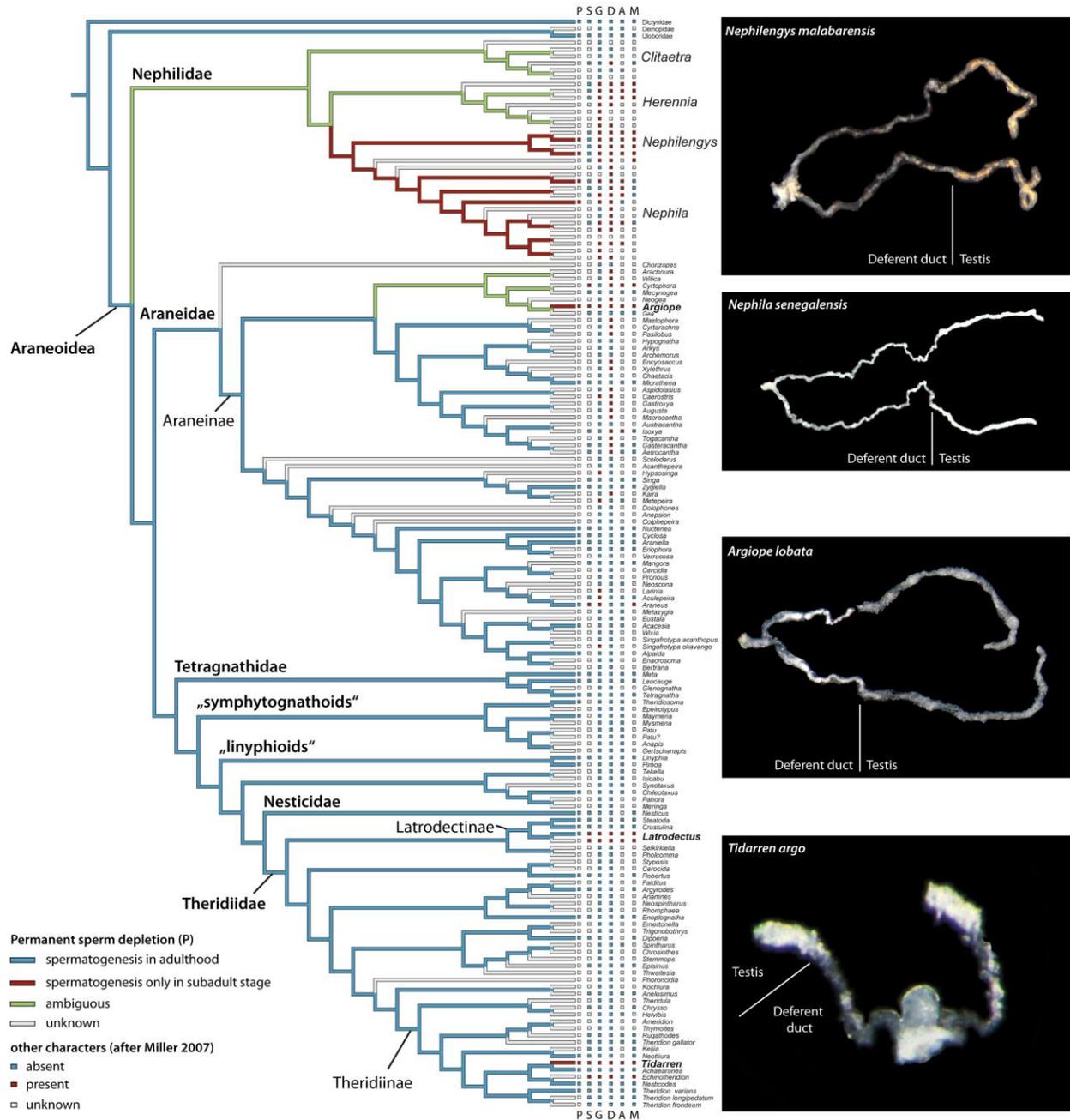


Figure 2-5. Evolution of PSD in araneoid spiders. PSD is optimized on the phylogeny of Araneidae according to Miller (2007; topology “Kuntner et al. ((TA)(EN))”). Note the ambiguous optimization in the Nephilidae node and within Araneidae. Boxes indicate states of the following characters: permanent sperm depletion (P), male sacrifice behavior (S), male genital mutilation (G), extreme sexual size dimorphism (D), male accumulation (A), and male monogamy (M). The pictures in the right column show examples from all genera where PSD is present indicated by the equal diameter of the deferent duct and the testis in the male reproductive system (*T. argo* from Michalik et al. 2010).

CHAPTER 3  
MALE DENSITY AFFECTS LARGE-MALE ADVANTAGE IN THE GOLDEN SILK  
SPIDER, NEPHILA CLAVIPES

**Background**

Understanding the factors that either constrain or maintain phenotypic variation is a fundamental puzzle in biology (Kingsolver & Pfennig 2007). The evolution of body size in particular is of interest because body size affects many aspects of survival and reproduction (e.g. Badyaev 2002; Lafferty & Kuris 2002; Hone & Benton 2005). For males, large-male advantage in male-male contests is common and plays an important role in body size evolution (Andersson 1994). However, male body size is a trade-off between male-male competition and other factors such as growth rate, mate searching efficiency, and predator avoidance. In many species, even though large males may have an advantage during contests, body size is under stabilizing selection, and variation in size is constrained because very large or very small sizes are selected against (Falconer & Mackay 1996; Blanckenhorn 2000). In contrast, in other species, male body size is highly variable, and a wide range of sizes coexist over evolutionary time (e.g. Schneider & Elgar 2005).

In sexually dimorphic species, selection pressures differ between the two sexes to maintain divergent optimal male and female sizes (Fairbairn 2007). However, stabilizing selection that maintains these size optima is predicted to decrease within-sex size variation. In contrast, in some dimorphic species, a broad range of male body sizes is maintained in a single population over time. One factor that could maintain male size variation is density-dependent selection, where large-male competitive advantage changes with density (Kneill 2009). Increased male density can have a positive or a negative effect on large-male advantage. In some cases, when male density is high and

male-male interactions are chaotic, females are difficult to monopolize, resulting in scramble competition that favors small males that find mates quickly (Conner 1989; McLain 1992) or efficiently (Fairbairn 2007). In other cases high male density excludes small males and favors large aggressive males (Zeh 1987; Bertin & Cezilly 2005), limiting small male reproductive opportunities to contexts where male interactions are infrequent. If density varies spatially or temporally, and large-male advantage is density-dependent, then selection will favor high variance in body size within a population. Size variation may be maintained because the optimal male size shifts with density or because extreme sizes are favored in different contexts (e.g. alternative tactics).

Spiders of the genus *Nephila* are regularly featured in studies that address the evolution of body size due to their strong sexual size dimorphism (e.g. Higgins 1992; Head 1995; Hormiga et al. 2000; Schneider & Elgar 2001; Foellmer & Fairbairn 2005b). In addition, male body size in *Nephila* shows unusually high variation (Vollrath 1980), as much as an order of magnitude in *N. edulis* (Schneider et al. 2000). Because larger male *Nephila* have a competitive advantage and typically win male-male agonistic encounters (Christenson & Goist 1979; Vollrath 1980), the persistence of a high degree of male size variation has received special attention. The prevailing hypothesis for the maintenance of variance in male body size in *Nephila* populations is that trade-offs associated with different male sizes result in an intermediate optimal body size (e.g. Vollrath 1980). However, stabilizing selection on an intermediate body size should erode genetic variation over time (Falconer & Mackay 1996), not maintain the high variance that is characteristic of *Nephila*. An alternative hypothesis for high variance in size is that seasonal variation in population density and operational sex ratio creates

density-dependent selection gradients on male size (e.g. Kasumovic et al. 2008). In addition to seasonal variation in population density, local male density (i.e. male group size on a single female's web) varies dramatically during the peak of the breeding season. Variation in male density could maintain the high degree of variation in male body size if increased density changes the strength or direction of sexual selection on male size (e.g. Danforth & Desjardins 1999; Emlen et al. 2005).

Here I investigate whether variation in local male density could contribute to body size evolution in male *Nephila clavipes*. In these web-building spiders, female webs are discrete competitive patches where males fight amongst themselves for opportunities to mate. I surveyed natural variation in male group size on female webs and tested how group size affected the reproductive success of males in a controlled mating experiment. I address how male group size affected male-male competition, the relative sizes of successful father(s), and female re-mating rates.

## **Materials and Methods**

### **Study Sites**

The male group size survey and mating experiment took place on a ranch in Jonesville, Florida (latitude 29.654, longitude -82.523). This site contains pasture as well as oak forest with palmetto understory and has a high density of *N. clavipes*. Survey data were collected along the perimeter of a hay field, which was bounded on all sides by oak forest. Females and males used in the mating experiment were collected from other forested sites on the property. The mating experiment was conducted in and around a covered but open 3.5m x 3m x 4m shed in the oak forest, which provided shelter from the rain and maintained humidity and temperature similar to the surrounding forest.

## **Male Group Size Survey**

I surveyed male group sizes on 1 August 2007 between 1300 and 1400 hrs., checking all webs along the perimeter of the hay field (N = 129). I chose this sampling method in order to get the best idea of male group size variation during the peak of the breeding season when adult males are in highest abundance (C. Rittschof, unpublished data). Because males do not change webs during the daytime, sampling at one time point during the day is an accurate measure of the group size on that day (Christenson and Goist 1979). To be included in my survey, webs had to: 1) contain either an adult female or a juvenile female within 1-2 instars of maturity; 2) be within 3 m of the ground; and 3) have at least one male present. I determined female maturity using genital morphology (Higgins 2000), and estimated the age of juvenile females by visually approximating their body size (about 5-6 mm cephalothorax width). I excluded smaller juvenile females because these webs typically do not contain males (Cohn et al., 1988). For each web, I recorded the number of males present.

## **Mating Experiment: Procedures**

I tested the effects of male group size on male reproductive success in a mating experiment conducted between 18 July 2007 and 23 September 2007. I conducted the mating experiment in cages to ensure that each female was a virgin at the start of the experiment. I introduced males to cages with females on the day of the female's terminal molt and left the males to mate for two days. I used two treatments, a 2-male group size and a 6-male group size. The 6-male group size was the maximum group size observed on the webs of virgin females (Farr 1977; T. Christenson, pers. comm.).

I collected penultimate-instar females (N = 47) and housed them individually in 90 cm x 90 cm wood frame cages 15 cm deep. Cages were built from pressure-treated

pine coated with Thompson's Water Seal<sup>®</sup> and covered with fiberglass screening (1 mm mesh size). One corner of the screen on one side of each cage was lined with Velcro<sup>®</sup> so that I could detach the screen to feed the spiders and add and remove males. Females built and maintained normal prey capture webs in these frame cages. I sprayed a water mist on webs daily and fed each female 1 large mealworm (approximately 2 cm in body length) per day for the duration of the experiment (Christenson & Cohn 1988).

Through most of the season, I collected mature males for the mating experiment from juvenile female webs. After their terminal maturation molt, males have dark body coloration and sclerotized pedipalps (Myers & Christenson 1988). Because males were collected from juvenile females' webs, I could ensure they had not mated with the current female web-owner, but I could not ensure virginity because males travel between webs (Cohn et al. 1988), and a male could have mated with a different female prior to collection. Towards the end of the season, juvenile females and males became scarce, and so two trials from each treatment used males collected from adult female webs. Although mating experience may have affected male behavior, due to my method of collection, any effects of mating experience are randomized across the two treatments.

Each day male collection was required, I collected a minimum of 8 males at a time (enough males for 1 trial from the 2-male treatment and 1 from the 6-male treatment) in order to standardize any effects of collection date across the two treatment groups. I collected a range of male body sizes, and males were assigned to treatments in the order of collection. Because previous studies have shown that male dominance

on female webs is a function of relative and not absolute body size (Christenson & Goist 1979) I did not control for absolute body size in my treatments. Male body size was determined by measuring cephalothorax width, and each male within a treatment trial was assigned a size rank starting with rank 1 for the largest male. The length of the first tibia-patellar (TP) leg segment can influence the outcome of male contests in spiders (Foellmer & Fairbairn 2005b). However, I validated that TP length for all legs scales linearly with cephalothorax width for males of this species (C. Rittschof, unpublished data). This suggests that cephalothorax width alone is adequate to describe the size differences among males, regardless of which element of body size gives large males a competitive advantage. Every male was uniquely marked with dots of Testor's® enamel paint.

I housed males individually in 946 mL Tupperware® containers with a portion of the lid cutout and replaced with fiberglass screening. I sprayed the males with water daily until the initiation of the mating experiment (mean waiting time = 3.1 d, S.E. =  $\pm$  0.06). Because mature males do not build prey-capture webs and eat only occasionally as adults on female webs (Cohn & Christenson 1987), I did not feed the males between capture and the initiation of the mating experiment. No males died while awaiting the beginning of the mating experiment.

Every morning, I checked female cages to determine if any females had molted. If a female molted, I verified she was mature by genital morphology (Higgins 2000) and initiated the experiment between 0800 and 1200 hours. As females matured, I assigned them to a treatment group, alternating between the 2-male and 6-male treatment. When more than one female molted on a single day, I started one trial at a time in order to

observe and record male behaviors for the first hour after males were introduced to the female's cage. I introduced males sequentially in order from smallest to largest in less than 1 min.

Males were left in the cage for 48 h to mate with the female. I did not feed females during the experiment, since the presence of food can alter males' mating behavior (Christenson et al. 1985). If a male died before the experiment was completed, I removed him and froze his body immediately. After 48 h, I removed all remaining males and killed them by placing them in a -20° C freezer for tissue preservation. After mating, females were housed in their cages and fed and misted as they were prior to the mating experiment until they laid their first clutch of eggs. Immediately after clutch production each female was collected, killed, measured, and preserved in the same way as the males. I left egg clutches where they were laid and collected them after the offspring hatched. Most clutches were collected and preserved in 95% ethanol after the offspring molted to their second instar. However 6 clutches were preserved before their second instar because they were at risk of predation by ants.

Paternity analysis was performed using 3 polymorphic microsatellite markers developed for this species. The loci had 2, 3, and 18 alleles (see Appendix B). I used the maximum likelihood calculation from the program GERUD 2.0 (Jones 2005) to determine paternal genotype(s) using the known maternal genotype and the genotypes of 24 progeny per clutch (see Appendix B).

### **Mating Experiment: Behavioral Observations**

I recorded male-male aggression and mating behavior for the first hour of each experimental trial. A behavior was considered an aggressive act only when the male performing the behavior was oriented in the direction of another male. Using this

criterion, any behaviors or interactions directed towards the female were excluded.

When males encounter one another, one or both males perform a series of behaviors that escalate from long-range threats (when males are as much as 20 cm apart) to physical contact (Christenson & Goist 1979). There are three basic stages of escalation (adapted from Christenson & Goist 1979): (1) strand plucking, where one male turns to face the other male, shakes his body from side to side and jerks the web with his front pair of legs (Christenson et al. 1985); (2) chasing, where the aggressor moves forward with his front legs raised in response to another male who either flees from or turns toward the aggressor in preparation for a fight; (3) grappling (Cohn et al. 1988) which is where males come into contact and hit one another with the front pairs of legs, attempt to bite, and push with the front legs to knock the opponent off of the web (Christenson & Goist 1979). Other male behaviors, such as avoidance or running away from other males were not tallied as aggressive behaviors. I recorded any male-male interaction as a “challenge”, and so each challenge could include one or a combination of the three escalating threatening and fighting behaviors. This general definition of male fighting behavior was necessary because the transition from strand plucking through grappling can occur in a matter of seconds. I recorded the male who initiated each challenge, the identity of the male targeted in the challenge, and the winner and loser of the challenge. The male who initiated the challenge was the first male to behave aggressively in response to the presence of another male, i.e. the male that first shook the female’s web, or chased another male as he approached the female (after Christenson & Goist 1979). The loser was the male that first turned away or fell off of the web during a grapple. I defined a “behaviorally dominant” male as the male who initiated the most

challenges within his group. In order to test if males in the 6-male treatment behaved more aggressively than those in the 2-male treatment, I calculated the number of male-male challenges per male.

I measured mating behavior for the first hour of each trial. During mating, a male climbs onto the ventral surface of the female's abdomen and inserts his pedipalps into her epigynum (Christenson et al. 1985). Because pedipalp insertions were difficult to see through the cage screen, I used mounting as a measure of mating attempts. A male was considered mounted when he climbed onto the ventral surface of the female's abdomen. I recorded the latency to any male mounting the female, the identity of the first male to mount, and the cumulative time a male spent in the mounted position. A dismount was scored if the male lost contact with the female.

### **Statistical Analyses**

Both behavioral and paternity analyses comparing treatment groups are unpaired analyses. For standard parametric and non-parametric statistical tests (Wilcoxon Signed-Rank test, T-tests and Chi Squared test) I used the program JMP 7.0 (SAS Institute, Cary NC). However, in order to determine if body size affects either male dominance or paternity success in a 6-male group, I needed to test whether large males were dominant males (or fathers) more often than would be expected if size had no effect on dominance and paternity. Due to non-independence, I could not directly compare male size ranks for dominant and non-dominant males (as well as fathers versus non-fathers) within each treatment group using a standard non-parametric test. Instead, I tested whether, across all 6-male trials, the sum of the body size ranks for dominant males (or fathers) was different from a sum of size ranks sampled randomly.

The mean ( $m$ ) of six ranks is 3.5 with a variance ( $v$ ) of 3, and the distribution of the sum of  $N$  number ranks is approximately normal (confirmed by simulation). I tested the sum of the ranks for behaviorally dominant males (or fathers) in the 6-male treatment against a normal distribution of sums with mean  $N*m$  and standard deviation the square root of  $N*v$ . I computed a one-tailed  $p$ -value for the hypothesis that dominant males (or fathers) fathers have a larger rank than the random expectation.

## Results

### Male Group Size

On juvenile female webs ( $N = 75$ ), male group size ranged from 0-6 males (Mean = 1.48, SE =  $\pm 0.16$ ; Fig. 3-1 top), 65.3% of webs had 0-1 males, 17.3% had two males, and 17.3% had 3 or more males. On adult female webs ( $N=54$ ), male group size ranged from 0-16 males (Mean = 2.4, SE =  $\pm 0.36$ ; Fig. 3-1 bottom), 46% of webs had 0-1 males, 24% had 2 males, and 30% had three or more males. Overall there were significantly more males on adult female webs (Wilcoxon Signed-Rank Test,  $Z = 2.29$ ,  $P = 0.022$ ).

### Experiment: Behavioral Analysis

Due to observation-time constraints on the day of the females' maturation molt, there are behavioral data for 35 total mating trials across the 2-male and 6-male treatment groups, and there are paternity data for 29 trials in which females laid viable clutches (Table 3-1).

Males used in the mating experiment spent 0-11 days in captivity before they entered the experiment, and there was no difference between the two treatments in captivity time prior to use in the experiment (Mean<sub>2-male</sub> = 3.2 d, S.E. = 1.0 d, Mean<sub>6-male</sub> = 2.9 d, S.E. = 0.81 d, two-tailed T-test,  $t = -0.18$ ,  $P = 0.86$ ). Time in captivity did not

affect male behavior. There was no relationship between the number of days males spent in captivity and the total number of male-male challenges ( $F_{1,33} = 0.012$ ,  $P = 0.91$ ). In addition, the time before first mount was independent of time in captivity ( $F_{1,33} = 1.43$ ,  $P = 0.08$ ), and the total time spent mounted was independent of time in captivity ( $F_{1,33} = 1.15$ ,  $P = 0.21$ ).

Within the first hour of observation, challenges occurred in 83% of 6-male trials ( $N = 15$  of 18) and 35% of 2-male trials ( $N=6$  of 17), a significant difference (Chi Squared Test,  $P < 0.0021$ ). The number of challenges in the 2-male group ranged from 0 to 6 (Mean = 1.1, S.E. = 0.46), and in the 6-male group from 0 to 12 (Mean = 4.9, S.E. = 0.9). Across all trials ( $N = 35$ ), the total challenges calculated per male did not differ between the treatment groups (Mean<sub>2-male</sub> = 0.56, S.E. = 0.23, Mean<sub>6-male</sub> = 0.82, S.E. = 0.15, two-tailed T-test;  $t_3 = 0.97$ ,  $P = 0.34$ ).

Male body size had a unimodal distribution and ranged from 1.3 mm to 3.45 mm (Mean = 2.25, SE =  $\pm 0.04$ ,  $N = 139$ ; Fig. 3-2). The range of male body sizes represented within a single trial did not differ between treatments (Mean<sub>2-male</sub> = 0.71, S.E. = 0.09, Mean<sub>6-male</sub> = 0.94, S.E. = 0.09, two-tailed T-test,  $t_{33} = 1.9$ ,  $P = 0.07$ ). In addition, absolute male body sizes within each 6-male treatment trial were evenly distributed across the size range represented in the trial; the average difference in absolute body size between each size rank within a trial was 0.20 mm (S.E. = 0.02 mm; range = 0.08 mm to 0.3 mm).

Across all challenges observed in this study, I observed 39 challenges in which there were clear winners. Of these contests, larger ranked males won contests more often than smaller ranked males (Chi Squared Test,  $X^2 = 11.3$ ,  $P = 0.0008$ ). However,

because males did not always respond to challenges, a winner was determined for only 39 of 76 total challenges observed across all trials (51%). In the 6-male treatment group, behaviorally dominant males (N = 11) were larger ranked males than predicted under the hypothesis that male size has no effect on dominance ( $P < 0.001$ ). The mean rank for dominant males was 1.9 (S.E. = 0.47) and for non-dominant males the mean rank was 3.8 (S.E. = 0.21). In the 2-male treatment, the dominant male was the larger male in all cases in which male interactions occurred (N = 4 trials where a dominant male was identified).

