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Effects of mating experience on subsequent reproductive behaviour and sperm release in the spider *Nesticodes rufipes*

YAMILE MOLINA* & TERRY CHRISTENSON†
*Department of Psychology, University of Washington
†Department of Psychology, Tulane University

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During a mating season, individuals encounter potential mates that have varied copulatory experiences. Such experience may influence the likelihood and nature of subsequent reproductive behaviour and sperm release within a female/male dyad. We examined the influence of an individual's sexual experience on its subsequent mating activities and that of a potential partner in a cobweb spider, *Nesticodes rufipes*. Our results suggest that an individual's prior sexual experience influenced its own subsequent motivation, but had little effect on that of the potential partner. For females, their prior experience decreased receptivity and increased aggressiveness towards the second male, while having little effect on the subsequent male's motivation (i.e. approach). Specific aspects of a female's initial mating experience (i.e. plug formation) did however influence the nature of subsequent copulation, which in turn influenced sperm release. For males, their prior experience decreased the latency to approach the female and increased the efficiency of pedipalp insertion, but male experience had little effect on the behaviour of the female. Our results suggest that mating experience may benefit both sexes in that (1) females that are not sperm limited may incur high costs from remating (time) and (2) males show experience-related improvement in copulatory efficiency.

Keywords: cobweb spider; copulatory plug; female receptivity; mating history; *Nesticodes rufipes*; sperm competition; spider

Under natural conditions, individuals in a population vary in mating experience (reviewed in: Andersson 1994; Birkhead 2000). Experience can influence an individual's subsequent mate choice and mating behaviour as well as the cryptic processes related to sperm release and egg fertilization. For example, mated females may be less receptive than virgin females because they have sufficient sperm stores and there are costs associated with copulation (Ortigosa & Rowe 2003; Fleischmann & Sakaluk 2004), or because seminal products are released during the first mating (Wolfner 1997; Estramil & Costa 2007). Mated males may be more selective with a second mate, if their sperm stores or ejaculate vehicles are relatively depleted (Markow et al. 1978; Dewsbury 1982; Wolcott et al. 2005). Experience may enhance a male's ability to determine female receptivity and thus reduce his risk of predation or energy expenditure (Wedell & Cook 1999).

An individual's experience may also influence a partner's subsequent behaviour. Males may prefer virgin females (Bonduriansky 2001; Gaskett et al. 2004), and thus, consequences of mating experience, including plugs from male and/or female glandular sections as well as male body parts, may influence the likelihood of a subsequent male's copulation (Eberhard 2004; Huber 2005). Male experience may also influence the female's behaviour because detecting such experience could help her avoid mating with a male with reduced sperm stores (Michereff et al. 2004; Torres-Vila & Jennions 2005).

Previous experimental designs investigating mating experience have generally focused on behavioural differences between virgin and mated animals (Bateman &

Correspondence: Y. Molina, Department of Psychology, University of Washington, Box 351525, University of Washington, Seattle, WA 98195, U.S.A. (email: ymolina@u.washington.edu). T. Christenson is at the Department of Psychology, Tulane University, 2007 Perceival Stern Hall, New Orleans, LA 70118, U.S.A.

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Ferguson 2004; Torres-Vila & Jennions 2005). Labelling an animal as mated does not account for the fact that copulatory behaviour can serve several functions, including sperm transfer (Simmons 2001), stimulation of sperm uptake, storage and utilization by the female (Eberhard 1996) and reducing subsequent copulation (e.g. components of seminal fluids: Wolfner 1997; or plugs: Eberhard & Huber 1998). Differential experience related to specific phases of a copulatory sequence may therefore have specific effects on subsequent mating efforts. We examined the effects of components of a spider copulatory sequence on individual and partner behaviour. Spiders offer a good model because the copulatory sequence is complex, with components influencing sperm transfer and uptake (Bukowski & Christenson 1997) and plugging (Eberhard 2004). In addition, the reproductive organs of spiders are discrete, allowing for removal and quantification of sperm, enabling studies linking behavioural changes to cryptic reproductive processes (i.e. sperm release).

