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# The adaptive advantage of prolonged mating: a test of alternative hypotheses

**CHRISTOPHER D. LINN*, YAMILE MOLINA*†, JAMIE DIFATTA* & TERRY E. CHRISTENSON***
*Department of Psychology, Tulane University, U.S.A.
†Department of Psychology, University of Washington, U.S.A.

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When a female can mate with more than one male, males are thought to reduce intrasexual competition by prolonging mating, that is, mating longer than necessary for sperm release. Two principal hypotheses have been proposed to explain the advantage of prolonged mating. The extended mate guarding hypothesis suggests that with longer mating, the female is less likely to be inseminated by another male. The ejaculate transfer hypothesis suggests that with longer mating, more of the male's sperm are stored. To our knowledge, this is the first study to test these alternatives simultaneously by manipulating duration of mating and examining effects on sperm release, storage and female polyandry. We found support for the mate guarding hypothesis in the golden orb-weaving spider *Nephila clavipes*; the longer the mating with the first male, the lower the likelihood of the female copulating with another male. This was not due to influence on female receptivity but on the ability of subsequent males to couple. Inconsistent with the ejaculate transfer hypothesis was the observation that duration of mating with the first male did not linearly correlate with release or storage of its sperm. We suggest that prolonged mating does not result in sexual conflict in this species.

*Keywords*: mate guarding; mating; *Nephila clavipes*; sperm competition; spider

Females of many taxa mate with more than one male (Watson 1991; Birkhead & Møller 1998; Arnqvist & Nilsson 2000; Birkhead 2000). Males use various means to reduce resulting competition between their sperm and those of other males (Parker 1970), including mating longer than necessary for sperm release (Bukowski & Christenson 1997; Simmons 2001; Snow & Andrade 2004). Prolonged mating may serve as extended mate guarding if it reduces chances of the female gaining sperm from a subsequent male, through, for example, reduced female receptivity or likelihood of copulating with another male (Parker 1970; Simmons 2001). If there were a more or less linear relationship between mating duration and sperm transfer and storage, then prolonged mating would serve to facilitate ejaculate transfer (Laird et al. 2004). Teasing apart these two possible functions is difficult, requiring knowledge of mating natural history and sperm release and storage (Simmons 2001).

We tested the extended mate guarding and ejaculate transfer hypotheses in an orb-weaving spider. Spiders provide an interesting model for studying sexual selection (Eberhard 2004a; Huber 2005): females are often polyandrous; sperm competition is intense (Austad 1984; Elgar 1998); and male copulatory organs, pedipalps, and female storage organs, spermathecae, are discrete and removable, so sperm contents can be quantified (Bukowski & Christenson 1997). There is evidence in some species (Jackson 1980; Elgar 1998) that longer matings reduce the likelihood that the female shall copulate with another male. In relation to the ejaculate transfer hypothesis, males of some species might release sperm quickly after intromission, as the female can pose a predatory risk (Elgar 1995; Snow & Andrade 2004) or because other males can threaten takeover (Austad 1982). The few studies in which sperm have been counted show that release and storage occur soon after intromission (Bukowski & Christenson 1997; Snow & Andrade 2004). However, in some species, a linear

*Correspondence and present address: C. D. Linn, Department of Psychology, Southern University at New Orleans, New Orleans, LA 70126, U.S.A. (email: clinn@suno.edu). Y. Molina is at the Department of Psychology, University of Washington, Seattle, Washington 98195, U.S.A. J. DiFatta and T. Christenson are at the Department of Psychology, Tulane University, New Orleans, LA 70118, U.S.A.*

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relationship between mating duration and sperm transfer has been suggested (Elgar et al. 2000; Schneider et al. 2000).