Across both treatments (N = 35), males mounted within the first hour in 16 trials ( $N_{2\text{-male}}=7$ ,  $N_{6\text{-male}}=9$ ). There was no difference among treatment groups in latency to mount (Mean<sub>2-male</sub> = 32.1 min., S.E. = 5.9 min., Mean<sub>6-male</sub> = 26.9 min., S.E. = 5.2 min.; two-tailed T-test,  $t_{14} = -0.67$ ,  $P = 0.51$ ) or in total time spent mounted (Mean<sub>2-male</sub> = 16.0, S.E. = 4.6, Mean<sub>6-male</sub> = 10.3, S.E. = 3.9, two-tailed T-test,  $t_{14} = -0.95$ ,  $P = 0.36$ ). For the trials where males mounted within the first hour and a behaviorally dominant male could be determined ( $N_{2\text{-male}} = 4$ ,  $N_{6\text{-male}} = 8$ ), the behaviorally dominant male mounted first in 100% (2-male) and 75% (6-male) of the cases. In 85% (N = 13) of all trials where males mounted and paternity could be determined, the first male to mount became the father. This included 75% of 6-male trials (N = 8) and 100% of the 2-male trials (N = 5).

The overall rate of multiple paternity was low; one male fathered all offspring in 83% of clutches (N = 29). There was no difference in the rate of multiple paternity in 2-male (N = 2) and 6-male (N = 3) groups (Chi-Square Test,  $X^2 = 0.33$ ,  $P = 0.56$ ). In 4 trials from the 2-male treatment and 2 trials from the 6-male treatment, the father died before

the end of the experiment, but even among these trials there was only 1 case of multiple paternity (in a 6-male trial).

Across both treatments, behavioral dominance corresponded to paternity in 80% of all trials (N = 15). Behaviorally dominant males were fathers 73% of the time (N = 11) in 6-male trials and 100% of the time in 2-male trials (N = 4). However, across all 2-male trials where paternity was determined, there was no difference in the probability that the smaller or larger male was the father (N = 15, Chi-Square Test,  $X^2 = 0.529$ ,  $P = 0.47$ ), while fathers in the 6-male treatment were larger males than predicted under the hypothesis that male size has no effect on paternity (Mean rank for fathers = 2.6 (S.E. = 0.4); Mean rank for non-fathers = 3.7 (S.E. = 0.2); N = 14,  $P < 0.006$ , Fig. 3-3).

The number of offspring produced per female was not correlated with the size of the father ( $F_{1,17} = 0.70$ ,  $P = 0.41$ ), it did not differ among trials with one (Mean = 560.7, S.E = 25.2 offspring) versus two fathers (Mean = 606.6, S.E. = 55.2 offspring), and it was not significantly different between the 2-male (Mean = 552.6, S.E. = 31.9 offspring) and 6-male (Mean = 585.5, S.E. = 33.0 offspring) treatment groups ( $F_{1,27} = 0.52$ ,  $P = 0.48$ ). The number of offspring produced was positively correlated with female size ( $R^2 = 0.19$ ,  $P = 0.007$ ) and female size was negatively correlated with maturation date ( $R^2 = 0.71$ ,  $P < 0.0001$ ).

## Discussion

*Nephila clavipes* males gather in groups of variable sizes on females' webs and compete to mate (Fig. 3-1). In contrast to some other density-dependent systems where smaller, non-competitive males find females and mate faster when at high densities (Knell 2009), larger males mated first in *N. clavipes* only when group size was large (Fig. 3-3). There was no large-male advantage when group size was small and male

interactions were infrequent. Thus, on many webs (Fig. 3-1), small males face no competitive disadvantage. This may allow small body sizes to persist in the population. In the mating experiment, the frequency of male-male challenges increased with increased male group size, and across all trials, larger males typically won challenges. Male group size did not affect the rate of multiple paternity, which occurred in 17% of all trials. Because there was typically only a single successful father per male group, male reproductive success was determined by his ability to reach the female first; in 85% of all trials the first male to mount was the father.

In the wild, males compete on juvenile, virgin, and non-virgin adult female webs (Christenson et al. 1985). However, in the mating experiment, I staged male-male competitions on virgin female webs to enable accurate paternity analysis. In addition, I chose to use virgin females because they are the most likely to be sexually receptive (Christenson et al. 1985; Cohn et al. 1988). Because neither the intensity nor the outcome of male contests changes with female type (Cohn et al. 1988), the positive relationship between male density and large-male advantage found on virgin female webs in this experiment may also apply to contests on juvenile and non-virgin adult female webs.

*Nephila clavipes* males arrive and compete to establish dominance on older juvenile webs in order to mate with the female once she has matured (Christenson et al. 1985), but males are also attracted to attempt to mate with adult females (Fig. 3-1, bottom; Christenson et al. 1985). The survey of juvenile female webs (Fig. 3-1, top) shows the degree of male-male competition over females that are close to their maturation molt. Because only 17.3% of juvenile webs had three or more males,

competition is low on most webs, and small and large males may be equally likely to mate with a large percentage of virgin females in the population. This range of male group size is comparable to group sizes reported in other studies in this species (Farr 1977; Vincent & Lailvaux 2006). In contrast, the range of male group sizes and the frequency of groups with three or more males are higher on adult female webs (Fig. 1). Group sizes of three or more occur on about 30% of these webs, which suggests that large male advantage affects male reproductive success to a higher degree on adult webs compared to juvenile webs.

Although the frequency of highly competitive webs is below 50% for adult females, males are not uniformly attracted to these females, and group size is positively correlated with female condition (Vincent & Lailvaux 2006; C. Rittschof unpublished data). Thus females with large groups of males may be higher quality mates compared to females with small groups. While male body size and density may be important determinants of male mating success, the quality of the female mate ultimately affects total male reproductive potential. Future work should address male dynamics on non-virgin webs, including the causes for variation in group size, and the relative fitness pay-offs for males that mate with females that attract large versus small male groups.

Male encounters and contests were infrequent in the 2-male treatment group. This outcome may have occurred because on the large webs characteristic of *N. clavipes*, males are unable to detect one another when group sizes are small. An alternative explanation is that males behave less aggressively when group size is small. Male aggression can vary as a function of operational sex ratio or the number of competitors present. For example, male guppies interfere with rivals' courtship displays

at higher rates as the operational sex ratio becomes increasingly male-biased (Jirotkul 1999). Similarly in the mosquitofish, decreased female density results in increased male-male competition (Smith 2007). Thus a minimum number of rival males could be required to stimulate aggressive interactions between males, and when these interactions occur, large males, who typically win contests, have a reproductive advantage.

Two other factors that could affect male reproductive success in a size-dependent manner are the order of male arrival to the web and male competitive endurance over the course of his tenure on the web (e.g. Blanckenhorn 2000). Because the timing of the female's maturation molt is difficult to predict, I introduced males to the female web simultaneously when I initiated the mating experiment. This method does not account for the order of male arrival to the web or male endurance. However, because in many cases in the mating experiment males required more than an hour to reach and mount the female, and because multiple males arrive to a web within hours of each other in the field (Rittschof & Ruggles 2010), my method approximates competitive scenarios that occur on female webs in the wild.

Females rarely re-mated within the time frame of this experiment, even in 5 trials where the single male mate died before the end of the experiment. Prior work suggests this result could be because females are initially unreceptive to mating after their first copulation (Christenson & Cohn 1988; Cohn et al. 1988). In all other experiments that report immediate second-male mating, the experimenters present food to the female in order to facilitate mating (Christenson & Cohn 1988). In my experiment, females were not fed during the 48 hrs. males were present, which may have prevented second-male

mates from gaining access to the female. In this 2-day time frame, the factor most critical to male reproductive success is his ability to mate first, which is a function of male body size and male density.

It is possible that the 6-male group size used for my high density treatment was not adequately large to overcome large-male advantage (Knell 2009). However, certain features of *N. clavipes* could facilitate a large-male competitive advantage even at very high densities. First, female *N. clavipes* spend most of their time in the center of the web. She moves only to collect prey that is caught in the web (Christenson & Goist 1979; Cohn et al. 1988). Males that lose male-male encounters move to the periphery of the web while contest winners move closer to the center and defend only the small area around the female. Second, males threaten one another at a distance by shaking the web. These long-range contests allow males to defeat multiple intruders in succession without physical contact. Third, the spider web is a two-dimensional surface, and unlike animals that fly or burrow underground, male intruders can only approach the female on the plane of the web. The small defendable central area, long-range threatening behaviors, and two-dimensional structure of the web could allow males to monopolize females even at high densities.

Body size in *N. clavipes* has a genetic component and a degree of plasticity in response to environmental conditions and food availability (Vollrath 1983; Higgins 1992). However, stabilizing selection on a single body size optimum would select against plasticity that could result in a body size that deviates from the optimum. Conversely, if changes in male density facilitate variation in reproductive success for males of different sizes, body size variation could be maintained by spatial variation in

the strength of sexual selection. Local variation in the strength or direction of sexual selection can be considered a heterogeneous mosaic of selection intensities. In the case of the damselfly *Ishnura elegans*, spatial variation in the density of females and the frequency of female morphotypes changes the strength and direction of selection on male body size (Gosden & Svensson 2008). Spider webs are discrete habitat patches (Agnarsson 2003) with different male densities. As a result, there is spatial variation in the strength of sexual selection on male body size. My results are similar to findings in *Nephila plumipes*, where seasonal changes in population density and operational sex ratio result in temporal variation in selection gradients on male size (Kasumovic et al. 2008).

Variation in male body size in web-building spiders has been explained in several different ways. For example, Elgar and Fahey (1996) emphasized an advantage to small size due to reduced sexual cannibalism. Moya-Larano et al. (2009) showed an intermediate optimum attributable to climbing speed. However, these studies suggest that stabilizing selection is acting to optimize male body size, which predicts decreased, not increased size variation (Falconer & Mackay 1996). In *N. edulis*, Schneider et al. (2000) showed that females prefer small males during the physical act of copulation, and small males typically copulate longer and father more offspring than large males. The authors speculate that some other variable is required to maintain large male sizes in this species, for example high or variable male densities.

An additional alternative hypothesis for the maintenance of small male sizes and size variation in *N. clavipes* is that large-male competitive advantage is balanced by a small-male advantage in mate-search efficiency, particularly if female density is low

(Blanckenhorn 2000; Fairbairn 2007). This hypothesis predicts that small males arrive to a web and mate before large males are able to reach the web. However, if small males arrive to a web first, this does not exclude the possibility that these males are avoiding competitive environments and choosing webs with few competitors. Furthermore, many studies in spiders have tested for an association between male body size and mate-search efficiency and have failed to find a relationship (e.g. Prenter et al. 1998; Foellmer & Fairbairn 2005b; Kasumovic et al. 2007; De Mas et al. 2009). In *N. clavipes*, there is evidence that rather than small males, intermediate sized males have the highest survival rate during mate-search (Vollrath 1980). Thus stabilizing selection on male size would result from the trade-off between male competition that favors larger size and mate-search efficiency that favors intermediate size. Such selection should limit variation in male size (Falconer & Mackay 1996).

Because females occur at such high densities, mate-search efficiency may not limit male mating opportunity in *N. clavipes* (Farr 1977; Hodge & Uetz 1992). Furthermore, adult webs in close proximity contain variable male group sizes. Males appear to assess some aspect of female webs through a period of residence time on the web. They arrive at a web and stay for variable periods of time depending on their success at competing with other males to mate (Christenson & Goist 1979). The fact that males are able to assess webs after they arrive and in some cases leave suggests movement between different webs may not be a strong constraint on male reproductive behavior.

Conversely, assessing the female's web upon arrival could provide information about the level of competition on the web and allow males to decide to stay or leave

depending on their own relative body size. Small males might avoid highly competitive webs while large males might target those webs because they have a competitive advantage in large groups. Such divergent mating tactics often contribute to variation in sexually-selected traits such as body size (Brockmann & Taborsky 2008). Thus density-dependent selection on body size could maintain male size variation and facilitate the evolution of size-dependent mating tactics. In addition, if the level of competition on a web is correlated with female value, these tactics carry different risks and pay-offs. Future studies could explore the contexts for male alternative mating tactics, specifically the relationship between female value, male competition intensity, and male reproductive success with respect to body size.

Table 3-1. Sample sizes across data types

	Number of Trials			
	2-male	6-male	Total	
Behavioral		17	18	35
Paternity		15	14	29
Both		13	13	26

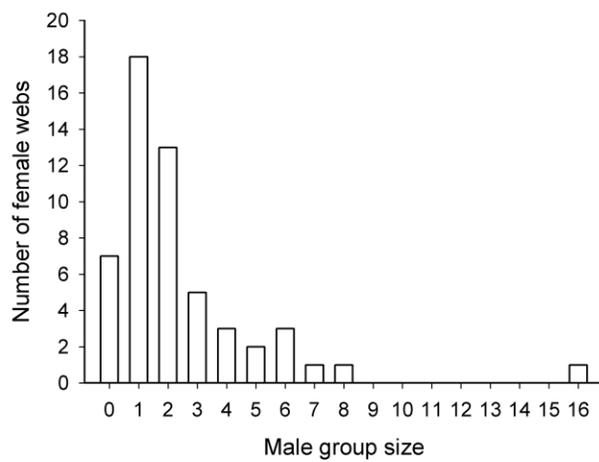
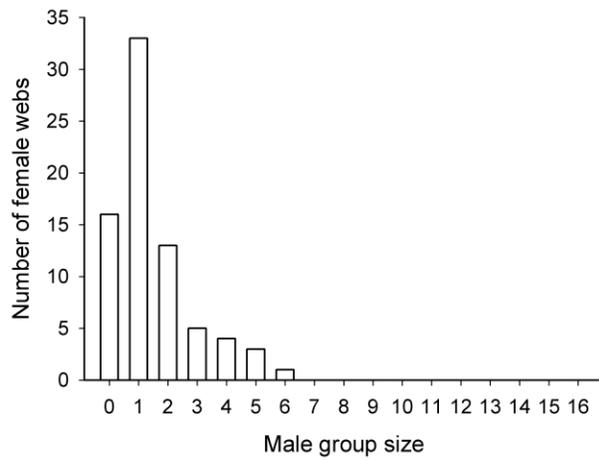


Figure 3-1. Frequency distributions for male group sizes from field surveys of juvenile (top panel) and adult (bottom panel) female webs.

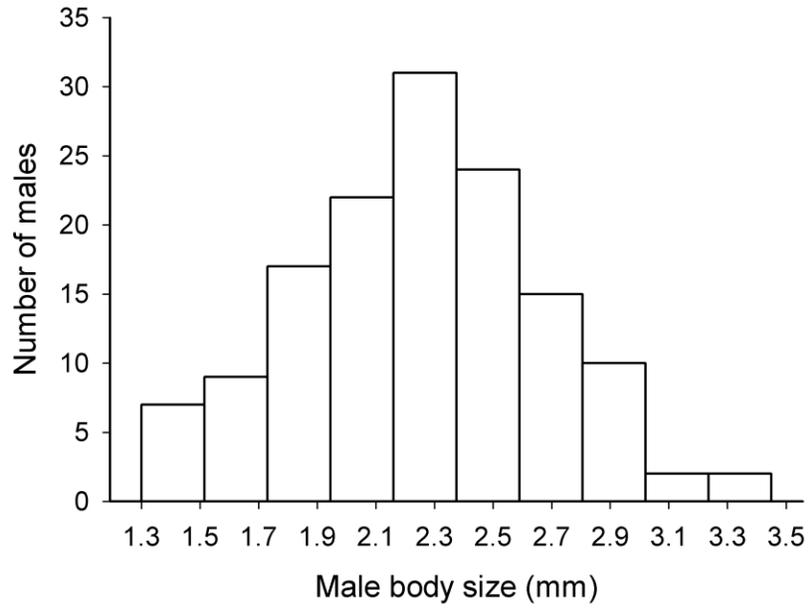


Figure 3-2. Distribution of body size (cephalothorax width, mm) for male *N. clavipes* in the mating experiment (N=139).



Figure 3-3. Size ranks for fathers versus males that were not fathers in the 6-male treatment group. Lower ranked males are larger.

CHAPTER 4  
MORTALITY RISK AFFECTS MATING DECISIONS IN THE SPIDER *NEPHILA*  
*CLAVIPES*

**Background**

For animals, decision-making involves risk-assessment (Seger & Brockmann 1987; Austad 1989; Creel 1993; McNamara et al. 2006; Beaumont et al. 2009; Wiklund & Friberg 2009; Beauchamp 2010; Nevoux et al. 2010). For example, many studies have addressed how males and females navigate the risks associated with searching for, competing for, and winning mates, including predation risk (e.g. Hedrick & Dill 1993) and sperm competition risk (Parker et al. 1997). For males, one element of risk that is rarely considered (Dunn et al. 2001) is that a female mate may not survive to offspring independence, resulting in total reproductive failure. This risk factor would be particularly important (a) when males, due to limited resources, mate with only one or a few females in their lifetime; and (b) when there is high variance in breeding success among reproductive females because of high and variable adult mortality. These traits are common in species with short life spans and few reproductive opportunities because there is strong selection on individuals of low quality to reproduce despite their low chances of success (Parker 1983; Roff 1992). In contrast, in species with longer reproductive life spans, low-quality individuals typically forego a reproductive cycle if they can improve their quality as non-breeding individuals, and thus improve their lifetime fitness by passing up a risky reproductive opportunity (e.g. Angerbjorn et al. 1991; Vanheezik et al. 1994).

If female mortality risk is a significant source of variation in female reproductive output, males who choose low risk females will have a fitness advantage. Although sperm competition and sperm precedence patterns are common sources of variation in

male reproductive success (Parker et al. 1997), female fecundity differences are often the greatest source of variation, and as a result, choosy males pay attention to indicators of fecundity (Bonduriansky 2001). However, regardless of a female's potential fecundity, a female must survive to offspring independence for males to gain any fitness at all. Therefore, if males can assess female mortality risk, they might benefit by choosing low-risk females even at a trade-off to other features that affect fecundity or number of potential offspring sired, e.g. female body size or sperm competition risk.

Few studies have considered whether males use female survivorship as a mate choice criterion, even though there is evidence that males can choose females using traits that predict survivorship. For example, in the seaweed fly, males choose females on the basis of vigor and vigorous females are more likely to survive the long period between mating and oviposition in this species (Dunn et al. 2001). In a number of taxa including fish and insects, there is evidence of male choice for gravid females (Bonduriansky & Brooks 1998; Katvala & Kaitala 2001; Benson 2007), although most of these studies argue that male preference for gravid females is a consequence of last-male sperm precedence patterns (Bonduriansky & Brooks 1998; Katvala & Kaitala 2001; Benson 2007). Gravid females, who are close to oviposition, are most likely to survive to offspring independence, and thus are a lower risk mate choice for males than females earlier in the egg development process. Particularly in cases where females store sperm over their adult lifetime, survival probability and proximity to oviposition may be stronger predictors of offspring number (and male success) than sperm precedence patterns. In this study I evaluate the role of mortality risk in male mate choice decisions

using a study system that allows me to separate risk from other likely mate choice criteria, including female body size and sperm precedence patterns.

In the golden orb-web spider, *Nephila clavipes*, males are attracted to and attempt to mate with both virgin and non-virgin females even though there is first-male sperm precedence (Christenson & Cohn 1988). Furthermore, prior studies have suggested that male attraction to non-virgin females increases as females approach oviposition, but that attraction is not related to female size, which predicts clutch size (e.g. Vincent & Lailvaux 2006; Rittschof 2010). Females that are close to oviposition may have a better chance of surviving to produce a clutch of offspring compared to females who are earlier in the process of egg development. If males prefer to mate with females close to oviposition regardless of female body size and reproductive costs due to sperm competition, then this result would support the hypothesis that males choose female mates on the basis of survival probability.

### **Study System**

Female *Nephila clavipes* mature sometime between July and late September and live approximately 3-4 months as adults (Christenson & Cohn 1988; Rittschof 2010). They require 30-40 days between maturation and first oviposition and lay at most 5 clutches of eggs in a laboratory setting (Christenson et al. 1985; Higgins 2000) before dying at the onset of winter. No females over winter in temperate populations of *N. clavipes* (Higgins 2000).

Mature males spend adulthood (estimated to be 3 weeks) searching for mates (Brown 1985), and can visit multiple female webs in their lifetime. Males are sperm-limited in this species. In spiders, males induct and store sperm in paired prosomal appendages called pedipalps (Foelix 1996). *Nephila clavipes* males induct sperm into

their pedipalps only once (Michalik and Rittschof, in press). Males adjust the amount of sperm they transfer during a copulation event, and so they may be able to mate multiply (Christenson & Cohn 1988), but once males deplete the sperm in their pedipalps, they cannot replenish it. Males are attracted to and attempt to mate with virgin females, and males use all of their lifetime sperm stores when mating with virgins (Christenson & Cohn 1988). However, males also mate with non-virgin females, and when they do so, they use only a portion of their sperm stores (Christenson & Cohn 1988).

There is some evidence in this species that males are attracted to females who are close to oviposition. Despite first-male sperm precedence patterns (Christenson & Cohn 1988), the number of males found on non-virgin adult females' webs increases with increased female abdomen size (Vincent & Lailvaux 2006; Rittschof 2010). Abdomen size is considered a measure of gravidity or time to oviposition in spiders (Ortlepp & Gosline 2008). In contrast, female cephalothorax width, which determines clutch size in this species (Higgins 2000; Rittschof 2010), does not correlate with an increase in male attraction (Vincent & Lailvaux 2006). In this study, I test the hypothesis that males are attracted to adult females who are close to oviposition, irrespective of female body size. Even though the largest females have the highest potential fecundity, males that choose females close to oviposition may maximize offspring number because these females are more likely to survive to lay a clutch of eggs.

In a male removal field experiment, I verify that males are consistently attracted to some females over others, i.e. that males make mate choice decisions. In a second experiment I determine how male mating behavior, sperm usage, and subsequent reproductive success change as a function of female time to oviposition. Finally, with

these same females, I determine that female abdomen size is strongly correlated with time to oviposition, and that the closer a female is to oviposition, the less likely she is to die before laying her first clutch of eggs.