We examined the mating activity of a cobweb spider, *N. rufipes* (*Theridion rufipes* Lucas), a species well suited to determine the effects of specific aspects of the mating experience on an individual and its partner. Newly moulted adult males build a sperm web to induct or transfer sperm from testes to the copulatory organs, the pedipalps. They then abandon their cobweb and search for females. With a given female, males will interrupt copulation, construct a sperm web and reinduct sperm to their pedipalps. This is repeated several times during a mating sequence (Knoflach 2004). After a point, the male dabs a white material onto the female's genital orifice (epigynum) with the pedipalps. The male subsequently reinducts several times and reapplies material that eventually hardens over the epigynum (Molina 2005). The mating sequence may thus be divided into three phases: (1) sperm web construction, (2) initiation of dabbing and (3) complete plug formation.

Most of the relevant studies we cite have also generally focused on the effects of either male or female mating history on subsequent experience (e.g. Lodder 1976). Selective pressures may however be working on both sexes. We test several predictions based on sex-specific differences in species' natural history. As female *N. rufipes* allow plugging by the first male and do not appear to be sperm limited (Molina 2005), and mating activities require a significant amount of time, we predicted that sexual receptivity would decrease with increased previous experience. Conversely, given the predatory nature of the relatively large female (Molina 2005) and the difficulties that male spiders in general face in moving about and finding receptive females (Christenson 1990; Prenter et al. 2006), we predicted that nonvirgin males would modify their approach and mating behaviours to become more efficient at attaining copulation. We also examined effects of mating experience on the partner's behaviour. Across the three phases of mating in this species, both the degree of sperm competition and the potential difficulty in attaining copulation increase. If males can monitor variation in female experience, then males should copulate less frequently and release less sperm with females that have experienced more copulatory phases. In terms of fecundity, females would not be expected to discriminate among males if mated males are capable of sperm reinduction and insemination of a second female.

METHODS

Subjects

Subjects (Table 1) were housed in Stern Hall at Tulane University, New Orleans, Louisian, U.S.A. Each spiderling was housed in a 20 ml scintillation vial, watered daily, fed a single, small *Tenebrio molitor* larva weekly and checked daily for moulting. Within a day after maturation, females were moved to clear plastic tubing (7 cm high, 8 cm in diameter). This species is sexually dimorphic in size: females (17.4 ± 0.6 mg) weigh more than males (5.7 ± 0.1 mg; Molina 2005).

Procedures for Staged Matings: Behaviour

Subjects were assigned to conditions such that female and male age and weight were counterbalanced across groups. Males generally began to mate within 7–45 min of introduction (Molina 2005). If a male did not interact with a female within an hour, it was replaced. We define copulation as a single insertion of a pedipalp. The term mating sequence refers to the period from first insertion of a pedipalp until completion of the plug or termination of the test. When we allowed the male to fully plug the female, we removed the plug after 30 min of inactivity. We recorded the following male behaviours: approach, mount, attempt to insert and pedipalp insertion. We noted the following female behaviours: solicit (pluck silk strands with legs I and II; see Knoflach 2004), approach, vibrate and attack.

Table 1. Sets of subjects used (total $N = 218$), according to mating status prior to trials and copulatory phase completed within trials

	Mating status		Copulatory phase	*N*	
Group	Female	Male	completed	Female	Male
A	Virgin	Virgin	EP	40	40
B	Virgin	Virgin	SW	6	6
C	Virgin	Virgin	DI	10	10
D	Virgin	Virgin	EP	10	10
E	Mated	Virgin	SW	(B)	6
F	Mated	Virgin	SW	(C)	10
G	Mated	Virgin	SW	(D)	10
H	Virgin	Virgin	EP	20	20
I	Virgin	Mated	SW	10	(10 of group H)
J	—	Virgin	—	—	10
Total *N*				**96**	**122**

Initial female mating experience was manipulated: some females were allowed to mate until the beginning of sperm web construction (SW), until dabbing on the epigynum was initiated (DI), or until an external plug was formed (EP).