We examine mating duration in an orb-weaving spider, *Nephila clavipes*. When more than one male is present on a female's web, males compete through chases and occasional brief fights. Interactions are almost always resolved in relation to size (Christenson & Goist 1979; Vollrath 1980). The largest male defends a position near the female while smaller males generally remain on the web periphery (Christenson & Goist 1979; Vollrath 1980). During the 2 days following the female's moult to adulthood, the largest male and female copulate several hundred times (Christenson et al. 1985). Individual copulations can last up to 14 min. When not copulating, the male is in guarding position. During this time other males may attempt to approach the female, sometimes disrupting copulation. The mating male defends his position and eventually resumes copulation. Rarely can a peripheral male copulate and then only very briefly (Christenson & Goist 1979). During these first few days after the female's moult, daily census records of marked unrestrained animals indicate that turnover in dominance relationship of males on the web can occur (Cohn et al. 1988), and during staged encounters at this time, other males may approach the female and copulate (Linn 2006). Males release their lifetime supply of sperm (Christenson 1989) within the first few hours of mating (Brown 1985). After 2 days of mating, most first males guard the female until she abandons the web, which occurs from 2 to more than 14 days after her final moult (Cohn et al. 1988). Other males may appear on the female's first and subsequent webs. Mating with these males occurs infrequently, and when it does occur, it is usually when the female is feeding (Christenson et al. 1985; Cohn et al. 1988). To test the extended mate guarding and ejaculate transfer hypotheses, we allowed newly moulted, virgin females to mate with a first virgin male for various durations. Some females were allowed the opportunity to mate with a second virgin male. Sperm release by males and uptake by females was assessed through examination of pedipalps and spermathecae.

## METHODS

### Subjects

Animals were observed in the field at Tulane University's Hebert Center in Louisiana, July–September 2002. Penultimate-instar females were collected and housed individually in boxes (30 × 30 cm and 30 cm high) constructed of wood and fibreglass screening. They built an orb web and were lightly sprayed with water daily and fed mealworm larvae. Virgin adult males were collected from their own webs, with status determined by coloration and presence of sperm webs (Myers & Christenson 1988). They were housed four to seven in a similar box containing twigs to facilitate sperm web construction and sperm induction, transfer of sperm from testes to pedipalps. Males were so kept for 5 days to ensure induction (Myers & Christenson 1988).

### Procedures for Mating

Females were checked several times daily, and within a few hours after the female's final moult, a first male was dropped onto the support strands of its web. Copulation typically began within 15 min. Older, adult nonvirgin females are more likely to mate when feeding (Christenson et al. 1985; Cohn et al. 1988), so a medium-sized mealworm was dropped onto each day 4 female's orb, providing second males the opportunity to mate. Mating with first males was terminated according to assigned duration and ceased for second males when females finished feeding, from 1.6 to 4.5 h. The term mating refers to copulatory experience of a male and female dyad and could include more than one copulation. All pairs were observed and a serial record was kept by hand of the following: thrust: brief placement of the pedipalp conductor to the female epigynum without insertion; intromission: insertion of the pedipalpal conductor into the epigynum; copulation: a single intromission of the pedipalp conductor, accompanied by haematodochal bulb expansions and contractions; and fend: female brushing off the male with the third pair of legs.

### Treatments

Female and first male dyads were allowed one of three mating durations: two natural pedipalpal copulations (one with the left and one with the right pedipalp, each lasting about 14 min), 2 h (during which numerous copulations with both pedipalps occurred), or 48 h (the natural, unrestrained duration). For the two-intromission group, 36 males and females were tested. All first males and 21 females were killed for sperm quantification. The remaining 15 females were presented a second male (equal in size to the first) to determine the likelihood of remating. All mated second male and female dyads ($N = 14$) were killed for sperm counting. For the 2-h group, 10 males and females were tested. All males were killed, all females were presented a second male, and mated dyads ($N = 9$) were killed. For the 2-day group, 42 males and females were tested and all males and 13 females were killed. Second males were added to the webs of 29 nonvirgin females and all mated dyads ($N = 8$) were killed.

Introduction of all second males onto nonvirgin females' webs occurred on day 4 postfemale moult, when it is more likely that a naturally occurring second male might have a mating opportunity. In nature, second males are not likely to mate during the 3 days following the female's moult. Fifty-nine marked, unrestrained census females were observed to moult and immediately commence mating with the first male (1978–1982, unpublished census data). Another male was present on the female's web during the 3-day postmoult in 16 (27%) cases. In 14 of these cases, dominance relationships remained stable and the peripheral male did not copulate. In two cases the status of the males switched and the newly dominant males were not observed to copulate. This was at least in part because the females were not observed to be feeding. About one-third of the marked census females abandoned