## **Methods**

### **Male Removal Field Experiment**

In this experiment I evaluated whether the occurrence of large groups of males on certain female webs could indicate male mate choice. Because female *N. clavipes* have long web tenure (Rittschof & Ruggles 2010), and males usually do not follow females when they change web sites, even over short distances (Cohn et al. 1988), the number of males found on a female's web could simply be a function of the length of time a female has been at a web site. To demonstrate that males in the field are consistently attracted to certain females and not others, I performed a male-removal experiment. On August 23, 2008, 71 mature female webs were identified and females were marked with enamel paint (Testors<sup>®</sup>) and measured following Rittschof and Ruggles (2010). On this day (day 0), the number of males on each web was recorded (hereafter the number of initial males). Males were then removed and released at least 5 m from the nearest treatment web. Each subsequent day for 14 days (or until the female abandoned her web site), I returned to each web, counted the number of males present, and removed males as I had on day 0. Due to time constraints, I did not mark males, and so some males may have returned to their original web or appeared on more than one web. While the short release distance could bias the removed males to return to their original web by chance, female density is very high during this peak time of the breeding season (Christenson et al. 1985), and because females are often found within 1 m of one another (Moore 1977), males choosing webs at random are unlikely to

reappear on a females' web by chance. Thus any patterns of male accumulation on webs would suggest a male web preference. For each female web, I totaled the number of males observed across all days of the experiment and divided this value by the number of days the female was observed on her web. This calculation is the average number of males present on the web per day.

## **Mating Experiment**

### **Animal collection and housing**

The mating experiment was conducted from 6 July 2009 to 21 August 2009. Females and males were collected about twice a week throughout this time period from three locations, the Ordway-Swisher Biological Station in Melrose, Florida (Putnam County, latitude 29° 42' 32.4", longitude -82° 2' 60.0"), Payne's Prairie State Preserve (Alachua County, latitude 29° 36' 36.0", longitude -82° 17' 60.0"), and San Felasco Hammock Preserve State Park (Alachua County, latitude 29° 43' 11.9994", longitude -82° 27' 0"). The mating experiment was conducted at the University of Florida Bee Biology Unit (Alachua County, latitude 29° 37' 14.5158", longitude -82° 21' 18.99"), which is a fenced and protected research area.

In order to ensure virginity, females were collected during their sub-adult instar. Female maturity was determined by inspecting the epigynum, which is black with two distinct openings in mature females, and smooth and maroon colored in sub-adult females (Higgins 2000). Females were housed outside in wooden cages (90 cm x 90 cm x 15 cm) and fed two large mealworms and sprayed with a mist of water daily. Females built and maintained normal prey capture webs in these frame cages (after Rittschof 2010). The cages were arranged along a chain-linked fence, and shielded from the sun and rain with a tarp awning. I collected mature males for the mating

experiment from juvenile female webs. After their terminal maturation molt, males have dark body coloration and sclerotized pedipalps (Myers & Christenson 1988). Because males were collected from juvenile females' webs, I could ensure they had not mated with the current female web-owner, but I could not ensure virginity because a male could have mated with a different female prior to collection. However, because I could not identify which males may have mated previously, male experience was evenly distributed across treatments.

### **Treatments**

The goal of the mating experiment was to determine whether males that mate with non-virgin females close to oviposition copulate more frequently, transfer more sperm, and father more offspring than males that mate with younger non-virgin females. To do this, I designed an experiment with two treatments that differed in the timing of the second male (hereafter P2) mating. For both treatments, females were allowed to mate with a first male mate (hereafter P1). Then, for females in treatment 1 (early treatment), a second male was introduced and allowed to mate with the female immediately following the removal of the first male. For treatment 2 (late treatment), I waited until the female was close to laying her eggs (see below) before introducing the P2 into the female cage.

I initiated each mating experiment trial on the day that the female molted to maturity (day 1), which was usually within one week of female collection. Females were checked daily from 0700 hrs. to 0900 hrs. If a female was mature, I introduced the P1 into the female's cage at the corner of her web. P1 remained in the female cage for three full days and was removed on the morning of day 4. Because females typically do

not eat immediately after their maturation molt (Christenson & Cohn 1988), I did not feed females on day 1. However, I resumed the normal feeding on day 2.

I assigned each female to a treatment once she matured, alternating between treatment 1 (early) and treatment 2 (late). In the early treatment, I introduced P2 to the corner of the female's web on the morning of day 5 (24 hrs. after P1 was removed). P2 was left for three full days to mate with the female, and females were fed normally throughout this time period. In the late treatment, after P1 was removed, females were left alone until they reached a body condition index of 1.5 (see below). The morning of the day the female reached this body condition index value, P2 was introduced and allowed three full days to mate with the female. Because male body size affects sperm number and could influence male sperm competition (Christenson & Cohn 1988; Cohn 1990), for each replicate, P1 and P2 males were matched for body size. Male body size was determined by measuring the tibia-patellar length of the 4<sup>th</sup> leg after it was removed for DNA extraction (see below). I fed the females normally during the time that the P2 males were with the female. For both treatments, after P2 was removed, females were kept and fed as they were prior to the start of the experiment and allowed to lay 2 clutches of eggs as in Rittschof (2010).

Female body condition index was defined as the abdomen height (the dorsal-ventral height of the abdomen measured just posterior to the epigynal slit; Vincent & Lailvaux 2006; Rittschof & Ruggles 2010) divided by the cephalothorax width (measured at its widest point). Cephalothorax width was measured using dial calipers. I measured abdomen height from digital photographs taken daily using a PowerShot A400 Digital Camera (Canon, Lake Success, New York). Photographs enabled me to

obtain accurate measurements of abdomen height without removing the females from their cages. I held a ruler in the plane of the abdomen directly above the female to provide a scale for each photograph. I viewed each picture using Windows Picture and Fax Viewer<sup>®</sup> (Microsoft, Redmond, WA) and measured the abdomen height and the scale by holding a ruler flush with the computer screen. From these measurements I calculated female abdomen height.

Each day a male was present, I checked the cage to observe whether or not the male was copulating with the female. Although males copulate throughout the day, they are most likely to copulate with the female while she is eating (Christenson et al. 1985), and so I checked for copulation 30 min. after food was introduced to the female web in order to estimate copulation effort. When a male is copulating with a female, he mounts the ventral side of her abdomen and inserts the distal part of his pedipalp (the conductor) into her epigynal opening (Christenson et al. 1985). I checked for copulation after feeding each day that the male was present in the cage (3 days). I report whether or not the male was observed copulating on any days of the mating trial.

Females laid egg sacs on the walls and screening of their cages. Initially, clutches were left to hatch where they were laid within the cages. However, due to high rates of parasitism by mantisflies (Neuroptera: Mantispidae), all un-hatched clutches were removed on 19 September 2009 and placed in 946 mL screened boxes in a screened shed. Any clutch laid after this date was also moved immediately and treated in the same way. The shed kept the eggs at ambient temperatures and humidity, but provided protection from parasites. Eleven females were selected at random and their clutch sizes were determined by counting the total number of offspring and eggs laid.

## **Male sperm counts**

In *N. clavipes*, a male's total lifetime sperm are stored in the pedipalps (Michalik and Rittschof, in press). It is not possible to determine the number of sperm in the male pedipalps without sacrificing the male. However, a count of the number of sperm remaining in the pedipalps after copulation provides a basis to estimate the number of sperm used during copulation (Christenson & Cohn 1988), which is useful for comparing sperm usage across treatment groups. Once males were removed from female cages following the mating experiment, male pedipalps were assessed for sperm number. Two assistants who were blind to male mate order and treatment group scored male sperm counts. Males were placed in a -20° C freezer for five minutes in order to anesthetize them, and then both pedipalps were removed by pinching the femur with forceps. The pedipalps were placed in a 1.5 mL microcentrifuge tube with 50µl of tap water and pulverized with a tissue grinder for 3 min. The samples were then vortexed for one minute, and centrifuged for one minute. Because centrifugation pulls the contents of the tube into a pellet at the bottom of the tube, the pedipalp solution was homogenized before counting by drawing the solution in and out of the pipette tip until the pellet was no longer visible. A 10 µL sample of the sperm solution was dispensed onto a hemacytometer (Fisher Scientific) and the sperm were counted using a light microscope (Leica, Wetzlar, Germany). Two 10 µL samples were counted for each male, and total sperm density per mL was calculated.

## **Parental genotypes**

I assessed paternity using microsatellite markers developed for this species (Rittschof 2010) which show low variability but reliable allelic peaks. In order to obtain full paternity exclusion and maximize the level of precision for paternity analysis, I pre-

screened male and female genotypes in order to match the P1 and P2 males and the female for full exclusion at one locus. To do this, I removed one leg per individual for DNA extraction and genotype analysis prior to the mating experiment. I chose one 4<sup>th</sup> leg from each male and one 1<sup>st</sup> leg from each female. To remove a leg, I pinched the femur between a pair of forceps until the individual autotomized its leg, which I transferred immediately to cell lysis buffer (Gentra Puregene Tissue Kit, Qiagen, Valencia, CA). No males or females died as a result of the leg-removal treatment. I extracted parental DNA and amplified and scored three polymorphic microsatellite loci for each individual (Rittschof 2010). For each mating trial, I chose males whose genotypes allowed for full paternity exclusion at one locus.

### **Offspring analysis**

For most of the clutches included in paternity analyses, offspring were preserved in 95% ethanol once they had molted to their second instar. However, due to parasitism and poor egg sac construction, 5 clutches began to desiccate before all offspring had hatched or molted to their second instar. In these clutches, offspring were preserved in developmental stages including embryos and first and second instar juveniles.

Twenty offspring from each clutch were selected for paternity analysis. Offspring were chosen at random by two assistants, blind to the mother's treatment group. DNA was extracted following Rittschof (2010). In the 5 cases where offspring were preserved at different developmental stages, both embryos and hatched offspring were included in the paternity analysis. To improve DNA yield, embryo DNA was extracted using the QIAamp DNA Microkit (Qiagen, Valencia, CA). Within this small sample group however, first and second male paternity share did not differ among the different offspring developmental stages.

## **Condition Index and Egg Development in Wild-Caught Females**

Because female abdomen size is a consequence of both egg development and the amount of food a female has consumed, I collected wild caught females across a range of abdomen sizes in order to test for a relationship between abdomen size and ovary mass (a measure of the degree or state of egg development). A positive relationship would suggest that females with larger abdomens are closer to oviposition time. Sixteen females were collected between 27 July 2010 and 12 September 2010. Females were anesthetized and the ovary was removed within 1 day of collection. To anesthetize the females, each female was placed for 7 minutes in a  $-20^{\circ}$  C freezer. After this period, female abdomen height was photographed with a measurement scale, and abdomen height was determined by measuring these photographs (see above). I measured cephalothorax width using dial calipers. The ovary was dissected out of the abdomen in 10mM PBS and preserved in 95% ethanol. Prior to assessing mass, ovaries were removed from ethanol and dried in plastic weigh boats in a drying oven at  $37^{\circ}$  C.

## **Results**

### **Male Removal Experiment**

There was a weak positive correlation between the number of initial males found on a female's web and her body condition index ( $N = 71$ ;  $R^2 = 0.08$ ,  $P < 0.01$ ). There was no correlation between cephalothorax width and the number of initial males found on the web ( $N = 71$ ;  $R^2 = 0.003$ ,  $P = 0.65$ ). When removed, the rate at which males returned to female webs was positively correlated with the number of initial males present on day 0 (Fig. 4-1;  $N = 71$ ,  $R^2 = 0.48$ ,  $P < 0.0001$ ).

## Mating Experiment

Female body condition index was negatively correlated with the number of days remaining before oviposition (Fig. 4-2;  $R^2 = 0.73$ ;  $P < 0.0001$ ). Females in the gravid treatment ( $N = 19$ ) reached a body condition index of 1.5 (the day on which the P2 male was introduced) an average of 24.4 days after their maturation molt (S.E. = 0.75 days), and 7.7 days before first oviposition (S.E. = 0.64 days).

For wild-caught females, abdomen size ranged from 4.4 to 11.9 mm (Mean = 9.2 mm; S.E. = 0.55 mm), and condition index ranged from 0.68 to 1.95 (Mean = 1.47; S.E. = 0.09). Ovary mass ranged from 0.0006 g to 0.2987 g (Mean = 0.095 g; S.E. = 0.03 g). Because of this broad range of masses relative to the range of abdomen sizes and condition indices, ovary mass was log transformed for analysis. There was a strong positive relationship between ovary mass and abdomen size ( $R^2 = 0.80$ ,  $P < 0.0001$ ) as well as ovary mass and condition index ( $R^2 = 0.76$ ,  $P < 0.0001$ ).

Twenty-two females in the early treatment successfully mated with both the P1 and P2 males, and in the late treatment 19 females mated with two males. The percentage of trials in which the P1 male was observed mating did not differ between the two treatment groups (Fig. 4-3; Early = 82%, Late = 74%;  $\chi^2 = 0.39$ ,  $P = 0.53$ ). However, a significantly higher frequency of P2 males mated in the late treatment group compared to the early treatment group (Fig. 4-3; Early = 34%, Late = 84%;  $\chi^2 = 9.6$ ,  $P < 0.002$ ). Across both treatment groups where sperm counts were obtained for both the P1 and P2 males ( $N_{\text{Early}} = 20$ ,  $N_{\text{Late}} = 18$ ), 100% of P1 males were completely sperm depleted after copulation regardless of treatment. However, P2 males from the early treatment group had significantly more sperm remaining compared to P2 males from the late treatment group (Two-tailed T-test,  $t_{36} = -3.4$ ;  $P < 0.002$ ).

Twenty-three females across both treatment groups laid viable first clutches of eggs ( $N_{\text{Early}} = 12$ ,  $N_{\text{Late}} = 11$ ). In the first clutch, a higher percentage of P2 males fathered offspring in the late treatment compared to the early treatment (Fig. 4-4; Early = 17%; Late = 73%;  $X^2 = 7.3$ ,  $P < 0.007$ ). When P2 males fathered offspring ( $N_{\text{Early}} = 2$ ,  $N_{\text{Late}} = 8$ ), paternity ranged from 44-100% (Mean = 79%; S.E. = 7%). There were 19 females that laid two viable clutches. For 10 of these females (all in the early treatment), clutch 1 had a single father, and this father was the P1 male. In these 10 cases, clutch 2 also had a single father, which was the P1 male. For the remaining 9 females that laid 2 viable clutches (1 female from the early treatment, 8 females from the late treatment), there was mixed paternity in clutch 1. Among these females, P2 paternity ranged from 50-100% (Mean = 78%; S.E. = 6%) in clutch 1. However, P2 paternity in clutch 2 was significantly different than clutch 1 (Fig. 4-5; Two-tailed paired T-test,  $t = -4.3$ ,  $p < 0.003$ ). In all but one case, P2 paternity share was lower in clutch 2 than in clutch 1, and the mean difference was 39% (S.E. = 9%).

Of 47 females that matured during the course of the experiment, 11 females died before oviposition (23% mortality). Six females that died mated only once (i.e. they did not make it to the body condition index required before the P2 male could be introduced), 4 females that died successfully mated with both the P1 and P2 males in the early treatment, and 1 female that died successfully mated with both the P1 and P2 males in the late treatment. Thus from a male's perspective, the risk of reproductive failure for mating with a virgin female is approximately 23% (11 of 47 mature females); the risk for mating with a non-virgin female early in adulthood is about 18% (4 of 22 early-treatment females); and the risk for mating with a gravid female is about 5% (1 of

19 late-treatment females). Because these data are from the male's perspective, and each female progresses from virgin to mated to gravid in her lifetime, these categories are not mutually exclusive in terms of the calculations of risk. For example, the gravid female that died was also tallied for the total calculation of risk for mating with a virgin female (this female just happened to die at the gravid stage). For this reason, it is not possible to compare mortality risk statistically across female categories.

Female egg number ranged from 357 to 908 (averaged across clutch 1 and 2 for each female,  $N = 11$ , Mean = 673, S.E. = 28) and was positively correlated with female body size (cephalothorax width;  $R^2=0.49$ ,  $P<0.025$ ). Clutch 2 trended towards having fewer offspring compared to clutch 1 (Mean<sub>clutch one</sub> = 711, S.E. = 26.5; Mean<sub>clutch two</sub> = 634, S.E. = 48; Two-tailed paired T-test,  $t = -1.86$ ;  $P = 0.09$ ). The mean difference was 76 offspring (S.E. = 41 offspring), which corresponds to a mean difference of 11% (S.E. = 6%).

## Discussion

The age and reproductive state of non-virgin females significantly affects male mating behavior. Field data show that males are consistently attracted to some mature females over others (Fig. 4-1) and that high levels of attraction are correlated with female abdomen size, which is an indicator of time until oviposition (Fig. 4-2). In contrast, males are not differentially attracted to females with large body sizes (cephalothorax width), even though body size predicts average clutch size (see results). In the mating experiment, when paired with non-virgin females close to oviposition, males mated at higher frequencies (Fig. 4-3), transferred more sperm, and fathered more offspring in the first clutch (Fig. 4-4) compared to males paired with younger non-virgin females. These findings support the hypothesis that males invest more

reproductive effort in gravid females who are close to oviposition compared to younger non-virgin females. Furthermore, at least within the controlled conditions of this mating experiment, mating with a female close to oviposition (i.e. a gravid female) decreased a male's risk of total reproductive failure due to female mortality. Gravid females had a mortality rate of 5%, a value much lower than the rate for both virgin females (23%) and young non-virgin females (18%). More work is needed to evaluate differences in survivorship among females in the wild, although mortality in general is probably even greater because food availability is variable and females are subject to predation. Male preference for females that are close to oviposition suggests that female survival probability could be a more important predictor of offspring number than other fecundity parameters like body size in this species.

Partner survivorship could be an important mate choice criterion in species where there is delayed reproductive pay-off after copulation, i.e. species where females store sperm. However, almost no studies have suggested a role for partner mortality in mate choice. In long-lived species, poor-condition individuals can forego reproduction for a season or breeding cycle in order to optimize their lifetime reproductive success (Roff 1992). The result is that yearly adult survival rates are high because individuals at risk do not enter the mating pool (e.g. Vanheezeik et al. 1994). However, in short-lived species with few reproductive opportunities, there is high variation in reproductive output (Roff 1992), and there is a high cost associated with reproductive failure for both sexes because in some cases individuals have as little as one opportunity to reproduce. In cases where male reproductive investment ends at sperm deposition, female mortality risk directly affects male reproductive output. In these species (e.g.

*Nephila clavipes*), males could incorporate female mortality risk into their choice criteria by choosing females on the basis of probable survivorship (Dunn et al. 2001).

Similar to the current study, other studies in insects and fish demonstrate male choice for gravid females who are close to egg deposition (Bonduriansky & Brooks 1998; Katvala & Kaitala 2001; Benson 2007). In these cases, as opposed to increased probability of female survival, the benefit of mating with gravid females is attributed to last male sperm precedence patterns. Sperm precedence studies assess whether ejaculates stored by a female fertilize eggs disproportionately due to mate order. As is evidenced by the current study, sperm precedence studies should be interpreted with caution because in most cases, it is difficult to assess differences in the number of sperm transferred by each male. Because *N. clavipes* males have a limited number of sperm, and all sperm are contained in the pedipalps (Christenson 1989), the sperm remaining in the pedipalps after copulation provides an estimate of sperm usage by males (Christenson & Cohn 1988). P2 males in the late mating treatment used more sperm and were more likely to father offspring (Fig. 4-4) than P2 males in the early mating treatment, a finding that emphasizes the importance of sperm numbers in paternity outcomes.

Previous findings in *N. clavipes* have suggested that there is first male sperm precedence, presumably a consequence of the conduit shape of the female reproductive tract (Christenson & Cohn 1988). However, my findings suggest that paternity patterns may reflect a complex relationship between the number of sperm transferred and mate order. Regardless of treatment, P1 males always transferred more sperm than P2 males, and across multiple clutches (e.g. clutch number 2), the male that

transferred more sperm (P1 male) showed an increase in paternity (Fig. 4-5). This finding is similar to the predictions of a fair raffle (e.g. Ball & Parker 2000). However, within the first clutch, sperm number and mate order affect paternity share, and the P2 male has an advantage over the first male even though he transfers fewer sperm. This disparity could be due to post-copulatory processes within the reproductive tract of the female, although the mechanisms are unknown.

Other studies in spiders have suggested that sperm precedence patterns are not simply a function of female genital morphology (reviewed in Huber 2005). In *Nephila* species specifically, multiple factors affect the number of sperm transferred by males, as well as the sperm stored and used for fertilization by females. Second male paternity share is affected by copulation duration and frequency in *N. edulis* and *N. plumipes* (Schneider et al. 2000; Elgar et al. 2003; Schneider & Elgar 2005). In addition, in *N. plumipes*, the number of sperm transferred and stored by the female does not show a simple positive correlation with paternity share, which suggests a role for sperm manipulation by the female prior to fertilization (Schneider & Elgar 2001). In spiders generally, it is common for post-copulatory processes, influenced by both males and females, to affect sperm storage and usage (Parker 1990; Bukowski & Christenson 1997a; Eberhard 2004; Huber 2005; Aisenberg 2009; Schneider & Lesmono 2009; Welke & Schneider 2009; Burger 2010; Peretti & Eberhard 2010). In some cases, these processes are associated with copulation interval and the female's mated status (Schneider & Elgar 2001; but see Jones & Elgar 2008). The current study shows that in *N. clavipes*, both the amount of sperm transferred to the female as well as mate order play an important role in determining the number of offspring sired by a male.

