Original Article

Mortality risk affects mating decisions in the spider *Nephila clavipes*

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Risk assessment is a fundamental component of any decision-making process, including mate choice. For males, one element of risk that is rarely considered is that a female mate may not survive to offspring independence, resulting in total reproductive failure. This element of risk could be particularly important in short-lived species where all individuals, regardless of condition, attempt to reproduce, and developing mature eggs is a time and energetically expensive process during which females are at risk of predation, starvation, and disease. I evaluate whether males use female survival probability as a mate choice criterion in the golden orb-web spider *Nephila clavipes* by assessing how male reproductive investment changes depending on female proximity to oviposition. Using a field experiment and a controlled mating experiment, I show that males mate at higher frequencies, transfer more sperm, and father more offspring in the first clutch when mated with nonvirgin females close to oviposition compared with younger nonvirgin females. Furthermore, females close to oviposition have a lower probability of mortality prior to egg deposition compared with virgin females and young nonvirgin adult females. These findings support the hypothesis that males invest more reproductive effort as a female approaches oviposition, possibly because these females are a low-risk mating investment. *Key words:* male mate choice, partner mortality, risk, sperm allocation, sperm competition. *[Behav Ecol 22:350–357 (2011)]*

For animals, decision making involves risk assessment (Seger and Brockmann 1987; Austad 1989; Creel 1993; McNamara et al. 2006; Beaumont et al. 2009; Wiklund and 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 and 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 1) when males, due to limited resources, mate with only 1 or a few females in their lifetime and 2) 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 nonbreeding 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, for example, 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 and Brooks 1998; Katvala and 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 and Brooks 1998; Katvala and 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 nonvirgin females, even though there is first-male sperm precedence (Christenson and Cohn 1988). Furthermore, prior studies have suggested that male attraction to nonvirgin females increases as females approach oviposition, but that attraction is not related to female size, which predicts clutch size

(e.g., Vincent and Lailvaux 2006; Rittschof 2010). Females that are close to oviposition may have a better chance of surviving to produce a clutch of offspring compared with 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 *N. clavipes* mature sometime between July and late September and live approximately 3–4 months as adults (Christenson and 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 2011). Males adjust the amount of sperm they transfer during a copulation event, and so they may be able to mate multiply (Christenson and 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 and Cohn 1988). However, males also mate with nonvirgin females, and when they do so, they use only a portion of their sperm stores (Christenson and 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 and Cohn 1988), the number of males found on nonvirgin adult females' webs increases with increased female abdomen size (Vincent and Lailvaux 2006; Rittschof 2010). Abdomen size is considered a measure of gravidity or time to oviposition in spiders (e.g., Ortlepp and 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 and 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, that is, 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.

MATERIALS AND 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 and 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) 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. Although 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 3 locations, the Ordway-Swisher Biological Station in Melrose, Florida (Putnam County, lat 29°42′32.4″, long −82°2′60.0″), Payne's Prairie State Preserve (Alachua County, lat 29°36′36.0″, long −82°17′60.0″), and San Felasco Hammock Preserve State Park (Alachua County, lat 29°43′11.9994″, long −82°27′0″). The mating experiment was conducted at the University of Florida Bee Biology Unit (Alachua County, lat 29°37′14.5158″, long −82°21″18.99″), which is a fenced and protected research area.

In order to ensure virginity, females were collected during their subadult instar. Female maturity was determined by inspecting the epigynum, which is black with 2 distinct openings in mature females, and smooth and maroon colored in subadult females (Higgins 2000). Females were housed outside in wooden cages (90 × 90 × 15 cm) and fed 2 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 and 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 nonvirgin females close to oviposition copulate more frequently, transfer more sperm, and father more offspring than males that mate with younger nonvirgin females. To do this, I designed an experiment with 2 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 1 week of female collection. Females were checked daily from 0700 to 0900 h. 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 3 full days and was removed on the morning of day 4. Because females typically do not eat immediately after their maturation molt (Christenson and 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 h after P1 was removed). P2 was left for 3 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 3 full days to mate with the female. Because male body size affects sperm number and could influence male sperm competition (Christenson and 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 fourth 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 epignal slit; Vincent and Lailvaux 2006; Rittschof and 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, NY). 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 (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 epignal 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 unhatched 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 2011). 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 and 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 5 min 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 50 µl of tap water and pulverized with a tissue grinder for 3 min. The samples were then vortexed for 1 min and centrifuged for 1 min. 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, Waltham, MA), 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 milliliter 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 prescreened male and female genotypes in order to match the P1 and P2 males and the female for full exclusion at 1 locus. To do this, I removed 1 leg per individual for DNA extraction and genotype analysis prior to the mating experiment. I chose one fourth leg from each male and one first 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 3 polymorphic microsatellite loci for each individual (Rittschof 2010). For each mating trial, I chose males whose genotypes allowed for full paternity exclusion at 1 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 2 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.