Mating with multiple non-virgin females or a single virgin female could be mutually exclusive mating strategies in this species. Male sperm use and mating effort indicate that males prefer to mate with either virgin females or gravid females close to oviposition (Fig. 4-3). Males may avoid mating with younger non-virgin females because these females have only slightly lower mortality risk compared to virgin females, and are costly to males in terms of sperm competition. Although mating with a virgin female is a high-risk strategy in terms of female mortality, this risk is offset by other potential advantages. First, if the female does not re-mate, the male could father 100% of her offspring. In a census survey in my population, females were visited 0-17 males over the course of their lifetime, which suggests female mating rate is variable. Second, because first male paternity share increases in the second clutch, the total reproductive pay-off for mating with a virgin female, even if she re-mates, may be higher than mating with a non-virgin female as long as the female lays more than one clutch (particularly if the clutches are of similar size; Higgins 2000). Female clutch number in the wild is highly variable, and can depend on factors including the time of the season at which the female matures (Higgins 1992, 2000). However, in my studies, most females easily lay 2 clutches in captivity, although three clutches is rare. Thus depending on the time of the season, local density, and operational sex ratio, the optimal male strategy may shift.

In addition to sperm competition risks and female mortality risks, male *N. clavipes* face other pre-copulatory challenges that could affect whether they successfully mate with a virgin or non-virgin female. In other spiders, male mortality risk during mate search appears to constrain male mating rates and presumably male choosiness (Andrade 2003; Andrade & Kasumovic 2005; Fromhage et al. 2007;

Kasumovic et al. 2007). Mate search mortality could be a function of female density and operational sex ratio, which also changes a male's re-mating probability (Fromhage et al. 2008). In addition, female webs differ in the number of male competitors present, which affects a male's probability of mating successfully (Elgar & Fahey 1996; Schneider & Elgar 2005; Rittschof 2010; Rittschof & Ruggles 2010). Variation in mating success may be highest on gravid female webs where male group sizes are largest (Vincent & Lailvaux 2006; Rittschof 2010). The probability of successfully mating, the ability to re-mate, and variation in female survival and clutch number could result in an evolutionarily stable state maintaining males in the population who adopt mating strategies that result in copulations with virgin and gravid non-virgin females (ESS; Brockmann & Taborsky 2008). Other work in *Nephila* suggests that these strategies could depend on male body size (Schneider et al. 2000; Schneider & Elgar 2001, 2005; Elgar & Jones 2008; Jones & Elgar 2008; Rittschof 2010).

Because there is conflict between the sexes over mating rate (Chapman et al. 2003), one alternative explanation for the results of this study is that females are in control of mating interactions, and that the increased frequency of copulation with gravid females occurred because females show increased sexual receptivity just before oviposition. Prior work in this species has suggested that, because non-virgin females are less receptive compared to virgins, male mating opportunity with non-virgin females is limited to times when the female is eating (Christenson et al. 1985; Christenson & Cohn 1988). Males presumably wait until the female is eating in order to avoid cannibalism (e.g. Fromhage & Schneider 2005), which suggests sexual conflict. Because cannibalism rates are uniformly low in *N. clavipes* (Christenson et al. 1985), it

is difficult to infer how cannibalism impacts male copulation behavior. Regardless however, the current study shows that, even when food is present, and as a result males have access to all non-virgin mates, males copulate with non-virgin females at different rates depending on female age, which demonstrates some role for male control during mating events.

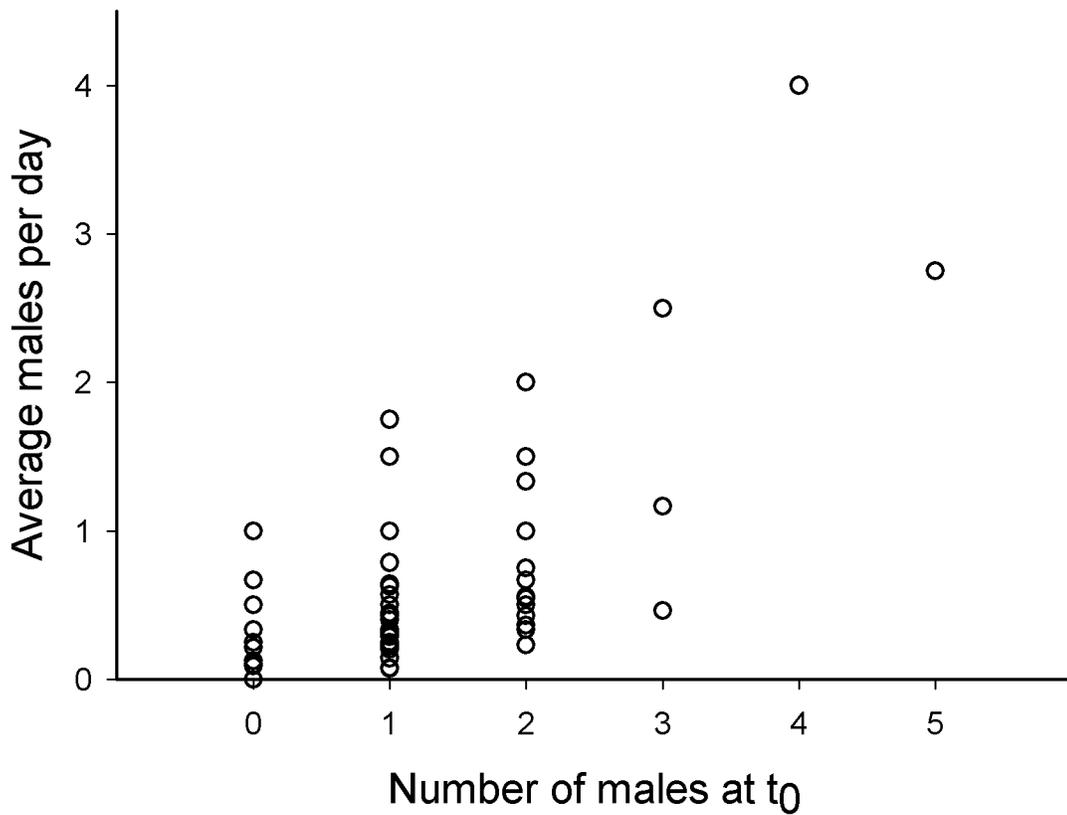


Figure 4-1. Average males per day versus males present at t<sub>0</sub>. The average number of males found on a female's web per day is positively correlated with the number of males present prior to the beginning of the removal experiment ( $R^2=0.48$ ,  $P<0.0001$ ). Each data point represents one female web (N=71) on which males were removed daily.

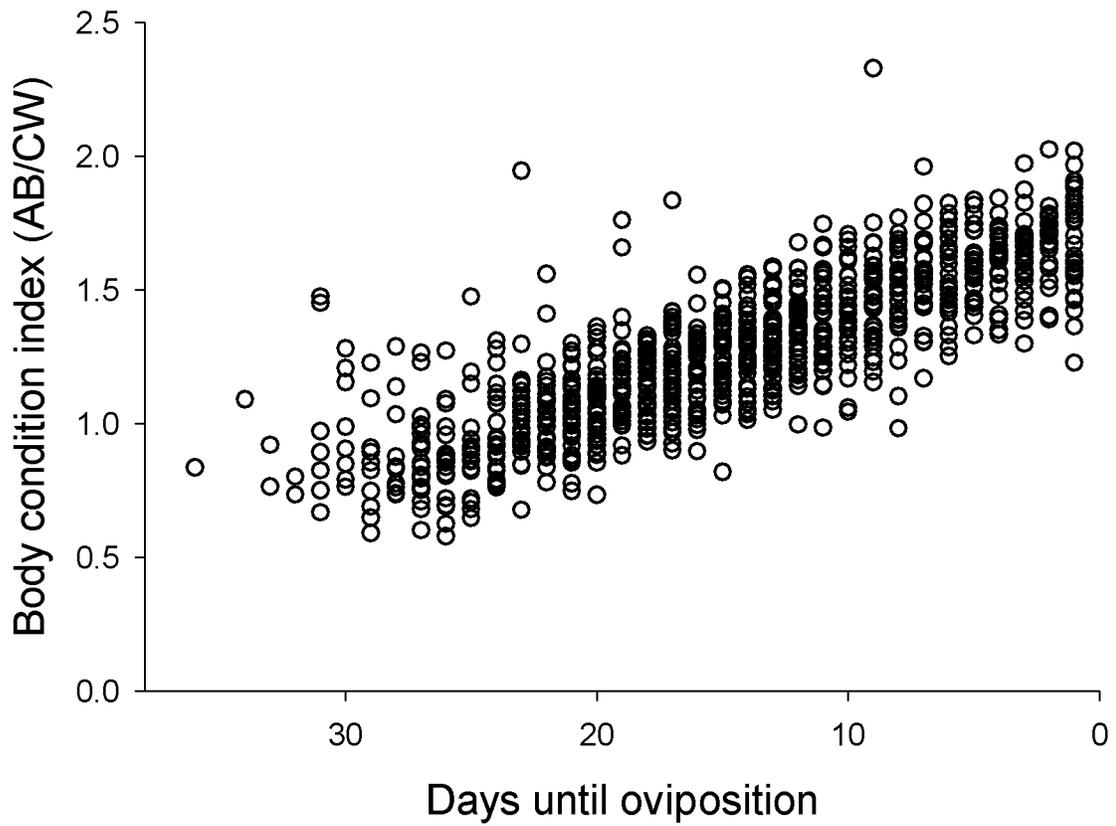


Figure 4-2. Body condition index versus days until oviposition. Female body condition index increases as the female approaches oviposition ( $R^2=0.73$ ,  $P<0.0001$ ). Each data point represents a single female on a given day of her reproductive cycle (N=35 females; N=938 total female-days).

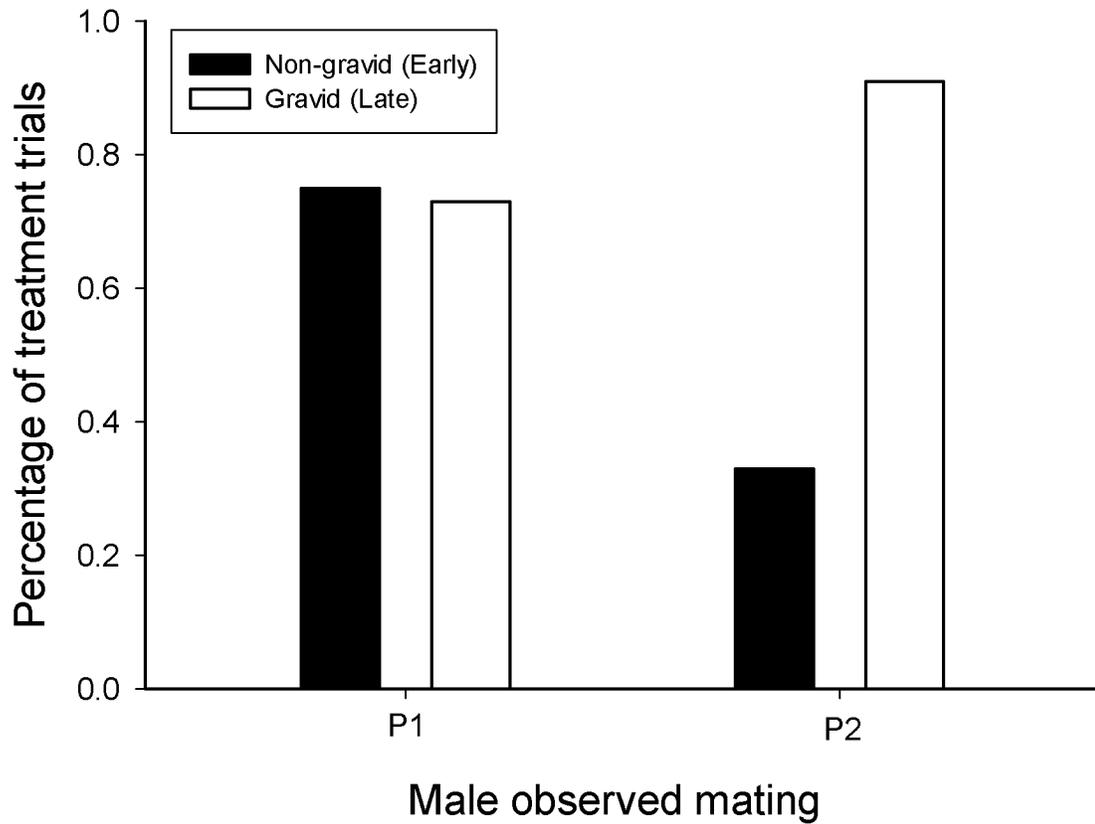


Figure 4-3. The proportion of P1 and P2 males observed mating for the non-gravid (early) and gravid (late) treatment groups. Mating frequency was similar for P1 males across treatments ( $P=0.53$ ), but P2 males mated with significantly higher frequency in the gravid (late) treatment group ( $P<0.002$ ).

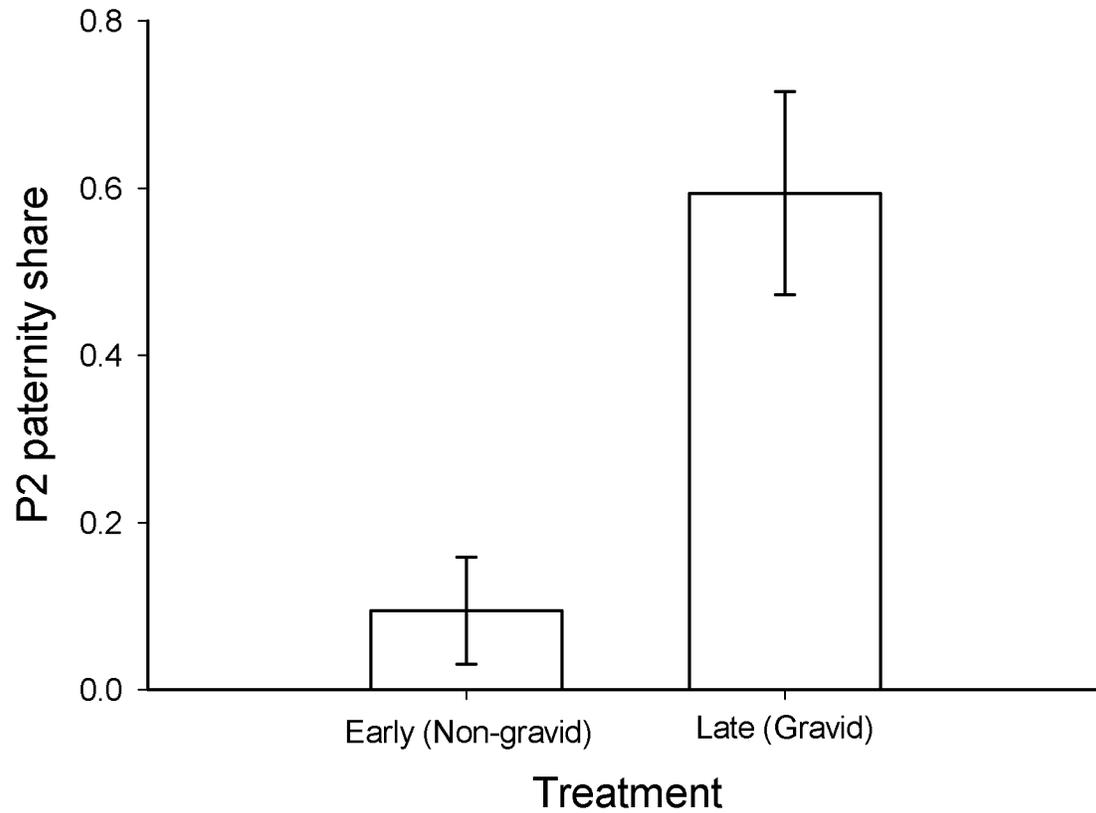


Figure 4-4. P2 paternity share versus treatment. P2 males fathered a greater proportion of offspring in the first clutch in the late treatment versus early treatment group.

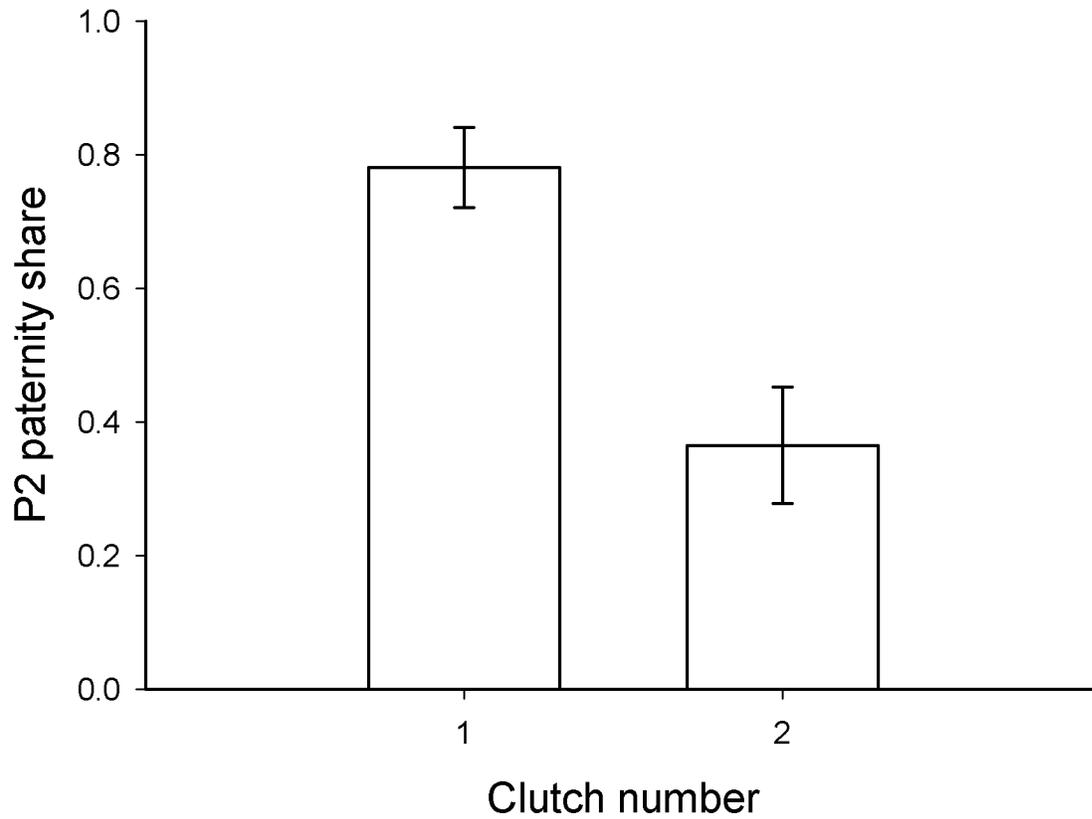


Figure 4-5. P2 paternity share versus clutch number. Pooled across both treatments, there were 9 cases where females laid 2 viable clutches and the P2 male fathered offspring in clutch 1 ( $N_{\text{Early}}=1$ ,  $N_{\text{Late}}=8$ ). Of these cases, P2 male paternity share was significantly lower in the second clutch compared to the first clutch ( $P<0.003$ ).

CHAPTER 5  
MALE MULTIPLE MATING AND ALTERNATIVE TACTICS IN THE GOLDEN ORB-  
WEB SPIDER *NEPHILA CLAVIPES*

**Background**

Recent empirical work shows that males and females often mate with multiple partners during a single reproductive episode (Arnqvist & Nilsson 2000; Jennions & Petrie 2000; Zeh & Zeh 2003). The discovery of high rates of multiple mating in females, typically considered the choosy sex, shows that rather than minimize their mate number, females optimize their mate number and mate choice depending on factors such as their condition, their genotype, their mate's genetic compatibility, and environmental conditions (Zeh 1997; Fox & Rauter 2003; Klemme & Ylonen 2010). In contrast, for males, it is widely accepted that fitness should increase with increased mate number (Bateman 1948). As a result, studies of male mating strategies have focused on factors that constrain male mate number, for example the number of available mates (Emlen & Oring 1977; Fromhage et al. 2008), dominance status (Emlen & Oring 1977), costs of obtaining a mate (Segoli et al. 2006; Oliver & Cordero 2009), and the costs of sperm production (Wedell et al. 2002; Smith 2009). However, empirical evidence shows that males do not always mate at the highest possible rate, which suggests that males, like females, follow decision rules to optimize their mate number (Wedell et al. 2002).

Male reproductive effort with a single mate can come at the expense of re-mating (Bonduriansky 2001; Matessi et al. 2009), and males use environmental cues as well as their own condition and competitive ability to optimize this trade-off. For example, when sperm competition risk is high, a male can increase his sperm allocation to one female, but potentially limit his future mating ability (Ball & Parker 2007; Teng & Zhang 2009).

Similarly, the switch-point between post-copulatory mate guarding, which prevents female re-mating, and searching for new mates can depend on male density, which affects the likelihood that a female will re-mate once she is abandoned (Schubert et al. 2009). Finally, a male's decision to provide parental care or forego care to attempt to mate with an additional female can depend on the male's own attractiveness, and thus his chances of winning an additional mate (Burley 1988). In all of these cases, it is beneficial for a male to adjust his reproductive investment and optimize his mate number in order to maximize total reproductive success. In extreme cases, male mate number optimization could result in the evolution of male monogamy (hereafter monogyny; Fromhage et al 2008).

Web-building spiders, particularly those in the genera *Argiope*, *Nephila*, and *Latrodectus*, have been the focus of empirical and theoretical studies addressing the evolution of monogyny. This is because members of these groups exhibit conspicuous behaviors like sexual cannibalism and genital mutilation during copulation, two traits that prevent males from re-mating (Andrade & Kasumovic 2005; Schneider et al. 2005a; Fromhage & Schneider 2006; Segoli et al. 2006; Uhl et al. 2010a; Michalik and Rittschof, unpublished manuscript). In contrast, the golden orb-web spider *Nephila clavipes* provides an opportunity to address male mate number optimization because, although males use all of their life-time sperm in a single mating in certain contexts (Christenson 1989; Michalik and Rittschof, in preparation), they are not always constrained to monogyny because they are rarely cannibalized (Christenson et al. 1985), and they do not consistently mutilate their genitalia during copulation. Thus males are able to mate multiply if they retain some sperm after insemination, and I

predict that males follow decision rules to optimize their mate number depending on their own condition as well as environmental factors. The constraints to reproduction faced by male *Nephila clavipes* are also found in a variety of other animal taxa. For this reason, *N. clavipes* can serve as a model system to address the relative importance of factors like sperm depletion, operational sex ratio, mate-search mortality risk, mate guarding, female value, and male competitive ability (e.g. body size), in determining male mating rate and mate number optimization.

In this study, I parameterize a dynamic state variable model for the spider *Nephila clavipes* (Clark & Mangel 2000) to (1) assess optimal mate number for males, and (2) examine the conditions that have the largest effect on male mating rate. In addition, because body size is highly variable in this species and is known to affect male reproductive outcomes (Christenson et al. 1985; Christenson & Cohn 1988; Rittschof 2010), I (3) assess whether males show size-dependent differences in strategies, tactics, and mating rates. The model generates a matrix of optimal male decisions for every value of male state considered. This decision matrix defines the male reproductive strategy. By solving the model using size-specific parameter values, I can compare strategies across different male sizes. After assessing differences in strategies, I simulate a theoretical population of males whose behavior is constrained to the optimal decisions generated by the model. This simulation gives the reproductive outcomes and behavioral patterns (including male mating rate) associated with a particular male strategy. I refer to reproductive outcomes and behavioral patterns generated by the simulation as tactics.

## Study System

Adult male *N. clavipes* travel between female webs at night and co-habit with mature females for one day to several weeks (Christenson et al. 1985). Females periodically change web sites and attract new males (Rittschof & Ruggles 2010), and field and empirical data suggest females in this species are likely polyandrous (C. Rittschof, in preparation). Females can store sperm for several months (Christenson et al. 1985) and lay their first clutch of eggs 30-40 days after their maturation molt (Christenson & Cohn 1988). Males can visit multiple webs (C. Rittschof, unpublished data), but mate search is risky, and male mortality across a 20-day period can be as high as 88% (Vollrath & Parker 1992). On a web, males fight among themselves to mate with the female. Larger males typically win male-male contests (Cohn et al. 1988; Rittschof 2010). Upon mating, males become permanently sperm depleted if they mate with a virgin female (Christenson 1989). However, if they mate with a non-virgin female, males retain some sperm and are capable of re-mating (Christenson & Cohn 1988). After copulation, males may continue to guard females, especially when mated with a virgin (Christenson et al. 1985).

Females vary in the degree of male competition on their webs as well as in the pay-off associated with a successful mating. Large male group sizes (5-6 males) are typically observed on virgin female webs (Moore 1977) and webs with non-virgin females (Rittschof 2010) that are close to oviposition (Vincent & Lailvaux 2006; C. Rittschof, unpublished manuscript). A male that mates with a virgin female fathers the majority of offspring in her first clutch (Christenson et al. 1985; Christenson & Cohn 1988), and males that mate with a non-virgin female fertilize approximately 20% of her clutch (Christenson & Cohn 1988). Thus, fertilization rates do not explain why large

numbers of males are attracted to non-virgin females close to oviposition (Vincent & Lailvaux 2006). Here I hypothesize that these females are attractive because they have a high probability of surviving to lay eggs. Female death before oviposition (hereafter female mortality risk) could occur because of random processes like predation, starvation, or injury. Generally, this risk is lower for females close to oviposition, but the risk increases overall at the end of the reproductive season when the ambient temperature may not be adequate for a female to complete egg development before she dies (Higgins 2000).

Considering both sperm competition and female mortality risk, in the model I define three types of females that correspond to three stages in adult female life: (1) newly-matured virgin females (hereafter virgin females), (2) non-virgin adult females that are more than one week from oviposition (hereafter mated females), and (3) non-virgin gravid adult females that are within a week of oviposition (hereafter gravid females). Because of first-male sperm precedence (Christenson & Cohn 1988), virgin females are the highest quality in terms of potential offspring sired, followed by mated and gravid females. Gravid females, who are closest to oviposition, have the lowest mortality risk, followed by mated and virgin females.

In the model I address how spatial variation in female quality and male-male competition influence males' decisions to change webs before copulation (i.e. pass up a mating opportunity) or after copulation (i.e. mate guard or not). I also evaluate how strategies and tactics change as a result of seasonal variation in female quality, operational sex ratio, and mate search mortality risk. Finally, because limited sperm

numbers are predicted to have a large effect on male mating rate and strategies, I manipulate male sperm availability across three versions of the model (see below).

## **Methods**

### **Overview**

In this study, I build a dynamic state model to evaluate male mating rate and the accompanying strategies and tactics. In order to evaluate the effects of male sperm limitation, I design three separate models that differ in the amount of sperm males have available for mating. The Basic Model is parameterized most realistically to *N. clavipes* using empirical data. In the Restricted Sperm Model, males use all of their sperm in a single copulation, regardless of female type, and are thus limited to monogyny. This situation is similar to other spider species where males mutilate their genitalia during copulation or are cannibalized and thus no longer able to mate after their first copulation (Andrade & Kasumovic 2005; Schneider et al. 2005a; Fromhage & Schneider 2006; Segoli et al. 2006; Uhl et al. 2010a; Michalik and Rittschof, unpublished manuscript). In the third model, the Unrestricted Sperm Model, males use fewer sperm than is typical during copulations with non-virgin females (see below), which increases their potential mating rate. This level of sperm availability is similar to species where males are able to re-induct sperm during or after copulation (Huber 1998; Knoflach 2004). All models are described in detail below.

### **The Basic Model**

#### **Model structure**

Each time step in the model ( $t$ ) corresponds to a single 24-hour day in which males can either (1) search for a female; or (2) stay on the current female web. In keeping with the spiders' natural history, when on a web, the male attempts to mate in

each time step until he has mated the female or he leaves the web. Because females mate immediately after molting to maturity (Christenson et al. 1985), if a male finds a virgin female web and does not mate within one time step, his web type changes from occupying a virgin web to occupying a mated web. However, within the time horizon of the model (21 days), females do not progress from mated to gravid (Cohn et al. 1988; C. Rittschof, unpublished data).

The time horizon ( $T$ ) is the lifespan of a male (21 days). Because all males die at the end of time ( $T$ ), the expected future fitness ( $F$ ) at  $T$  is zero. If a male does not have enough time to complete a mating, his fitness is discounted in proportion to the time shortage ( $\tau$ ). This is important when a male mates with a virgin female, because these matings require 48 hrs. (Christenson et al. 1985), while all other matings require only 24 hrs.

Four state variables affect male behavior and reproductive success: (1) web type ( $J$ ; a male can occupy one of three types of webs,  $J = 1-3$ , or no web at all,  $J = 4$ ); (2) energy level ( $E$ ); (3) available sperm ( $X$ ); and (4) mating history ( $I$ ; i.e. whether the male has mated or guarded the current female for at least one time step). Following the natural patterns, different types of females differ in the level of male-male competition ( $m_J$ ), the energetic costs of attempting to mate ( $c_J$ ), the sperm requirements for successful mating ( $\alpha_J$ ), and the pay-off to the male if he mates ( $f_J$ ; Tables 5-1, C-1, C-2).

Each male starts with 21 energy units ( $E$ ). Males lose energy at a rate of  $s$  per unit time (Table 5-1). Males also expend energy fighting with each other and mating with females ( $c_J$ ; Table C-2). Males rarely eat as adults (Christenson 1985), and so for simplicity, in the model they never gain energy. If energy falls below 1 unit, the male

dies. If a behavior requires more energy than a male has, the fitness gained from that behavior is discounted in proportion to the shortage of energy ( $\varepsilon$ ; Table 5-1).

Males start with pedipalps full of sperm (100%) ( $X$ ; Table 5-1). The minimum amount of sperm required to gain maximum fitness from mating with a given female type ( $\alpha_J$ ) is 100% for virgin females and estimated to be 40% for both mated and gravid females following empirical studies (Table C-1; Christenson & Cohn 1988; Christenson 1989). If a male does not have the required amount of sperm at the time of mating, his fitness from that mating is discounted in proportion to the shortage of sperm ( $\sigma$ ; Table 5-1). The mating history variable ( $I$ ; Table 5-1) tells whether the male has mated (if unmated,  $I = 1$ , if mated,  $I > 1$ ) and also tells the number of days the male has guarded the female and successfully prevented other males from copulating with her (number of successful guarding days =  $I - 2$ ). For each time step a male successfully guards his mate, he accrues additional reproductive success.

### Decision 1: search

If a male decides to search his expected fitness is:

$$F_{Search} = (1 - \beta) * \sum \begin{cases} r_1 * (m_1 * (C_{mate,J} + F'_{mate,J}(t', E', X', J', I')) + (1 - m_1) * F'_{notmated}(t', E', X', J', I)) \\ r_2 * (m_2 * (C_{mate,J} + F'_{mate,J}(t', E', X', J', I')) + (1 - m_2) * F'_{notmated}(t', E', X', J', I)) \\ r_3 * (m_3 * (C_{mate,J} + F'_{mate,J}(t', E', X', J', I')) + (1 - m_3) * F'_{notmated}(t', E', X', J', I)) \\ r_4 * ((1 - m_4) * F'_{notmated}(t', E', X', J', I)) \end{cases} \quad (5-1)$$

where  $\beta$  is male mortality risk for searching,  $r_J$  is the encounter rate for each female type,  $m_J$  is the probability of mating with the female encountered (Table C-2).

$C_{mate,J}$  is the fitness gained from mating, where

$$C_{mate,J} = (\min(\varepsilon, \sigma, \tau) * (y_J * f_J)) * d_J \quad (5-2)$$

$F'$  is the potential future fitness, and depends on whether the male successfully mates ( $F'_{mate,J}$ ) or not ( $F'_{notmated}$ ):

$$F'_{mate,J}(t', E', X', J, I) = F(t + tj_J + 1, E - s(tj_J + 1) - c_J, X - \alpha_J, J, 2) \quad (5-3)$$

$$\text{If } J = 1: F'_{notmated}(t', E', X', J, I) = F(t + 1, E - s, X, 2, 1) \quad (5-4)$$

$$\text{If } J > 1: F'_{notmated}(t', E', X', J, I) = F(t + 1, E - s, X, J, 1) \quad (5-5)$$

Male mortality risk per web movement ( $\beta$ ; Table C-2) is uniform across male body sizes in order to allow for the possibility that size-dependent mortality, as observed in census studies (Vollrath & Parker 1992), reflects a trade-off between lifespan and reproductive success corresponding to size-based alternative strategies. Overall, male mortality risk increases over the course of the season.

Female encounter rate ( $r_j$ ; Table C-2) is a function of the total density of females and the relative proportions of each type of female in the population. These proportions change over the season: total female density declines over the season; the density of virgin females peaks mid-season; and the density of gravid females peaks late in the season (C. Rittschof, unpublished data).

A male's probability of mating ( $m_j$ ; Table C-2) depends on the web type ( $J$ ) because male group size, and thus competition, changes across the three female web types (Christenson and Goist 1979; Rittschof 2010). Mating probability for each female type remains uniform because male group size on these webs is consistent throughout the season (C. Rittschof, personal observation). I assume that on virgin and mated webs, larger males have a higher mating probability (Rittschof 2010), but on gravid webs mating probability is equal with respect to male size because group sizes are very large which could eliminate large-male advantage (C. Rittschof, personal observation).

The fitness outcome of mating is measured in terms of the proportion of a female's first clutch a male sires ( $f_j$ ). At the time of mating, a male gains only a portion of his potential paternity share ( $y_j * f_j$ ; Table C-1) because successfully guarding the female is required to accrue the highest possible fitness from a mating (Linn et al. 2007; see below). Although female size (cephalothorax width) predicts the number of eggs laid by a female to some degree (Higgins 2000, Rittschof 2010), there is a lot of residual variation in offspring number that cannot be explained by body size. Furthermore, male attraction is not correlated with female body size (C. Rittschof unpublished manuscript). Thus I do not consider female body size in the model as a component that affects male mating strategies, and I measure male fitness in terms of proportion, not total number, of offspring sired.

Female survival probability ( $d_j$ ; Table C-2) incorporates female mortality risk due to random processes and seasonal risk. Random risk is constant throughout the season. However, at the latest of the three time periods within the season, risk increases due to declining temperatures. During the third period, virgin females have no chance of oviposition, mated females have a 30% chance of surviving, while gravid female mortality risk is the same as in early and middle season periods (98.8% survival).

### **Decision 2: stay**

A male's second option is to stay on his current web instead of searching. Most components of the structure of the model for this decision are very similar to the decision to search. If a male stays on a web and he has not mated with the female ( $I = 1$ ) he will attempt to mate again, and his expected fitness is:

$$F_{Stay} = \sum \left[ \frac{(1-w) * (m_J * (C_{mate,J} + F'_{mate,J}(t, E', X', J', I')) + (1-m_J) * F'_{notmated}(t', E', X', J', I'))}{w * F'_{aband}(t', E', X', J', I')} \right] \quad (5-6)$$

If the male has already mated with the female ( $l > 1$ ) and stays, he will attempt to guard the female and prevent her from re-mating:

$$F_{Stay} = \sum \left[ \frac{(1-w) * (k_J * (C_{guard,J} + F'_{guard,J}(t', E', X', J', I')) + (1-k_J) * F'_{notguard}(t', E', X', J', I'))}{w * F'_{aband}(t', E', X', J', I')} \right] \quad (5-7)$$

A male increases his fitness incrementally ( $z_J * f_J$ ; Table C-1) each day he successfully guards a female he has mated:

$$C_{guard,J} = (z_J * f_J) * d_J \quad (5-8)$$

He guards successfully with a probability  $k_J$  (Table C-1):

$$F'_{guard}(t', E', X', J', I) = F(t+1, E-s, X, J, I+1) \quad (5-9)$$

$$F'_{notguard}(t', E', X', J', I) = F(t+1, E-s, X, J) \quad (5-10)$$

However, there is a chance the female will abandon her web ( $w$ ) during the time step, and if she does, the male is forced to search in the next time-step (eqn 5-1).

$$F'_{aband}(t', E', X', J', I) = F(t+1, E-s, X, 4, 1) \quad (5-11)$$

Given the choice to search or stay, I can evaluate whether males decide to leave a web without mating (e.g. if they are choosy about their mates). As a limitation of the coding of the model (i.e. because a male always attempts to mate with a female if he is on her web), there is some probability a male will choose to search, find a web, and mate successfully during a single time step. In this case, the male does not have to opportunity to choose against the female whose web he finds.

### Size and season dependent strategies

I designed nine sets of parameters (Tables C-1 and C-2) to evaluate differences in male strategy associated with three male body sizes (small, medium, and large) at

each of three time periods of a single season (early, middle, late). “Small” males in our model correspond to males that range in cephalothorax width from approximately 1.3-2.0 mm, “medium” males from 2.1-2.7 mm, and “large” males from 2.8-3.5 mm. “Early” season corresponds to the last week in June through the month of July, “middle” season is approximately the month of August, and “late” season is September through early October in North Central Florida.

### **Model Manipulations**

In order to assess how male sperm limitation changes male reproductive strategies, I created two manipulations of the basic model where I increase and decrease male sperm limitation. These manipulations change a male’s potential mating rate, which is predicted to affect male mating strategies and tactics (Wedell et al. 2001). In the first manipulation, the restricted sperm model, I increased the value of  $\alpha_J$  so that males deplete their sperm stores after a single mating, regardless of female type. In the second manipulation, the unrestricted sperm model, I lowered the value of  $\alpha_J$  for mated and gravid females so that males only use half as much sperm (in comparison to the basic model) to successfully inseminate these females. The changes in sperm usage for both model manipulations affect potential mate number, but they do not change the proportion of the clutch the male sired, i.e., the pay-off associated with each female.

### **Data Analysis**

The solution to the above models is a state- and time-dependent series of optimal decisions and associated expected fitness values. These decision matrices can be considered male mating strategies. In order to assess how these strategies affect reproductive outcomes and behavioral patterns (including male mating rate), I

conducted Monte-Carlo forward simulations of a population of males adopting the state-dependent strategies identified in the Basic, Restricted, and Unrestricted models (Clark & Mangel 2000). From these simulations, I collected data on the reproductive success, mating experience, and behavior of 60 males in each size class and seasonal period.

Male reproductive success was calculated as proportion of clutch sired. Because most of the data distributions for reproductive success were non-normal and in some cases bimodal, I calculated the mean and 95% confidence limits using a bootstrapping method. To do this I re-sampled the data set 1000 times with replacement to generate a distribution of sample means. I calculated the mean of this distribution as well as the 95% confidence limits. Logistic regression and Chi-squared Tests were used for categorical data analyses. Tests using logistic regression are noted in the results. In all other cases with a  $X^2$  value listed, a Chi-squared Test was used.

Finally, I classified males as having employed one of six behavioral tactics that incorporate mate number (monogyny versus polygyny) as well as female type (virgin, mated, or gravid). Because polygynous tactics can encompass mates of more than one female type, I established a hierarchy for strategy assignment. If a polygynous male mated with a virgin female (regardless of the types of his other mates), he was assigned to the polygynous virgin category. If he did not mate with a virgin female and mated with at least one gravid female, he was assigned to the polygynous gravid category. If he mated only with mated females, he was assigned to the polygynous mated category. All monogynous males were assigned to categories based on their single female mate, monogynous virgin, monogynous mated, monogynous gravid.

## Results

### Male Mating Strategies

#### Basic model

I defined 9 strategies in the basic model, 3 different male body sizes (small, medium, and large) each at 3 different season periods (early, middle, and late). In a pair-wise comparison of the strategies, I tallied differences in the optimal decision associated with each combination of state values and found that the 9 strategies ranged from 0.8% to 19% different from one another overall. Differences among male sizes in the strategies predicted by the model were most pronounced in the early season period (11% different on average versus 8% different for the middle season and 5% different in the late season period), and large male strategies changed the most over the course of the season (12% difference in early versus late season strategies, compared to 2% difference for small males and 9% different for medium males). Because medium male strategies and middle season strategies were intermediate between small and large males and early and late season respectively, here I will compare only small and large males and the early and late season in order to simplify the results. However, the data for medium sized males and the middle time period are included in calculations of male mating rate and male reproductive outcomes from the Monte Carlo simulations (see below).

In general, across all strategies, if a male located a web, he remained on the web until mating successfully. There was evidence of gravid female abandonment towards the end of a male's lifetime in the early season. In these cases, males would arrive at the web and then leave without mating in favor of attempting to mate on a virgin or

mated female's web. Large males abandoned gravid females at a higher rate than small males (about 23% of the time for large males, and 2% for small males).

In the basic model, almost all differences among strategies corresponded to differences in male behavior after mating. If a male mated with a non-virgin female (either mated or gravid), he had to decide whether to leave the female after mating or stay behind to guard her. In the early season period, large males were very likely to stay behind to guard their non-virgin female mates (Table 5-2; Fig. 5-4). In contrast, in the late season period, large males behaved similarly to small males by becoming less likely to guard their mates.

### **Restricted sperm model**

In the restricted sperm model, males were limited to a single mate. Because a single mating depleted males of their sperm, there was no variation in post-copulatory guarding behavior among strategies (all males guarded; see methods). The major difference between male strategies in the basic and restricted sperm model was that in the restricted sperm model, for 63% of the state space when large males were unmated but on a mated female web, they abandoned the web without mating. This was the optimal behavior up until the last 3-4 days in a male's adult lifespan, where males reverted to the strategy of staying on the web until mating. Small male strategies in the early season did not differ from the basic model.

### **Unrestricted sperm model**

In the unrestricted sperm model, males used only half the sperm for each non-virgin mating compared to the basic model (see methods). The general trends in mating strategies were similar between the unrestricted sperm model and the basic model, although overall in the unrestricted sperm model, both small and large males spent a

lower proportion of the state space guarding non-virgin females compared to the basic model (Table 5-2).

## **Reproductive Outcomes**

### **Basic model**

**Male mating rate.** Across all body sizes and season periods 184 of 270 males successfully mated. Most males mated polygynously ( $N_{\text{Polygyny}} = 117$ ,  $N_{\text{Monogyny}} = 67$ ), and polygynous males mated with either two or three females. Mean reproductive success was higher for polygyny relative to monogyny (Fig. 5-1). Comparing males that had one, two, or three mates, mean reproductive success increased with mate number, although the difference between two and three mates was slight (Fig. 5-2A). This is because males used a minimum of 40% of their sperm at each mating, thus, males mating for a third time never achieved full pay-off with their last mate. The effect of mate number on mean reproductive success changed with female type (Fig. 5-3A). Males that mated monogynously with mated or gravid females had lower mean reproductive success compared to the other four strategies. Monogynous males that mated with virgin females had mean reproductive success comparable to polygynous males, although the variance in reproductive success was much lower for polygynous males who mated with mated and gravid females compared to males who mated monogynously with virgin females.

**Male size.** Of the 90 males simulated for each size class (across all season periods), 61 small males, 63 medium males, and 60 large males mated successfully ( $X^2_2 = 0.24$ ,  $P = 0.89$ ). There was no difference in mean reproductive success across male size classes ( $\text{Mean}_{\text{Small}} = 0.19$ , 95% CI: 0.16-0.24;  $\text{Mean}_{\text{Medium}} = 0.2$ , 95% CI: 0.16-0.24;  $\text{Mean}_{\text{Large}} = 0.18$ , 95% CI: 0.14-0.22). There was a trend towards a difference in

rates of monogyny and polygyny among male sizes where large males mated monogynously at a higher rate compared to other male sizes ( $X^2_2 = 3.6$ ,  $P = 0.17$ ).

Size differences in rates of monogyny do not reflect differences in male mate-guarding strategies with non-virgin females (see above). Large males guarded in 76% of mating events versus 12% for small males ( $X^2_1 = 80.1$ ,  $P < 0.0001$ ; Fig. 5-4A-B). However, mate guarding had no effect on the number of subsequent matings achieved comparing large males (who typically guard) to small males (who typically do not guard;  $X^2_2 = 0.89$ ,  $P = 0.7$ ). Difference in rates of polygyny among size classes could have occurred because large males mated with virgin females at slightly higher rates ( $X^2_2 = 5.2$ ,  $P < 0.075$ ; Table C-3) and became sperm-depleted after these matings.