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 (Figure 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 (Figure 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 (standard error [SE] = 0.75 days) and 7.7 days before first oviposition (SE = 0.64 days).
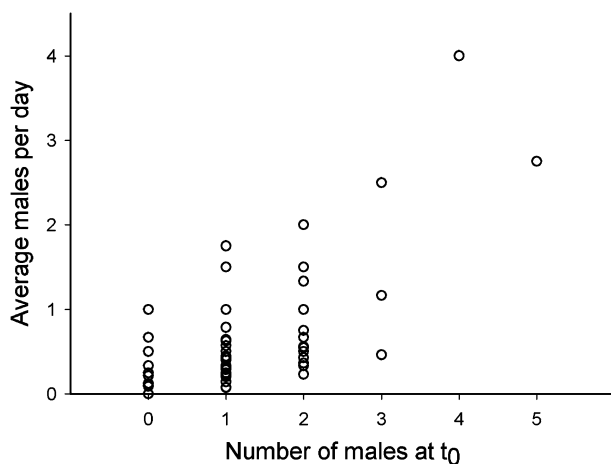

Figure 2
Female body condition index increases as the female approaches oviposition ($R^2 = 0.73$, $P < 0.0001$). Each data point represents a single female on a given day of her reproductive cycle ($N = 35$ females; $N = 938$ total female days).

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 2 males. The percentage of trials in which the P1 male was observed mating did not differ between the 2 treatment groups (Figure 3; Early = 82%, Late = 74%; $X^2 = 0.39$, $P = 0.53$). However, a significantly higher frequency of P2 males mated in the late-treatment group compared with the early-treatment group (Figure 3; Early = 34%, Late = 84%; $X^2 = 9.6$, $P < 0.002$). Across both treatment groups where sperm counts were obtained for both the P1 and P2 males ($N_{Early} = 20$, $N_{Late} = 18$), 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 with 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 ($N_{Early} = 12$, $N_{Late} = 11$). In the first

Figure 1
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 1 female web ($N = 71$) on which males were removed daily.
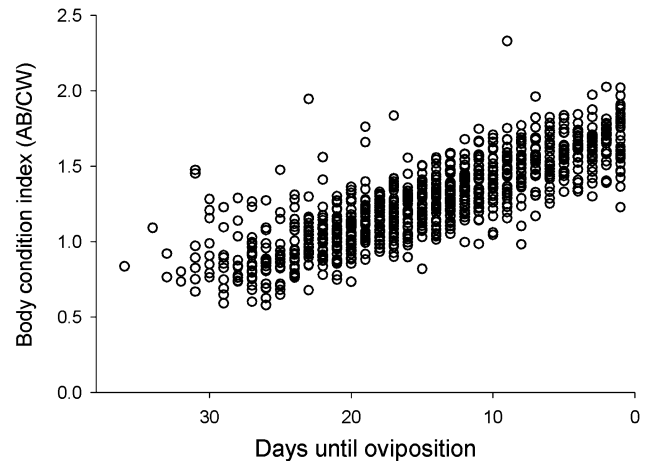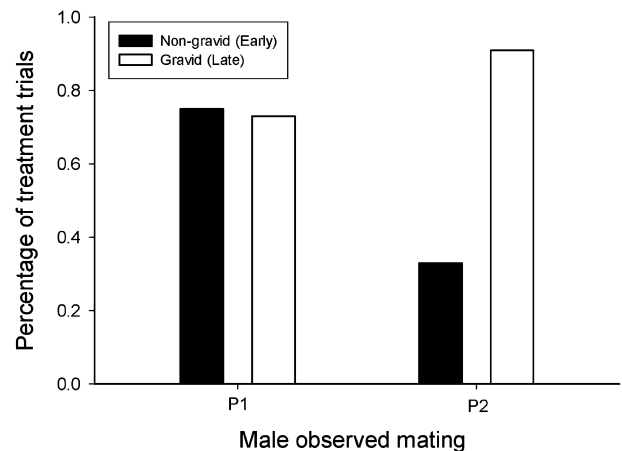