**Seasonal effects.** Male mean reproductive success declined across the time periods within the season for males of all body sizes (Fig. 5-5B). In addition, as the season progressed, a greater proportion of males adopted a polygynous strategy ( $X^2_2 = 6.1$ ,  $P < 0.047$ ). Guarding also decreased across time periods within the season ( $X^2_2 = 30.6$ ,  $P < 0.0001$ ; Fig. 5-4).

### **Model comparisons**

**Model similarities.** In the restricted sperm model, males were limited to a single mate (see above). In the unrestricted sperm model, males had twice the number of available sperm compared to the basic model when mating with non-virgin females. The basic model and the unrestricted sperm model were similar in terms of male tactics. In both models most males were polygynous (Basic = 65% polygynous, Unrestricted = 64% polygynous). Polygyny was not possible in the restricted sperm model because males were only able to mate once. In the basic and unrestricted sperm models, the mean reproductive success for each of the six strategies showed similar patterns (Fig.

5-3B). Combining the three time periods, equal proportions of small and large males successfully mated in all models (Restricted model:  $X^2_1 = 0.17$ ,  $P = 0.68$ , Unrestricted:  $X^2_1 = 0.85$ ,  $P = 0.36$ ), and there were only small size-based differences in mean reproductive success across models. Male mate guarding showed similar size-based patterns in the basic and unrestricted sperm models. However, in general, guarding rates were much lower in the unrestricted sperm model for all male sizes.

**Model differences: male mating rate.** Under reduced sperm limitation, the advantage of being polygynous increased (Fig. 5-1B), although mating monogynously had equal reproductive success to polygyny if the male mated with a virgin female (Fig. 5-3B). In the unrestricted sperm model, polygynous males mated with between two and five females, compared to a maximum of three mates in the basic model (Fig. 5-2A-B). Unlike the basic model where males lost reproductive success due to sperm limitation at the third mating, in the unrestricted sperm model, the relationship between mate number and reproductive success continued to increase without bound because males could mate with up to five non-virgin females in their lifetime with no sperm limitation (Fig. 5-2B). In the basic model, there was a trend towards increased monogyny with increased male size. However, there were no size-based differences in the rate of monogyny in the unrestricted sperm model ( $X^2_1 = 0.001$ ,  $P = 0.98$ ).

**Model differences: seasonal effects.** In contrast to the basic and unrestricted model where multiple mating was possible, there were seasonal differences in the number of small and large males that successfully mated in the restricted sperm model. In the early season, more small males successfully mated compared to large males ( $X^2_1 = 4.4$ ,  $P < 0.034$ ), and in the late season this trend was reversed, although the difference

was not significant ( $X^2_1 = 2.4$ ,  $P = 0.12$ ). Nonetheless, overall, across all models and season periods, large and small males had similar mean reproductive success (Fig. 5-5). Only in the late season period of the restricted sperm model were there suggestions of large male advantage (Fig. 5-5A).

## Discussion

I used a dynamic state modeling approach to (1) determine optimal male mating rate; (2) evaluate the factors that constrain male mating rate; and (3) determine whether males of different sizes adopt alternative mating strategies and tactics in the spider *Nephila clavipes*.

### Male Mating Rate

Model results suggest that there may be more than one mating rate optimum for male *Nephila clavipes*. In the basic model, males achieved high reproductive success from mating at a maximum rate (i.e. polygynously with 2 or 3 females; Fig. 5-2A), but they also achieved similar reproductive gain from mating monogynously with a female if she was a virgin (Fig. 5-3A). Due to female mortality risk, the variance in reproductive success was much higher for mating monogynously with a virgin female compared to mating polygynously with either mated or gravid females (Fig. 5-3A). These findings suggest that polygyny is a lower risk tactic compared to monogyny, even though the mean reproductive outcome between the two tactics is similar. Males could mate polygynously as a way to spread female mortality risk and minimize the risk of total reproductive failure.

There are patterns of reproduction in other species that suggest that males may benefit by mating multiply to distribute risk of brood failure. For example, in some species males exhibit a preference for novel females, which manifests as decreased

mating investment in a familiar female and increased investment in unfamiliar females (the Coolidge Effect; Wilson et al. 1963; Dewsbury 1981; Pierce et al. 1992; Pizzari et al. 2003; Koene & Ter Maat 2007). This preference for novelty is puzzling when it occurs despite intrinsic disparities in quality between the familiar and unfamiliar female. For example, in the burying beetle *Nicrophorus vespilloides*, males decrease their latency to copulate when presented with a novel female, regardless of her virginity status and therefore the level of sperm competition the male may face. Males choose females on the basis of novelty as opposed to reproductive pay-off (in terms of number of offspring sired), which could result in males mating with multiple low pay-off females instead of restricting their reproductive investment to a few high pay-off females (Steiger et al. 2008). The Coolidge Effect is expected in cases where there are diminishing returns from repeated copulations with a single female. These diminishing returns could occur because continuous mating results in physical exhaustion without increased sperm competition benefits (Jordan and Brooks 2010), or because sperm are limited, and so it benefits males to allocate sperm prudently (Wedell et al. 2001). Here I suggest that a preference for novelty in general may simply promote a risk-spreading mating tactic, beneficial in cases where mating at a higher rate with multiple females increases average reproductive success but also decreases variance in reproductive success that could result from female mortality or brood failure due to inadequate parental care.

## **Constraints on Mating Rate**

### **Mate-guarding**

One traditional factor thought to impose a constraint on male mating rate, mate search mortality risk (Vollrath and Parker 1992), did not constrain male mating rate in the model. However, similar to other species (Stockley et al. 1996; Zamudio & Sinervo

2000; Schubert et al. 2009), mate guarding affected male mating strategies, although it did not affect male mating rate. For males, mate-guarding is typically considered to be a trade-off between preventing female re-mating and achieving matings with additional females (Alcock 1994). However, in the model, male re-mating frequency was comparable between large and small males even though large males spent more time guarding (Fig. 5-4A). This could be because for large males there is little cost to mate guarding relative to small males because large males can guard and still have time to mate with the maximum number of females (3). However, small males, who are less competitive and have to wait longer once they reach a web to achieve successful copulation, do not have time to guard.

Female density affected male guarding behavior in the opposite way than predicted based on empirical studies in other species, where decreased mate availability increases guarding rates (Latty 2006). At the end of the season, female density (i.e. mate availability) was lowest, but the frequency of guarding also decreased (Fig. 5-4). Even with low female density and high mate search mortality risk (a 20% chance of failing to find a female coupled with 26% mortality risk per search), it was more beneficial for males to spend additional time searching for new mates in order to reach their maximum reproductive rate. Similarly, males with the greatest chances of re-mating, large males, actually spent the most time guarding, because they were able to spend less time waiting to mate compared to small males once a new web was found. These results could in part be a result of the fact that guarding successfully carried little additional benefit for males. However, the substantial differences in guarding behavior

between the basic and unrestricted sperm models (where the benefit of guarding were the same; Fig. 5-4) suggest that sperm availability may play an even greater role.

The unrestricted sperm model revealed that guarding frequency was strongly affected by potential reproductive rate. Changes in potential reproductive rate however, were not caused by decreased female density. Rather, because males had more sperm available for copulation, guarding frequency decreased in the unrestricted sperm model because males needed to spend more time searching and copulating in order to reach an optimal mate number (5 mates; Fig. 5-4B). Thus in general, it seems that males must have either an extremely low probability of re-mating or very high benefits from guarding in order to off set the potential benefits of mating with additional females. As a result, some other mating rate limitations (e.g. male sperm limitation, which is common across a number of taxa) may be required for guarding behavior to evolve. Sperm limitation could have a greater effect on mate guarding behavior than other traditional measures of male mating potential (e.g. female density and male competitive ability).

### **Male sperm limitation and choosiness**

Sperm depletion constrained male mating rate in general (Fig. 5-2A), but it also affected male reproductive tactics. For instance, there was a trend towards increased monogyny for large males compared to small males in the basic model. This difference was not a result of differences in male strategies, but rather a consequence of male sperm depletion: large males mated virgin females at a higher rate compared to small males (Table C-3), which depleted their sperm and eliminated re-mating ability.

Increased sperm limitation (restricted sperm model) also caused males to become choosy in some cases. Large males abandoned low quality mated female webs without mating in the early season period. This result is not surprising, because in

species in which females vary in quality, males are expected to be choosy about their reproductive investment, particularly when mating opportunities are limited (e.g. Hardling et al. 2008; Barry & Kokko 2010). However, female quality and number of mating opportunities are not the only important variables to consider when evaluating whether males should be choosy. In the basic model, even though males were sperm-limited, males were never choosy, which is a consequence of the low cost associated with mating with a non-virgin female, the reproductive benefits of mating multiply (Fig. 5-1-5-3), and the mortality risk associated with searching for a new mate. It is only in the restricted sperm model, when males are most severely sperm-limited (but all other parameters remain the same), do the benefits of choosiness outweigh the costs and risks associated with foregoing a mating opportunity.

There was a second form of male “choosiness” in the model results. Large and small males abandoned gravid female webs at a consistent rate across all three models during the early season period. Because males abandoned gravid females but not mated females in the basic and unrestricted models, it suggests that the reason for female abandonment was not that males were choosing against low quality females, but instead that males were attempting to re-locate to a web where their mating probability was higher. The mating probability on gravid webs is low for all males (due to large male group sizes on these webs). At the end of their lifetime, it is more beneficial for males to search for less competitive webs. This is especially true for large males who have a good chance of out competing other males on other webs types. In contrast to abandonment behavior with gravid females, only large males chose against low quality mated females in favor of higher quality females in the restricted model.

Choosiness, as defined as discrimination against females based on quality, was strongly dependent on a male's ability to win a new mate once abandoning a female. For this reason, choosiness was exclusively a large male strategy. Large males abandoned mated female webs in order to pursue a mating with a higher quality female (in the early season of the forward simulation, a virgin female). In contrast, choosiness was not beneficial for small males because the alternative to mating with a low quality female was to move to a highly competitive web of a different type where small males have a low chance of successfully mating compared to large males. Thus there are size-dependent costs and benefits of choosiness, a finding consistent with work in other species that suggests that male mate investment and mate choice strategies can change as a function of male condition, size, or ability to secure mates (Burley 1988; Hardling & Kokko 2005; Bel-Venner et al. 2008; Candolin & Salesto 2009). In spiders in particular, male reproductive behavior is affected by experience during male-male contests (Kasumovic et al. 2010) and so it is possible for males to assess their own competitive ability as well as the competitive environment on a web, and make decisions accordingly. In *N. clavipes* specifically, there is evidence that males arrive on webs and assess the competitive environment before deciding to move to a new web (Christenson & Goist 1979).

Because small males were unable to be choosy, and thus mated indiscriminately, I might expect a large male reproductive advantage when large males are able to be choosy. Indiscriminate mating with low quality mates compared to choosy mating with high quality mates could be perceived as a "best of a bad job" strategy (Lee 2005). Surprisingly however, the ability to be choosy did not provide a reproductive advantage

for large males. Instead, when choosiness occurred (the early season period of the restricted sperm model), this high-risk strategy resulted in significantly fewer large males achieving copulations compared to small males. In addition, due to the variance associated with mating with a virgin female, there were no significant size-based differences in male reproductive success during times where males were choosy (Fig. 5-5A). These findings suggest that choosiness and indiscriminate mating may represent stable alternative strategies (Fawcett & Johnstone 2003; Hardling & Kokko 2005).

### **Male Body Size Variation**

In my model, variation in female value coupled with constraints on male mating rate resulted in weak selection on male body size despite competitive advantages of large size. Past studies in other *Nephila* species have suggested that trade-offs associated with male size result in weak stabilizing selection (Vollrath 1980). For example, while there is a large-male advantage in contests (Christenson & Goist 1979), small males have lower rates of sexual cannibalism (Elgar & Fahey 1996), an intermediate size is favored in terms of climbing speed (Moya-Larano et al. 2009), and there can be seasonal fluctuations in the strength of selection on size (Kasumovic et al. 2008). However, here I also demonstrate that spatial variation in the strength of sexual selection on females' webs, which is in part a result of variation in female attractiveness, provides opportunities for small males to achieve lifetime reproductive success similar to that of large males.

The only scenario in which the model recovered body size differences in reproductive success was extreme sperm limitation, where each male could mate only once (Fig. 5-5A), and in this case, large male advantage only occurred in the late season period. This suggests that the ability to mate multiply coupled with variation in

female quality could lessen size-based differences in reproductive success, and weaken selection on male body size in species similar to *N. clavipes*. Within the genus *Nephila* there are interspecific differences in the degree of male body size variation (Elgar & Fahey 1996; Schneider et al. 2000; Rittschof 2010). In some species, males are constrained to a single mate because they are cannibalized during copulation (Schneider et al. 2000; which is somewhat analogous to our restricted sperm model; Kasumovic et al. 2007). If multiple mating weakens selection on body size, male body size variation should be more extensive in species where males potentially re-mate. This hypothesis could be tested through comparative studies of mating rates and body size variation among populations and species. For instance, two sympatric species of *Nephila* found in Australia show different rates of sexual cannibalism and different levels of male body size variation. In *N. edulis*, cannibalism is rare, giving males the potential to re-mate, whereas cannibalism is very common in *N. plumipes* (Schneider et al. 2000; Kasumovic et al. 2007). As I would expect from our model, the coefficient of variation for male body mass is 104% in *N. edulis* and only 31.7% in *N. plumipes* (Elgar & Fahey 1996; Schneider et al. 2000; Elgar et al. 2003).

### **Final Remarks**

In this study, I use a modeling approach to assess male mating rate optima and alternative reproductive tactics in the spider *N. clavipes*. Multiple mating rate optima are a function of female type and mate number. Mate guarding and male sperm depletion constrain male mating rate. Multiple factors contribute to whether a male will be choosy, including potential reproductive rate, female quality, benefits of multiple mating, and the costs of mate search. Different sized males adopt mating strategies and tactics that differ in terms of degree of mate-guarding and male choosiness. However, these

strategies and tactics result in similar reproductive pay-offs, which suggests that alternative tactics may be present in this species.

Table 5-1. Parameter terms for the dynamic state model.

Term	Definition
State variables:	
T	time horizon (21 days)
t	time step (1 day)
J	web status
E	energy level
X	proportion of sperm available for mating
I	mating history of the male at his current web
Factors affecting state:	
c <sub>J</sub>	cost of male-male competition
t <sub>J</sub>	time required to mate (days)
α <sub>J</sub>	proportion of total sperm used during mating event
s	daily senescence (energy) cost
Probabilistic events:	
β	male mortality risk (per search)
r <sub>J</sub>	probability of encountering a female
m <sub>J</sub>	probability of mating
d <sub>J</sub>	female survival probability
k <sub>J</sub>	probability of successfully guarding
w	probability that the female abandons her web
Fitness:	
f <sub>J</sub>	total fitness (proportion of clutch sired)
y <sub>J</sub>	proportion of fitness gained by mating
ε	fitness discount due to energy shortage
τ	fitness discount due to time shortage
σ	fitness discount due to sperm shortage
z <sub>J</sub>	proportion of fitness gained with each day guarding

Subscript (J) denotes variables that change with female type.

Table 5-2. Summary of male strategies by degree of male sperm limitation, size, and season.

Model	Size	Season	Choosiness	Guard
Basic	Small	Early	Yes (G)	+
	Large		Yes (G)	+++++
	Small	Late	No	+
	Large		No	+++
Restricted	Small	Early	Yes (G)	-
	Large		Yes (G,M)	-
	Small	Late	No	-
	Large		No	-
Unrestricted	Small	Early	Yes (G)	+
	Large		Yes (G)	++++
	Small	Late	No	+
	Large		No	++

For the "choosiness" category, males left female webs without mating. Males guarded females at different rates. "+" symbols refer to the percentage of mating events where males guarded at least one day: +++++ = >50%, ++++ = 40-50%, +++ = 25-40%, ++ = 10-24%, + = <10%. G denotes the gravid female type, M denotes the mated female type.

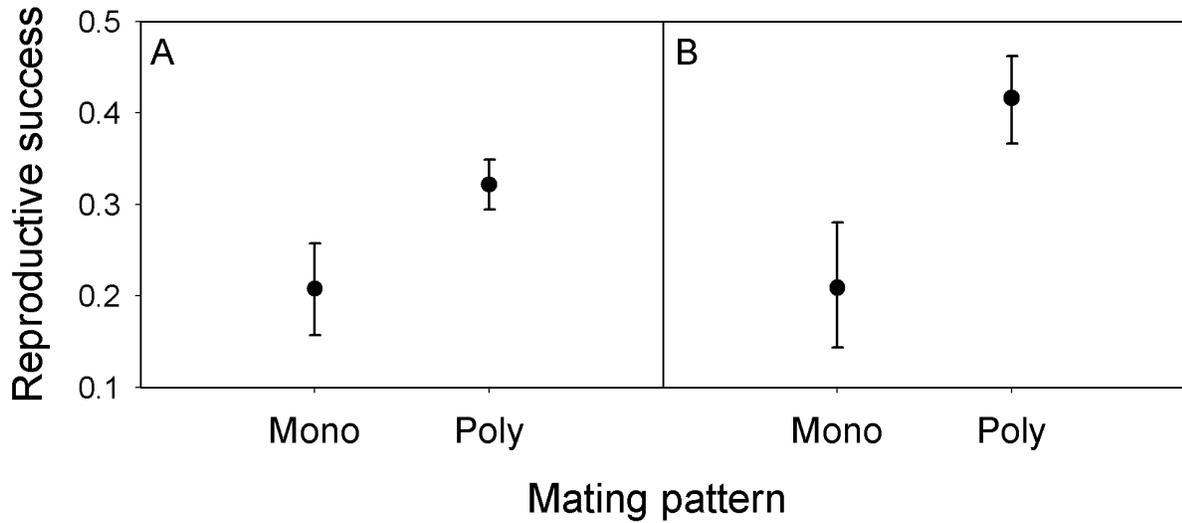


Figure 5-1. Mean reproductive success and 95% confidence limits are shown for polygynous (Poly) versus monogynous (Mono) mating in the basic (A) and unrestricted (B) sperm models. Mating polygynously yields higher mean reproductive success compared to mating monogynously in both models. However, the degree of difference in reproductive success between monogyny and polygyny is greater with weaker sperm limitation, i.e., in the unrestricted model (B versus A).

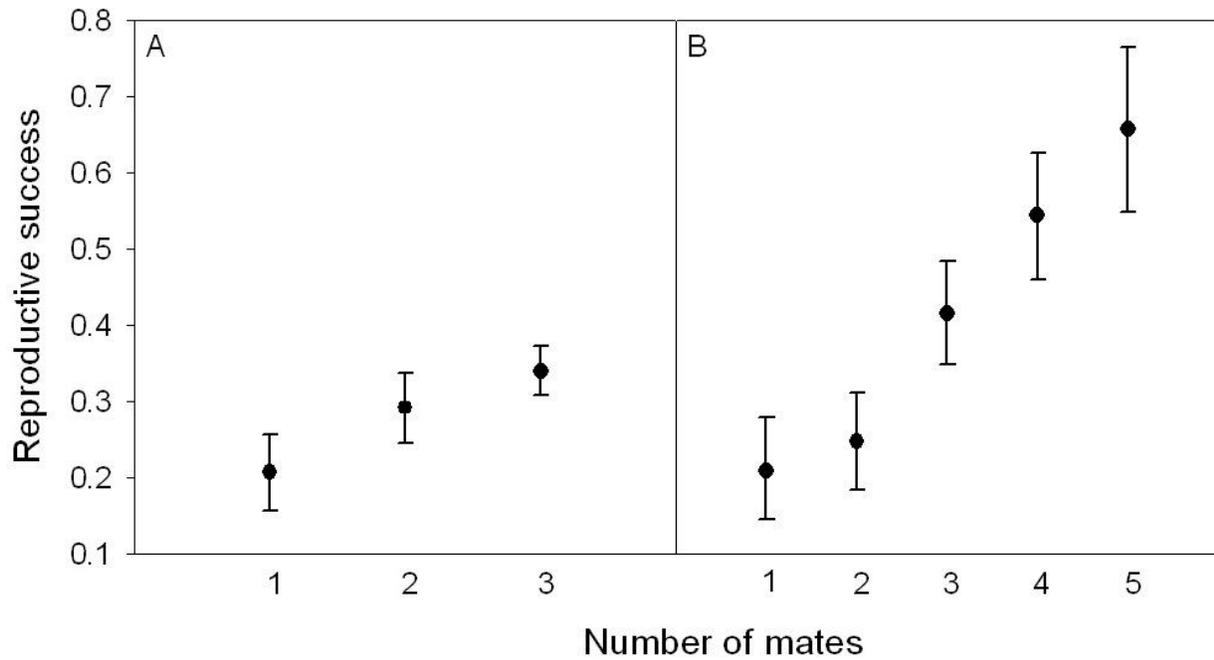


Figure 5-2. Mean reproductive success and 95% confidence limits as a function of mate number for the basic (A) and unrestricted sperm (B) models. In both models mean reproductive success increases with mate number. However, in the basic model, males are sperm-limited at the third mating, and reproductive success asymptotes (A) while reproductive success increases without bound in the unrestricted sperm model (B).