Figure 3
The proportion of P1 and P2 males observed mating for the nongravid (early) and gravid (late) treatment groups. Mating frequency was similar for P1 males across treatments ($P = 0.53$), but P2 males mated with significantly higher frequency in the gravid (late) treatment group ($P < 0.002$).

clutch, a higher percentage of P2 males fathered offspring in the late treatment compared with the early treatment (Figure 4; Early = 17%, Late = 73%; X^2 = 7.3, $P < 0.007$). When P2 males fathered offspring (N_{Early} = 2, N_{Late} = 8), paternity ranged from 44% to 100% (Mean = 79%; SE = 7%). There were 19 females that laid 2 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 and 8 females from the late treatment), there was mixed paternity in clutch 1. Among these females, P2 paternity ranged from 50% to 100% (Mean = 78%; SE = 6%) in clutch 1. However, P2 paternity in clutch 2 was significantly different than clutch 1 (Figure 5; two-tailed paired T-test, $t = -4.3$, $P < 0.003$). In all but 1 case, P2 paternity share was lower in clutch 2 than in clutch 1, and the mean difference was 39% (SE = 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 nonvirgin 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, SE = 28) and was positively correlated with female body size (cephalothorax width; $R^2 = 0.49$, $P < 0.025$). Clutch 2 trended toward having fewer offspring compared with clutch 1 (Mean$_{clutch\ 1}$ = 711, SE = 26.5; Mean$_{clutch\ 2}$ = 634, SE = 48; two-tailed paired T-test, $t = -1.86$; $P = 0.09$). The mean dif-
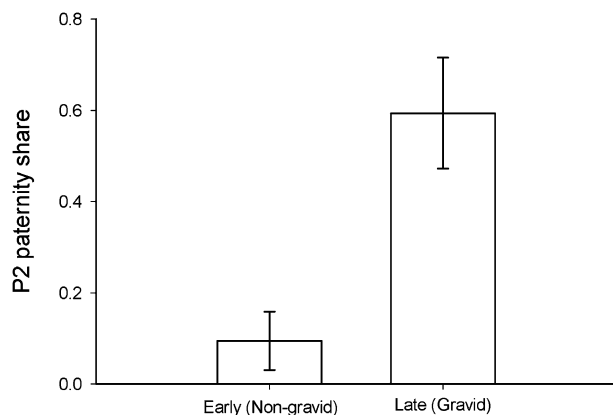
ference was 76 offspring (SE = 41 offspring), which corresponds to a mean difference of 11% (SE = 6%).

DISCUSSION

The age and reproductive state of nonvirgin females significantly affects male mating behavior. Field data show that males are consistently attracted to some mature females over others (Figure 1) and that high levels of attraction are correlated with female abdomen size, which is an indicator of time until oviposition (Figure 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 nonvirgin females close to oviposition, males mated at higher frequencies (Figure 3), transferred more sperm, and fathered more offspring in the first clutch (Figure 4) compared with males paired with younger nonvirgin females. These findings support the hypothesis that males invest more reproductive effort in gravid females who are close to oviposition compared with younger nonvirgin 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 nonvirgin 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.

Response to female mortality risk during mate choice may occur in other species of *Nephila*. Similar to my findings for *N. clavipes*, in the congener *N. edulis*, authors manipulated time between a female's first and second copulations and found that second males copulated longer with nonvirgin females who were close to oviposition compared with younger nonvirgin females. However, unlike the current study, there was no paternity gain associated with mating with older nonvirgin females (Jones and Elgar 2008). One possible
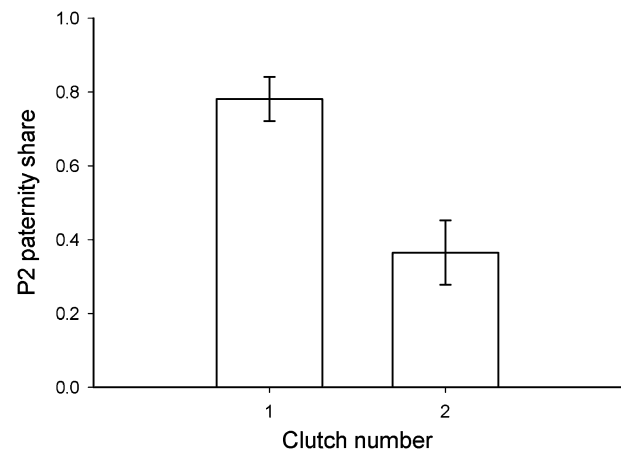

Figure 4
P2 males fathered a greater proportion of offspring in the first clutch in the late treatment versus early-treatment group.

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

explanation for increased mating effort in the absence of increased paternity share could be that males choose to invest more reproductive effort with older females simply because they are close to oviposition and a low mortality risk. Future studies should investigate this possibility in other spiders, a group in which sperm storage, as well as delayed oviposition, are common (e.g., Austad 1982).

Partner survivorship could be an important mate choice criterion in species where there is delayed reproductive payoff after copulation, that is, 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., Vanheezik 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., N. clavipes), males could incorporate female mortality risk into their choice criteria by choosing females on the basis of probable survivorship (Dunn et al. 2001).

Other studies in insects and fish demonstrate male choice for gravid females who are close to egg deposition (Bonduriansky and Brooks 1998; Katvala and 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; Michalik and Rittschof 2011), the sperm remaining in the pedipalps after copulation provides an estimate of sperm usage by males (Christenson and Cohn 1988). P2 males in the late mating treatment used more sperm and were more likely to father offspring (Figure 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 and 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 (Figure 5). This finding is similar to the predictions of a fair raffle (e.g., Ball and 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 postcopulatory 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 and 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 and Elgar 2001). In spiders, generally, it is common for postcopulatory processes, influenced by both males and females, to affect sperm storage and usage (Parker 1990; Bukowski and Christenson 1997; Eberhard 2004; Huber 2005; Aisenberg 2009; Schneider and Lesmono 2009; Welke and Schneider 2009; Burger 2010; Peretti and Eberhard 2010). In some cases, these processes are associated with copulation interval and the female's mated status (Schneider and Elgar 2001; but see Jones and 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 nonvirgin 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 (Figure 3). Males may avoid mating with younger nonvirgin females because these females have only slightly lower mortality risk compared with 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 off set by other potential advantages. First, if the female does not remate, 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 payoff for mating with a virgin female, even if she remates, may be higher than mating with a nonvirgin female as long as the female lays more than 1 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 3 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 precopulatory challenges that could affect whether they successfully mate with a virgin or nonvirgin female. In other spiders, male mortality risk during mate search appears to constrain male mating rates and presumably male choosiness (Andrade 2003; Andrade and 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 remating 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 and Fahey 1996; Schneider and Elgar 2005; Rittschof 2010; Rittschof and Ruggles 2010). Variation in mating success may be highest on gravid female webs where male group sizes are largest (Vincent and Lailvaux 2006; Rittschof 2010). The probability of successfully mating, the ability to remate, 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 nonvirgin females (evolutionarily stable

strategy; Brockmann and Taborsky 2008). Other work in *Nephila* suggests that these strategies could depend on male body size (Schneider et al. 2000; Schneider and Elgar 2001, 2005; Elgar and Jones 2008; Jones and 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 nonvirgin females are less receptive compared with virgins, male mating opportunity with nonvirgin females is limited to times when the female is eating (Christenson et al. 1985; Christenson and Cohn 1988). Males presumably wait until the female is eating in order to avoid cannibalism (e.g., Fromhage and 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 nonvirgin mates, males copulate with non-virgin females at different rates depending on female age, which demonstrates some role for male control during mating events.

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