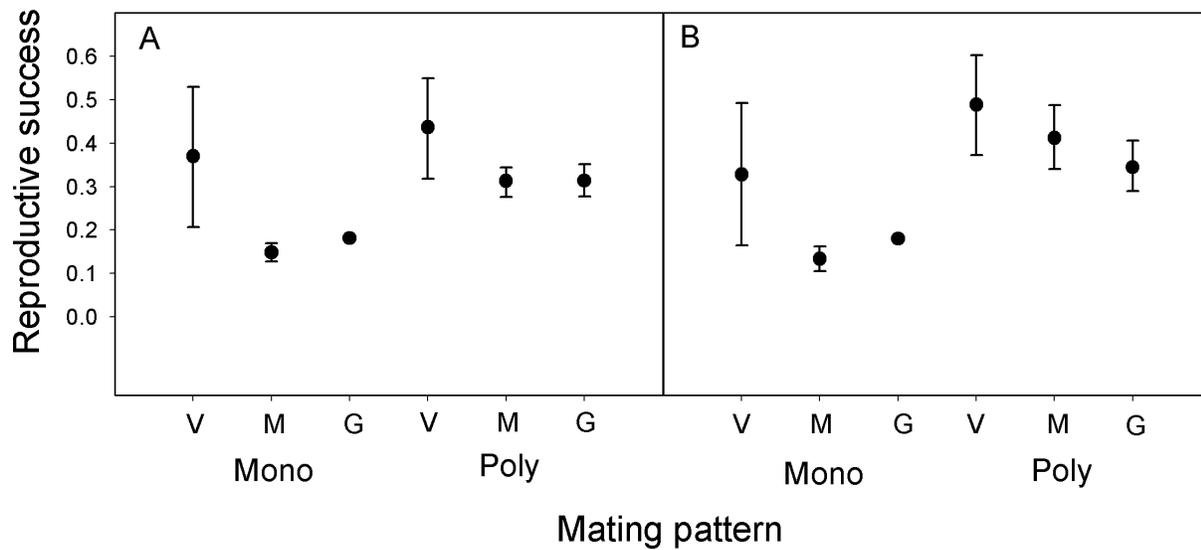


Figure 5-3. Mean reproductive success with 95% confidence limits for monogynous (Mono) and polygynous (Poly) mating outcomes divided by female type: virgin (V), mated (M), and gravid (G). Mean reproductive success for mating monogynously with a virgin female is similar to mating polygynously with lower quality (M or G) females.

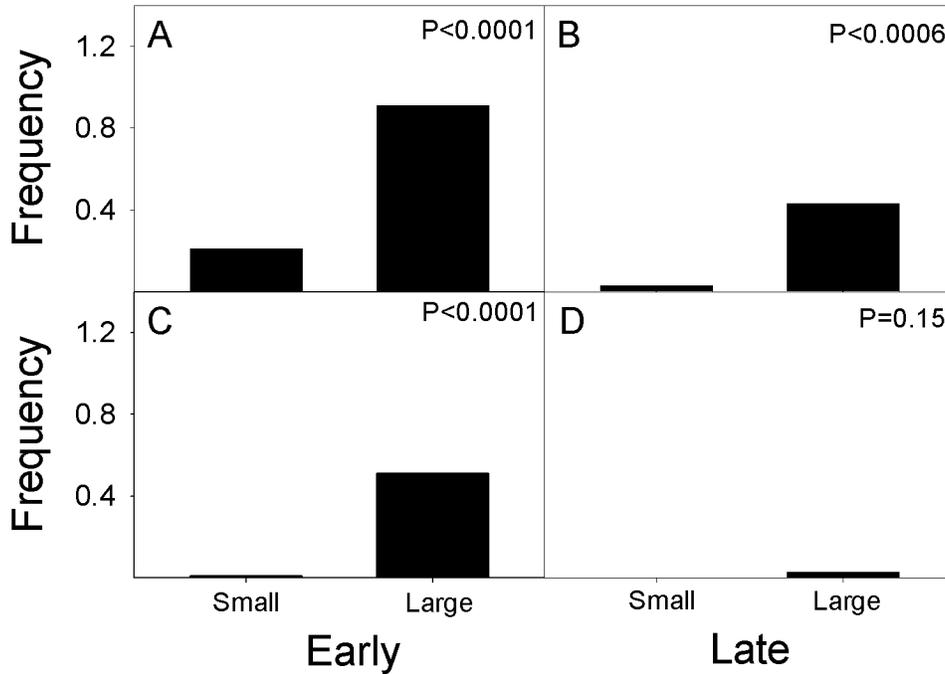


Figure 5-4. The frequency of mating events where males chose to stay and guard the female for at least one day after copulation. Data only include males that had sperm remaining after copulation (Basic model:  $N_{\text{Small, Early}} = 33$ ,  $N_{\text{Large, Early}} = 32$ ,  $N_{\text{Small, Late}} = 27$ ,  $N_{\text{Large, Late}} = 28$ ; Unrestricted sperm model:  $N_{\text{Small, Early}} = 81$ ,  $N_{\text{Large, Early}} = 77$ ,  $N_{\text{Small, Late}} = 68$ ,  $N_{\text{Large, Late}} = 67$ ). Results are divided by model and season: basic (A,B) and unrestricted sperm (C,D) models, early (A,C) and late (B,D) seasons. P values are the results of Chi-squared Tests comparing small and large males for each model and season period. Males are more likely to guard if they are larger, if its early in the season, and if potential reproductive rate is low.

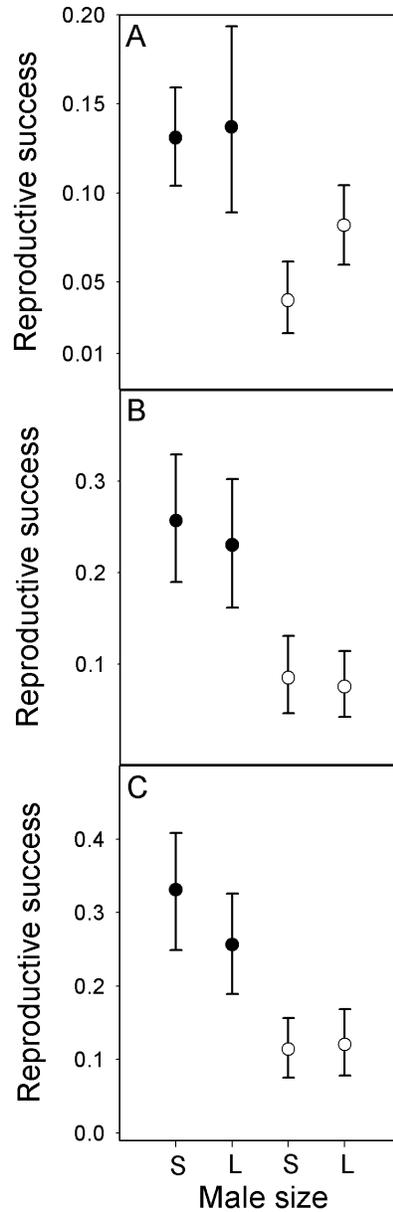


Figure 5-5. Mean reproductive success with 95% confidence limits for small (S) and large (L) males in each of the three models, restricted sperm (A), basic (B), and unrestricted sperm (C). Closed symbols correspond to values in the early season period, and open symbols correspond to the late season period. Across all models, reproductive success is lower in the late season period. However, size-based differences were only evident in the restricted sperm model (A), and significant differences occurred only in the late season period.

## CHAPTER 6 CONCLUSIONS

### Summary

In the golden orb-web spider, *Nephila clavipes*, multiple factors interact to affect male body size variation. Large males have an advantage during contests over females, but this advantage depends on the number of other competitors present on the web. Large males have an advantage when male density is high (Fig. 3-3), but large and small males copulate at equal frequencies when density is low (Chapter 3). Furthermore, male density on a web is highly variable (Fig. 3-1) which is, at least in part, a result of variation in female attractiveness. Males in the field are attracted to adult females who are close to oviposition (Chapter 4), and in a mating experiment, males mated at a higher rate (Fig. 4-3) and transferred more sperm when paired with non-virgin females close to oviposition compared to younger non-virgin females. Males that mated with gravid females close to oviposition or virgin females had the highest pay-off in terms of total offspring sired (Fig. 4-4, 4-5), although virgin females may be a high risk mating investment compared to gravid females because there is a long period of sperm storage between mating and oviposition, and female mortality is common during this time period (Chapter 4). Thus Chapters 3 and 4 show that male reproductive success is determined by a complex interaction between multiple measures of female quality and male competitor density. These interactions have implications for the strength of selection on male body size.

Using a dynamic state model (Chapter 5), I assessed how different sized males could optimize their reproductive success in a variable mating environment where females differ in quality and the rate of male attraction. In the model I account for factors

that could affect male mating strategies and reproductive success, including mortality risk while moving from web to web, and seasonal changes in male and female density. In addition, I consider male sperm depletion as a factor in the model because this unusual characteristic of this species has implications for male mating rate (Chapter 2). Male *N. clavipes* have a finite number of sperm, and once this sperm is depleted, they can no longer fertilize eggs (Chapter 2). I showed in Chapter 4 that males alter their sperm use depending on the quality of their mate, and the dynamic state model (Chapter 5) suggests that sperm limitation will only affect male reproductive strategies in very extreme cases (i.e. when males are limited to a single mate). Sperm depletion, however, could have implications for male body size evolution (Chapter 5; discussed below). The integrative dynamic state model showed that different sized males could employ subtly different strategies and tactics that optimize their reproductive success in response to a heterogeneous reproductive environment, resulting in similar levels of success across male sizes (Chapter 5). These findings suggest that environmental heterogeneity in selection pressure, coupled with male behavioral variation, could maintain male body size variation in *N. clavipes*.

### **Environmental Heterogeneity and Plasticity**

Environmental heterogeneity occurs across a variety of scales in space and time (Svensson & Sinervo 2004). On a large ecological scale, populations may be distinct, capable of local adaptation, and depending on the level of gene flow, speciation (Lande 1982; Arnqvist 1992; Johannesson et al. 1995; GarciaRamos & Kirkpatrick 1997; Kawecki 1997; Case & Taper 2000; Hendry et al. 2002; Doebeli & Dieckmann 2003; Nosil & Crespi 2004). Such inter-population dynamics can be complex, for example, environmental clines can result in stable hybrid zones between neighboring species or

populations (Hewitt 1988). However, environmental heterogeneity can also operate on very fine-grained spatial scales, and as opposed to clinal variation, it may be more accurately described as a mosaic (Svensson & Sinervo 2004; Gosden & Svensson 2008). In this context, where there are high levels of genetic mixing and poorly defined sub-populations and population boundaries, environmental variation remains a force that can spatially alter the strength or direction of selection and thus contribute to phenotypic variation (Levins 1968). The latter description of environmental heterogeneity applies to spider webs. Webs partition the environment into a mosaic of discrete patches, and in the case of *N. clavipes*, the characteristics of these patches vary (Chapters 3, 4; Agnarsson 2003; Rittschof 2010; Rittschof & Ruggles 2010), with implications for the evolution of male size. The resulting heterogeneous environment has continuity due to gene flow, but fine-scale differences in the strength of selection on male body size could maintain variation in this trait, which in this species has a broad, unimodal distribution (Fig. 3-2).

For a single population in a heterogeneous environment, several processes can result in a broad unimodal trait distribution, including multiple phenotypic optima (Lande 1982; Brooks 2002), frequency- or density-dependent selection (Gross 1991; Punzalan et al. 2005), phenotypic plasticity (Gotthard & Nylin 1995), and behavioral plasticity (West-Eberhard 2003). In *N. clavipes* I found evidence that all of these processes may contribute in part to the evolution of male size and the maintenance of a broad size distribution. In Chapter 3 I discuss how different webs could select for different optimal male sizes because large male advantage is density-dependent. In this section I focus on 1) the role of phenotypic and behavioral plasticity in the maintenance of size

variation in *N. clavipes*, and 2) whether size and behavior are co-evolving or are independent phenotypes, with consequences for the evolution of size.

### **Plasticity and Size Variation**

Phenotypic plasticity is a common adaptive response to environmental variation (Levins 1968). Many species show phenotypic plasticity for body size, maturing at the size best suited for the current environmental conditions (Indermaur et al. 2010). In *N. clavipes*, large male size is advantageous in high male densities (Chapter 3), but because there are typically costs to growing large in terms of growth rate and emergence time (Roff 1992), males would benefit if they could emerge at a smaller size when male density is low. There is evidence in other web-building species that individuals can respond during development to pheromone cues that signal density (Kasumovic & Andrade 2006; Kasumovic et al. 2009a) in order to mature at an optimal size. For this reason it is important to examine the possibility that male size variation in *N. clavipes* is a consequence of density-dependent phenotypic plasticity in adult size.

In the sister species *Nephila plumipes* (Kasumovic et al. 2009a), there is evidence that males assess local density cues during development (Andrade and Kasumovic 2006), and emerge at larger sizes when male density is high, and small sizes when male density is low. In *N. plumipes*, females live in stable aggregations and as an adult, a male joins an aggregation close to where the male matured. On a small spatial scale, males might sense and respond to changes in density. On a larger scale (>5m) however, Kasumovic et al. (2009a) found no relationship between male size and female and male density, and so it appears that males are unable to assess larger scale differences in density. In contrast to *N. plumipes*, the larger spatial scale is most relevant for *N. clavipes* because females are much more mobile in this species: they do

not have stable aggregations and they change web sites often (Rypstra ; Vollrath ; Vollrath & Houston ; Rittschof & Ruggles). In addition, due to frequent web movement and differential mate attraction, the number of males found on a female web (i.e. local male density) varies throughout a female's lifetime (Chapter 4), and within the lifespan of a male. Furthermore, males can travel large distances (C. Rittschof, unpublished data), and they do not always inhabit female webs found near their juvenile webs. Thus if male *N. clavipes* respond developmentally to changes in density, they should do so on a relatively large (>5 m) spatial scale.

On a larger spatial scale, in *N. clavipes* there are population-wide seasonal shifts in male density (from high to low), which means that large males could have a competitive advantage early in the season (Higgins). Similar shifts have been observed in the sister species *N. plumipes*, and in addition, in this species, average male size decreases over the course of the season (Kasumovic et al 2009a). Such a decrease could be a plastic response to a decrease in male density and an increase in the number of available females (Kasumovic et al. 2009a). In *N. clavipes* too, there appears to be a slight decrease in average male body size over the course of the season (C. Rittschof, personal observation). However, in both species, size variation within a particular time of the season (e.g. on a given day) is much greater than the degree of change in average size across the season. For instance, from the beginning to the end of the season, average male size in *N. plumipes* decreases by about 10%, however, within the population throughout the season the largest males are 110% the size of the smallest males. Kasumovic et al (2009a) conclude that seasonal variation in size explains only a small component of the total degree of male size variation. Similarly, in

*N. clavipes*, seasonal shifts in size do not explain the 2.5 fold variation in male size that is evident throughout the season. Furthermore, female *N. clavipes* have seasonal shifts in size that are similar to that of males, which suggests that both males and females may show decreased size as a result of a shared selection pressure. Perhaps at the end of the season all adults show decreased size as a result of accelerated development because of the short reproductive time period available to adults before the onset of winter (no individuals are capable of overwintering; Higgins 2000).

Although there are seasonal changes in male density in *N. clavipes*, the greatest source of variation occurs on a finer spatial and temporal scale. Neighboring webs differ greatly in the number of males present, and thus the intensity of male-male competition (Fig. 3-1). The degree of competition on a web can change in a very short time period due to female web movement or egg development (Chapters 3,4; Rittschof & Ruggles 2010), and thus *N. clavipes* males may not benefit as much from phenotypic plasticity as they would from targeting webs best suited for their size. This response to environmental heterogeneity, behavioral plasticity, is fine-tuned level and allows an organism to respond quickly to environmental change.

Individuals can respond to changes in the environment over time by adopting behavioral strategies that account for systematic or cyclical variation in environmental conditions (Levins 1968). However, strategies vary greatly in their degree of plasticity and in the cues individuals use to assess the environmental conditions and alter their behavior. For instance, strategies may be responses to seasonal shifts in the environment (Gotthard and Nylin 1995), or they may be responses to less predictable environmental changes, like shifts in prey availability, or conspecific or predator density

(Milinski and Heller 1978). At the simplest level, the behavioral repertoire of an organism allows the individual to respond to a complex environment in real time rather than wait for population-level heritable changes at the DNA sequence level (Milinski & Heller 1978; West-Eberhard 2003). Behavioral plasticity is a form of phenotypic plasticity, and may be particularly important for an organism that is navigating a spatially variable reproductive environment.

The dynamic state model (Chapter 5) assumes that *N. clavipes* males are capable of some level of adaptive behavioral plasticity. In this case, males alter their strategy depending on the level of sperm they have available, the type of female on the web they occupy (which affects reproductive pay-off), the time of the season (which is associated with changes in conspecific density and mate search mortality risk), their body size, and their energy level. In spiders generally, there is evidence that these assumptions about behavioral plasticity are valid. Spiders can change their behavior in response to past experiences (Whitehouse 1997; Kasumovic et al. 2009b; Kasumovic et al. 2010), which suggests that they may have knowledge about their own mating experience, and thus perhaps their sperm numbers or competitive ability. It is well known that male spiders can identify female reproductive state (and perhaps quality) using airborne and substrate-born chemical cues (reviewed by Gaskett 2007). Males can also sense female body weight through the silk, which would be an indicator of gravidity (Chapter 4). In general, a number of arthropods alter their reproductive behavior in response to their experience of conspecific density as a juvenile (e.g. Harrison 1980; Bouaichi & Simpson 2003; Higaki & Ando 2003; Poniatowski & Fartmann 2009), which means males may also have information about mate search

mortality risk or the level of male-male competition in the population at a given time. Given this ability for adaptive plasticity, I used the dynamic state model to attempt to describe the optimal strategy different sized males could use to maximize their reproductive success.

The dynamic state model revealed the types of strategies males should employ to maximize their fitness in a variable environment. Although there were some size-based differences in male strategies in *N. clavipes*, males in the basic model (which was the model parameterized in the way most relevant to the species' natural history) all had a similar basic strategy regardless of size or time of the season. Males, once a web was found, would remain on the web until they had the opportunity to mate. Males did not target certain types of females or exhibit choosiness in most cases (although small males did leave highly competitive webs in some cases). The biggest difference across sizes was in guarding behavior. Large males spent more time guarding females in order to prevent re-mating compared to small males, and small males mated, on average, at a slightly higher rate. Similar strategies across male sizes had different outcomes in terms of the types of females males successfully mated (Table C-3), but in terms of reproductive success, large and small males did equally well. Given that the assumptions of the model are true, male behavioral plasticity (whether size-dependent and size-independent) could be a potent force that affects the evolution of male size in this species.

The idea that males behave in size-dependent ways in order to optimize their behavior could explain the results of the Kasumovic et al. (2009a) study that found that male body size variation results from density-dependent phenotypic plasticity in *N.*

*plumipes*. Upon surveying adult male body size in the field, the authors found that adult males in female aggregations with high male densities were typically larger compared to males found in low male density aggregations. The authors assume that this is because males matured in proximity to these aggregations and used density cues to optimize their body size in response to local conditions. As an alternative however, large males may target or spend a longer time on webs or in aggregations with high male-male competition because they have a density-dependent competitive advantage (similar to *N. clavipes*; Chapter 3) when in these areas. In this case, when surveying only adult males, large males should be more common on webs with higher male densities. Thus size-dependent behavioral variation instead of phenotypic plasticity may also explain these results. A survey of both local sub-adult and adult males may reveal whether adult males are responding behaviorally or morphologically to local conditions.

### **The Link between Size and Behavior**

The connections between behavioral and morphological evolution can be difficult to ascertain, but it is clear that behavior influences and sometimes precedes the evolution of morphological traits (reviewed by West-Eberhard 2003). For instance, visual courtship displays typically consist of behaviors that are enhanced by exaggerated morphological traits (Candolin 2003). Thus a behavior may precede the evolution of morphology, but in some cases become evolutionarily linked to a particular morphological trait variant (Wcislo 1989) if the two traits are most beneficial when inherited together. However, behavioral plasticity can also be independent of any particular morphological trait (e.g. experience-based changes in behavior; Kasumovic et al. 2009b), particularly if behavioral plasticity itself is the target of selection (West-Eberhard 2003). If this is the case, a capacity for behavioral variation that allows

individuals to achieve similar levels of reproductive success regardless of morphology can weaken selection on that morphological trait. This latter concept can be considered a form of phenotypic accommodation (West-Eberhard 2003). In addition, there could be consistent and repeatable inter-individual differences in behavior that are not coupled to a particular phenotype, considered behavioral syndromes or personalities (Dall et al. 2004; Sih et al. 2004). Whether or not behavior and body size are linked in *N. clavipes* has important implications for the long-term evolutionary consequences of behavioral plasticity in this species.

In the case of *N. clavipes* males, it is unclear whether body size and behavior are co-evolving traits. The model (Chapter 5) assumes that body size is linked to behavior, because I developed the decision matrix (male strategies) using different parameterizations for different sized males. Similarly, for example, in many alternative tactics, behavioral polymorphism and phenotypic polymorphism are often strongly coupled (reviewed by Taborsky et al. 2008). In *N. clavipes*, it makes sense to couple behavior to size because there is evidence of size-dependent differences in reproductive outcomes in this species (Christenson and Goist 1979; Cohn 1989; Rittschof 2010), and if males are behaving optimally, they are predicted to incorporate size-based differences in reproductive outcomes into their strategies over evolutionary time. Body size and behavioral changes may also be related even if the two phenotypes are not linked in an evolutionary sense. For example, if males adjust their behavior in contests based on their own experience of winning, large males may be more likely to show increased levels of aggression compared to small males because they are more likely to win fights and learn that they have a competitive advantage (Kasumovic et al.

2010). It is difficult to evaluate the link between size and behavior empirically in *N. clavipes* because, as the model demonstrated, there may be only subtle differences in behavioral strategies among male sizes. Furthermore, male body size shows a continuous distribution. Are behavioral strategies distributed in a continuous manner? The dynamic state model is one approach to attempt to capture the true complexity of male strategies, and to provide a connection between size and behavior.

### **Evolutionary Consequences of Environmental Variation**

As discussed above, environmental variation corresponds to the degree of variation in a phenotypic trait under selection (Vanvalen 1965). This is because, over time, low environmental variation will increase the efficiency of selection, which decreases trait variation (Falconer & Mackay 1996). As a result, if environmental conditions change, historical processes, which have decreased trait variance, will limit the degree to which a species is capable of responding to their new environment (Lovette et al. 2002; Arbogast et al. 2006; Losos 2010; Mahler et al. 2010).

Because the evolutionary history of a species affects its capacity to respond to a changing environment (Travisano et al. 1995), lineages vary in their potential evolvability, or ability to exploit a diverse or novel environment (Lovette et al. 2002; Arbogast et al. 2006; Losos 2010; Mahler et al. 2010). The concept of evolvability has theoretical implications but also practical implications for conservation initiatives. For example, why are some species able to exploit the (novel) urban environment while others fail? Are certain species more vulnerable to habitat fragmentation than others? What evolutionary lessons can be learned from “pest” species, or successful invasive species? Interspecific differences in adaptability could be the result of evolutionary history, and the ability to predict a species’ resilience to change may be important given

the unprecedented and increasing levels of anthropogenic environmental change. These questions are particularly relevant for the field of behavior and the study of behavioral plasticity because behavior is often the first trait to respond to changed environmental conditions, and as a result, behavioral plasticity can buffer selection on other morphological traits by providing a mechanism for individuals to adjust to changes in conditions (Wcislo 1989; West-Eberhard 2003). To understand a species' evolvability in real time, it may be useful to attempt to examine plasticity (i.e. either morphological or behavioral plasticity within a species) and its mechanisms across multiple time scales. Short term plasticity, a product of molecular and physiological mechanisms functioning within a single individual, could have implications for large scale plasticity, linked to the capacity for ecological and evolutionary change in a species over time.

#### **Assumptions and Biases: Models Versus Data**

Even though the model allows for a high degree of male behavioral plasticity, male strategies are remarkably consistent. In almost all contexts, males remained on a female's web until successfully mating. Male strategies, as revealed in the model, can be considered fairly conservative, especially because there is some evidence in this species that males are choosy and forego reproductive opportunities in certain contexts (Chapter 4). Disparities between the model strategies and behavioral data reflect the model parameterization that I used, for example, I estimated the benefit of mating with low quality females to be too high for males to overcome the costs of passing up a mating opportunity in the model, or I set the mortality risk of mate search too high to reveal male choosiness. Such disparities are to be expected however, because I estimated parameters from empirical data, which were collected with certain assumptions and biases.

Although the model has parameter biases, inferences from the model also reveal interesting potential biases in empirical assessment of male tactics. In the model, despite strong similarities among male strategies, these strategies still resulted in differences in male reproductive outcomes. For example, the model showed some differences in rates of multiple mating for males of different sizes, and large and small males mated with different types of females at different rates (Table C-3). According to the model, these outcomes do not reflect major differences in decision rules and strategies among male sizes (e.g. certain sized males choose against certain kinds of females). However, a male who employs an optimal strategy is still subject to probabilistic events that affect the outcome and pay-off associated with the decision. Size based differences in outcome probabilities in this case yielded very different reproductive outcomes and tactics even though males were using similar decision rules.

In most systems, male mating tactics are defined from empirical and observational data (reviewed by Taborsky et al. 2008), and observed tactics are used to infer behavioral decision rules. For example, if I had observed large and small males mating with different types of females in the wild, I may have assumed these males were following different decision rules. If I also observed large males mating at higher rates with virgin females, I might have assumed large males have higher reproductive success compared to small males, and concluded that small males are adopting a “best of bad job” strategy. Model data shows that reproductive outcomes, which are often measured empirically as tactics, may not relate directly to differences in behavioral decision rules (Brockmann 2002).

In some ways a modeling approach may more accurately capture the fitness differences associated with alternative tactics. Brockmann (2008) reviews several reasons why it can be difficult to estimate and compare lifetime reproductive success across reproductive tactics accurately using empirical data. For example, the average success of a given tactic should include all animals that employ the tactic but it is easy to exclude animals that die without mating at all, because they were not observed (S.M. Shuster, as cited by Brockmann 2008). Similarly it can be difficult to assess accurately the outcome of certain lifetime fitness trade-offs associated with a tactic, for example trade-offs between lifespan and reproductive success for a given mating event, especially while observing individuals within a short time frame (Banks & Thompson 1985). Both of these problems could be resolved by monitoring all individuals performing a given tactic (or at least an accurate subset of individuals that encompasses the range of variation) across their entire lifetimes. These data can be difficult to obtain even though they may change the interpretation of the costs and benefits associated with different phenotypes or strategies. For instance, many studies have put forth arguments pertaining to the evolution of male body size and the maintenance of size variation in Nephilid spiders (e.g. Elgar & Fahey 1996; Schneider et al. 2000; Moya-Larano et al. 2009). However, the broad and complex array of factors that could affect reproductive outcomes in these species, and the inability to observe individuals over their lifetimes, have made it difficult to interpret the importance of body size in terms of male reproductive success and male strategies. However, a dynamic state modeling approach allows me to incorporate many of the standing hypotheses that attempt to explain male size, e.g. male mate search mortality risk.

My model of *N. clavipes* mating behavior incorporates the parameter male mate search mortality risk, which has been hypothesized to be an important factor that affects male reproductive outcome and presumably male strategies and body size evolution (Vollrath & Parker 1992; Higgins & Rankin 2001; Andrade 2003; Foellmer & Fairbairn 2005b; Fromhage et al. 2007; but see Fromhage et al. 2008; De Mas et al. 2009). Mate search mortality risk is difficult to account for in the wild, because it is unclear whether males who disappear are dead or simply unobservable. In addition, it is difficult to say whether size based differences in mortality estimated from field survey data (Vollrath & Parker 1992) result from size-based tactics that ultimately have equal pay-offs, or if they truly reflect a cost associated with a particular size. Finally, if mate search mortality contributes substantial variation in reproductive success, this factor in the model more accurately captures the variance in reproductive success associated with different male sizes. When mate search mortality risk and the resulting variation in reproductive success is accounted for, it suggests that there are only weak size-based differences in reproductive success.

In addition to describing male strategies more accurately and associated reproductive success, factoring in mortality risk also provides insight about the evolution of permanent sperm depletion in this species. Males have a finite volume of sperm (Chapter 2). However, if most males fail to use all of their sperm in their lifetime, there may not be strong selection on males to regenerate sperm. The percentage of males that die without ever reaching a female's web are important to factor into this calculation, because these males die with all of their sperm remaining. The model allows me to account for this group of males, which may be ignored in empirical studies

because males are extremely cryptic when they are not on a web. Model data suggests that most males in this species die with at least some sperm remaining, due in part to mortality during mate search (Chapter 2). Thus there may be very weak selection on males to regenerate sperm even if most males that mate do so with multiple partners (Chapter 5). A combination of modeling and empirical approaches may lead to the most accurate inferences about behavioral decision rules, and the complex interplay among factors that influence male reproductive success in this species.

### **Novel Insights for Behavioral Ecology**

#### **Sperm Depletion**

Sperm depletion is an unusual phenomenon, and its evolutionary causes and consequences in spiders are unclear (Chapter 2). *Nephila clavipes* is an unusual sperm-depleting species because unlike other nephilids males are not cannibalized during copulation, and they do not break off their pedipalps, and thus they presumably can re-mate if they have sperm remaining (Chapter 2, Discussion). It appears that sperm depletion is the ancestral state in the *Nephila*, and presumably evolved following the evolution of genital mutilation and male sacrifice behavior in this group (Chapter 2). One hypothesis to explain why male *N. clavipes* fail to regenerate sperm even though they retain functional pedipalps is that, unlike pedipalp breakage and cannibalism, which are context-dependent traits (see Chapter 2 for discussion), testes function may not be as evolutionarily labile, and once function is lost, it is hard to re-gain. An alternative hypothesis is that male *N. clavipes* are not under strong selection to regenerate sperm because most males mate only once or not at all due to ecological constraints of locating mates (e.g. mate search mortality risk). Even with limited sperm, males can mate multiply because they can prudently allocate sperm to females

depending on the quality of the female (Christenson & Cohn 1988; Chapter 4). Males may gain significantly from mating multiply in spite of their low potential mating rate (Chapter 5). *Nephila clavipes* males retain permanent sperm depletion even with the ability to mate multiply, and this could either be a result of evolutionary constraints, tradeoffs, or weak selection on sperm regeneration.

Sperm depletion may have implications for body size evolution in *N. clavipes* because it limits the potential reproductive rate of highly competitive males. In the model simulations, large males become sperm-limited early in their lifetime because they have a higher success rate of copulating with a female once a web is found. As a result, large males may run out of sperm, potentially before they encounter gravid females, which are rare but high pay-off mates. Because large male mating rate is constrained, small males are able to match their mating rate and their lifetime reproductive success. In addition, because they often fail to copulate with a female before she abandons her web, small males retain sperm for a longer portion of their lifetime compared to large males and therefore have a greater chance of encountering and copulating with rarer gravid females. Male sperm depletion, which imposes a constraint on large-male mating rate, may be one characteristic that results in equal reproductive success among different sized males.

The suite of mate-limiting traits found in spiders, including sperm depletion, genital mutilation, and sexual cannibalism, make spiders an interesting group to assess male costs of reproduction across multiple levels. One logical next step is to evaluate the physiological costs of sperm production in spiders. Although these costs are often assumed (Dewsbury 1982), or demonstrated to impact males behaviorally, few studies

have bridged the gap between the physiological costs of maintaining testes function and sperm production and the implications for male sperm use and mating strategies.

Comparing related species in which males cease sperm production altogether with ones that produce sperm throughout adulthood is a unique opportunity to directly address the energetic demands associated with sperm production.

### **Partner Mortality Risk**

I address how males may respond to female mortality risk during mate choice, a mate choice criterion that has only been considered in one other species (Dunn et al. 2001). Male mate choice criteria typically include female size or weight (indicators of potential reproductive output), and female age or mated status (indicators of sperm competition risk; Bonduriansky 2001). My findings in *Nephila clavipes* provide support for the hypothesis that males select females on the basis of mortality risk, in part because males appear to ignore female body size during mate choice, which is an indicator of fecundity in this species (Chapters 3, 4). Males instead select females on the basis of abdomen size, which can indicate condition and therefore fecundity (Higgins 2000), but is very strongly correlated with proximity to oviposition (Chapter 4). Model data (Chapter 5) and empirical data (Chapter 4) reveal two ways that males may account for female mortality risk in their mating strategies. Males can mate multiply and distribute mortality risk across multiple female mates (Chapter 5). Males can also be choosy, and allocate more sperm to low-risk females (Chapter 4). Both of these tactics can increase male fitness and decrease fitness variance in comparison to mating monogynously, and in *N. clavipes*, these two strategies are not mutually exclusive.

If mortality risk accounts for more variation in male reproductive success than factors like sperm competition and female size, males should choose low risk females at

the expense of other indicators of potential reproductive success. In *N. clavipes*, two factors suggest that males would benefit from selecting females on the basis of mortality risk. First, similar to the Dunn et al. (2001), in *N. clavipes*, female survival is variable and there is a relatively long period of time between mating and oviposition. These factors contribute to variation in female reproductive output, and are important regardless of female size. Second, mortality risk may be a better predictor of female fecundity than other factors like female size or sperm competition risk. Female body size, a typical indicator of fecundity, explains only a portion of the variation in offspring number among females (Higgins 2000; Chapters 3, 4). Furthermore, these data do not include females who die without reproducing at all. In addition, because females mate polyandrously, there is high variation in paternity share as a function of mate order (Christenson & Cohn 1988; Chapter 4). For instance a male that mates with a virgin fertilizes 0-100% of her offspring in the first clutch if the female re-mates, regardless of the number of sperm transferred. The range of reproductive success for the second male is similarly variable (range 0-100%), and on average the first and second male split paternity almost equally in the first clutch (Chapter 4). Therefore males may not benefit from selecting against previously mated females without also accounting for female mortality risk, which could have a more predictable impact on male offspring number compared to sperm competition risk.

Mortality risk could influence male reproductive outcome in any species where females store sperm. However, the length of time between maturation and oviposition relative to female lifespan may be the key feature that predicts whether males respond to this risk factor. In *N. clavipes*, at least one quarter of a female's adult lifespan is

devoted to maturing and laying her first clutch of eggs. However in spiders generally, there is a long period of time between maturation and oviposition. For instance, in the bowl and doily spider, time between maturation and first oviposition can range from about 7% to 30% of an adult female's lifespan (Austad 1982). In comparison, in *Drosophila melanogaster*, where adult females can live for roughly 45 days (Sun & Tower 1999), first oviposition can occur within 24-48 hrs., only 2-4% of an adult female's lifespan, a relatively short period of time. Thus female mortality risk may be particularly important for male spiders to consider because females in this group have a characteristically long time interval between maturation and oviposition. Although other species may face a similar reproductive delay (e.g. the seaweed fly; Dunn et al. 2001), such long periods before oviposition may not be common in other sperm-storing species, e.g. insects.

### **Final Remarks**

Here I have investigated how environmental heterogeneity may contribute to male body size variation in the spider *Nephila clavipes*. The high degree of variation in local density on female webs creates a variable reproductive environment for males. One phenotypic characteristic, behavioral plasticity, may be critical in allowing males to achieve similar levels of reproductive success regardless of size in this species. Future work should study the mechanistic coupling between body size and behavior, the factors, for example experience, that guide male behavior as it changes across a male's lifetime, the link between short-term behavioral plasticity and long-term evolutionary change in morphology, and the ways in which evolutionary history constrain a species' ability to adapt to a novel environment, particularly in terms of behavior.

APPENDIX A  
SUPPLEMENTARY MATERIAL FOR CHAPTER 2

Table A-1. Real age (number of days after penultimate or final molt when sacrificed) and standardized age (age relative to the youngest sub-adult male) for all males in the study

Subadults																
Age	5	7	15	23	32	42	43	46	51	59	61	62	66	69	70	72
Standardized age	5	7	15	23	32	42	43	46	51	59	61	62	66	69	70	72

Adults																	
Age	0,04	1	2	3	4	5	6	7	8	9	12	15	16	17	19	28	34
Standardized age	72.04	73	74	75	76	77	78	79	80	81	84	87	88	89	90	99	104

## APPENDIX B SUPPLEMENTARY MATERIAL FOR CHAPTER 3

In order to determine paternity, I developed three polymorphic microsatellite markers (Table B-1) using protocols adapted from Fleischer & Lowe (1995) and Kandpal et al. (1994). Genotyping was done on a 3730 Automated Sequencer (Applied Biosystems) using fluorescent-labeled primers. GeneMarker (Version 1.75) was used to determine allele sizes, and each score was verified visually. I screened for polymorphism, estimated allele frequencies, and tested for neutrality and linkage disequilibrium using 190 parents from the mating experiment. Each locus was tested for neutrality using the Hardy-Weinberg Exact Test and for linkage disequilibrium using Fisher's Method in the program GENEPOP version 3.2 (Raymond & Rousset 1995).

The three microsatellite loci used in paternity analysis (NC\_F, NC\_R and NC\_BB) did not differ significantly from Hardy-Weinberg equilibrium (HW Exact Test,  $P=1.0$ ,  $P=0.61$ ,  $P=0.12$  for NC\_F, NC\_R, and NC\_BB, respectively), and were not in linkage disequilibrium (Fisher's Method,  $X^2=0.99$ ,  $P=0.61$ ,  $X^2=1.4$ ,  $P=0.51$ ,  $X^2=0.21$ ,  $p=0.90$ ). With the female parent known, the three polymorphic loci had a combined exclusion probability of 0.72.

Mothers, candidate fathers, and 24 spiderlings for each experimental trial were genotyped at the three loci using fluorescent-labeled primers. DNA was extracted from adult and spiderling tissue using the Gentra Puregene Tissue Kit (Qiagen). I adjusted the amount of tissue used in DNA extraction with sex and age because of variation in extraction efficiencies. For adult females, one of the third pair of legs was used, for adult males 2-3 full legs, and for all spiderlings the entire body.

Each microsatellite locus was amplified using polymerase chain reaction (PCR), and the products were pooled together in order to genotype all three loci simultaneously. Each 10 $\mu$ L PCR reaction mixture contained 4.96 $\mu$ L of H<sub>2</sub>O, 10X Taq polymerase buffer at 1X concentration, 1mM MgCl<sub>2</sub>, 200mM of each of four deoxynucleotide-triphosphates (dNTPs), 0.3mM of each forward and reverse primer, 0.2 U of Taq DNA polymerase (New England Biolabs), 2  $\mu$ L of template DNA (approximately 10 ng). PCR conditions were similar across loci, although annealing temperatures varied (Table B-1): 94° for 5 min.; 94° for 30 s; variable (see Table B-1) 60 s; 72° for 60 s; repeat steps 2-4 for 39 cycles; 72° for 10 min. Each genotype was scored using GeneMarker and verified visually. The 24 offspring genotypes were scored blind to the genotypes of the mother and candidate fathers.

Once all genotypes were scored, the number of fathers responsible for each clutch was determined using the maximum likelihood approach in the program GERUD 2.0 (Jones 2005). The likelihood calculation is based on Mendelian segregation probabilities and population-level allele frequencies (Jones 2005). Given the progeny genotype array, GERUD 2.0 subtracts the maternal genotype and calculates the most likely genotype(s) for the father(s) of the progeny. If the likelihood calculation determines there is one paternal genotype for the progeny array, GERUD 2.0 lists this genotype. In this case, the genotype listed is the sole father of the progeny. In cases of multiple paternity, more than one paternal genotype is required to explain the progeny array, and GERUD 2.0 lists the paternal genotypes as well as the likely number of offspring attributed to each father. In some cases of multiple paternity, more than one

combination of paternal genotypes is consistent with the progeny array. If this is the case, the program calculates a likelihood score for each paternal genotype combination.

I compared the parental genotype outputs from GERUD 2.0 to the genotypes of the candidate fathers in each experimental group in order to determine the identity of the father(s). If GERUD 2.0 listed more than one paternal genotype solution for a progeny array, I chose paternal genotype solution with the highest likelihood score that was also consistent with genotypes for the candidate fathers within the experimental group.

Table B-1. Microsatellite loci used in paternity analysis

Locus	Motif	Length (bp)	N	Temp (°C)	Primer Sequence
NC_F	CA	247	2	62.1	5'ACCCATCTTGGGACCTTTTC 3' 5'AGAAAAAGCCAAGACCCAGA 3'
NC_R	AT	162	3	54.0	5'AAAAATCTGTGATACCCACTGC 3' 5'TGTGTTGCGTTGTCCAAAAT 3'
NC_BB	CT	289	18	55.4	5'GGAGAAATTACAGTTTAGATGCTTGA 3' 5'TCGTGTTAAGGAGCTTGGATTT 3'

N is the number of alleles at each locus, and Temp refers to the annealing temperature for each locus.

APPENDIX C  
SUPPLEMENTARY MATERIAL FOR CHAPTER 5

Table C-1. Parameter values that change with female type

		Female Type		
		Virgin	Mated	Gravid
$t_{J^*}$	time required to mate	2	0	0
$\alpha_J$	proportion of sperm used at mating	1	0.4	0.4
$f_J$	reproductive success	0.8	0.2	0.2
$y_J$	proportion of $f_J$ gained by mating	0.8	0.9	0.9
$k_J$	probability of successfully guarding	S	0.5	0.25
		M	0.61	0.37
		L	0.87	0.76
$z_J$	proportion of $f_J$ gained by guarding per t	0.01	0.005	0.005

\*Value changes across model manipulations. Small, medium, and large denote male body sizes.

Table C-2. Parameter value that change with season and female type

		Early			Middle			Late		
		Virgin	Mated	Gravid	Virgin	Mated	Gravid	Virgin	Mated	Gravid
$\beta$										
Male searching mortality risk			0.21			0.23			0.26	
$r_J$										
female encounter probability		0.25	0.35	0	0.2	0.4	0.2	0.2	0.4	0.2
$m_J$	S	0.17	0.25	0.17	0.17	0.25	0.17	0.17	0.25	0.17
mating probability	M	0.25	0.5	0.17	0.25	0.5	0.17	0.25	0.5	0.17
	L	0.5	0.75	0.17	0.5	0.75	0.17	0.5	0.75	0.17
$d_J$										
female survival probability		0.828	0.885	0.988	0.864	0.909	0.988	0	0.3	0.988
$c_J$	S	1	1	1	1	1	1	1	1	1
cost of male-male competition	M	3	1	3	3	1	3	3	1	3
(E)	L	1	1	1	1	1	1	1	1	1

Early, middle, and late denote season periods, virgin, mated, and gravid denote female types, and S,M,L refer to three male sizes, small, medium, and large.

Table C-3. Of males that mated, the proportion of males within each size class that mated with each of three types of females.

Size	Virgin	Mated	Gravid
<i>Basic Model</i>			
Small	0.07	0.87*	0.32
Medium	0.11	0.94*	0.30
Large	0.22	0.77*	0.15
<i>Restricted Sperm Model</i>			
Small	0.05*	0.89*	0.06*
Large	0.16*	0.61*	0.23*
<i>Unrestricted Sperm Model</i>			
Small	0.16*	0.84	0.63
Large	0.43*	0.71	0.51

\* Significant differences among male size classes ( $P < 0.05$ ).

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## BIOGRAPHICAL SKETCH

Clare was born and raised in Morehead City, a small town on the coast of North Carolina. She attended a local Catholic School before transitioning to public school for junior high and high school. Clare spent a lot of time assisting her father, who is a marine biologist, and later his graduate students, in fieldwork. This mostly involved finding and collecting local marine animals including crabs, fish, and mollusks, and leading student tours of the local estuary at night. Clare entered Cornell University in the fall of 2002 as a biology major.

As her first research experience during college, Clare spent a summer working on butterflies in Owen McMillan's ecological genetics lab in Puerto Rico. During this time she was mentored by Durrell Kappan, a post-doctoral fellow, who encouraged her to consider research science as a career goal. Unsure of her field of interest, Clare returned to school that fall where she took two formative classes, Animal Behavior and Spider Biology. The following semester, Clare began working on social huntsman spiders with Linda Rayor at Cornell. That summer, she switched to working on honeybee swarming behavior in Tom Seeley's lab as part of an HHMI summer program. Clare continued her research on bees through her senior year. Following these experiences, Clare decided to pursue a degree in zoology, applying to work in Jane Brockmann's lab on questions related to alternative reproductive tactics. In her future work, she plans to explore the mechanisms of behavioral plasticity and experienced-based changes in behavior in honeybees in Gene Robinson's lab at the University of Illinois.