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Seminal compounds, female receptivity and fitness in the almond moth, *Cadra cautella*

KATHRYN B. McNAMARA, MARK A. ELGAR & THERÉSA M. JONES
Department of Zoology, University of Melbourne

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Seminal compounds variously affect female receptivity, fitness and longevity. Typically, they are delivered simultaneously with sperm. Thus, for most species, it is difficult to disentangle their relative contribution in altering female physiology and/or behaviour. However, the timing of seminal compound delivery is known for a few species, allowing direct manipulation and assessment of their effect on female reproduction. We examined the effect of seminal compounds on female fitness and longevity in the almond moth, where seminal compounds are delivered before the transfer of the sperm-containing spermatophore. We permitted females either one or two matings and manipulated the dose of seminal compounds received by interrupting half the copulating pairs after seminal protein transfer, but before spermatophore transfer. Females that received a double dose of seminal compounds laid more eggs regardless of whether they also received a spermatophore, suggesting this effect is independent of any effects of sperm on oviposition. The dose of seminal compounds did not affect female receptive behaviour, but females that received seminal compounds copulated less frequently than virgins. Moreover, females that received an entire ejaculate remated less frequently than females receiving only seminal compounds, and larger females were more likely to remate. Thus, the mechanical distension of the female reproductive tract may provide a proximate mechanism for inducing nonreceptivity in this species. Finally, females receiving an entire ejaculate lived longer when they received an additional dose of seminal compounds. We discuss factors promoting the development of seminal compounds and their role in the maintenance of monandry.

Keywords: almond moth; *Cadra cautella*; monandry; polyandry; sexual conflict; spermatophore

There is growing empirical and theoretical evidence indicating a conflict between the sexes over a female's optimum mating frequency (reviewed in Arnqvist & Rowe 2005). While females may derive a variety of benefits from mating multiply (Arnqvist & Nilsson 2000; Jennions & Petrie 2000), males secure greater paternity success by preventing their mates from copulating again. This disparity over preferred female mating rate has driven the evolution of a suite of male pre- and postinsemination adaptations that reduce or prevent females from remating. These traits can confer substantial costs to females (Arnqvist & Rowe 2005), and, thus, such adaptations may have profound consequences for male and female fitness traits (Arnqvist & Rowe 2005).

Correspondence: K. B. McNamara, Department of Zoology, University of Melbourne, Melbourne 3010, Australia (email: k.mcnamara@pgrad.unimelb.edu.au).

At a postinsemination level, male seminal compounds that are transferred along with sperm during mating have been implicated in the modification of the physiology, behaviour and life span of females in a range of species (Chapman et al. 1995; Cordero 1995; Gillott 2003; Arnqvist & Rowe 2005). The role of seminal compounds in inducing such changes has been studied intensively in *Drosophila melanogaster*; nearly 100 proteins and peptides have been identified and implicated in altering female reproductive processes, such as increasing female oviposition rate and fecundity and, in association, reducing female longevity (reviewed in Chapman 2001; Wolfner 2002). Species in which females are largely, but not completely, monandrous are interesting models to explore the evolution and maintenance of seminal compounds as they are systems in which the conflict over female remating frequency is potentially still contested, and because experimentally restricting such females to monandry

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or inducing polyandry is within the naturally occurring mating frequency of the species.

In the Lepidoptera, postmating receptivity is governed by a number of mutually nonexclusive mechanical and physiological proximate factors. In most moths, receptive females adopt a 'calling' posture and produce pheromones to attract potential mates. In a number of species, male-transferred seminal proteins induce cessation of pheromone production (pheromonostasis; Kingan et al. 1993, 1995) thus reducing a mated female's future mating opportunities. However, receptivity may also be reduced by the mechanical presence of a spermatophore (Sugawara 1979). Males exploit this mechanical stimulus controlling female nonreceptivity by transferring large quantities (approximately 70–90% of the sperm in the ejaculate) of relatively cheap, anucleate, nonfertilizing 'filler' sperm into the female's spermatheca (Cook & Wedell 1999; Wedell 2001). As a result, female size may affect the duration of nonreceptivity in species in which there is an allometric relationship between female body and reproductive tract size (Torres-Vila et al. 1997). Studies examining the specific effect of seminal compounds for lepidopterans typically focus on the initiation of pheromonostasis by injecting accessory cell proteins directly into the body of the female (Bali et al. 1996; Jin & Gong 2001), or examining the effect of specific proteins on dissections of the female's pheromone gland (Eliyahu et al. 2003). While these studies have found that male-transferred substances reduce female receptivity, they do not reflect natural processes and are unable to assess subsequent measures of female fitness. Furthermore, while a role for seminal compounds in altering female reproductive processes may be suggested by comparing the behaviour of virgin and mated females, such studies are unable to disentangle the role of sperm in female nonreceptivity, fitness and longevity from the effects of the seminal compounds themselves.

The almond moth (also known as *Ephestia cautella*; family: Pyralidae; subfamily: Phycitinae) is a stored product pest. When given an opportunity to remate with multiple partners, approximately 20% of females mate more than once and may mate up to five times (McNamara et al. 2008). However, only 68% ($N = 75$) of virgin females will mate when first presented with a male in an experimental setting (K. B. McNamara, unpublished data). Female almond moth receptive ('calling') behaviour is clearly distinguishable; females remain stationary and elevate their abdomen and extrude their pheromone gland. Calling is an important precursor to mating; 65% of once-mated females ($N = 43$) that called after their first mating subsequently remated (K. B. McNamara, unpublished data). Males are polygynous, although their reproductive investment, but not fertilization success, is dramatically reduced after the first copulation (McNamara et al. 2007). During copulation, a male first transfers a gelatinous mass of seminal compounds from his lower unpaired gland into the female's bursa copulatrix, followed approximately 30 min later by a membrane-bound spermatophore (K. B. McNamara, unpublished data) consisting of sperm and additional seminal compounds (as does the closely related Indian meal moth, *Plodia interpunctella*; Norris 1932). The transfer of a gelatinous mass prior to the transfer of sperm has also been reported for other species, such as *P. interpunctella*, the spruce budworm, *Choristoneura fumiferana*, and the obliquebanded leafroller, *C. rosaceana* (Delisle & Simard 2002). Once spermatophore transfer commences, the male and female are locked together until copulation is complete. The function of the seminal compounds delivered prior to the spermatophore is unknown. They may act as a paternal investment that increases either female reproductive output or longevity, and/or a form of male mating effort, with the mass of seminal compounds either mechanically or physiologically reducing female postmating receptivity (Simmons & Parker 1989; Vahed 1998). The role of the seminal compounds contained within the spermatophore is unclear, and the relative importance of the two sources of seminal compounds has never been investigated.

In this study, we examined the potential role of prespermatophoric and spermatophore-contained seminal compounds on postmating receptivity, reproductive output and longevity in the female almond moth. To achieve this, we manipulated both the dose of seminal compounds and the number of spermatophores that a female received.

Given both the effect of seminal compounds on mediating female reproductive processes and the use of these compounds in female somatic maintenance in other insect species, we predicted a dose-dependent relationship between the quantity of seminal compounds received and female receptivity, fitness and longevity parameters.

METHODS

Adults and larvae of *C. cautella* were obtained from the CSIRO, Canberra, in June 2004. This population, initially sourced from a wild Queensland population, had been maintained in the laboratory for 4 years (approximately 36 generations). Larvae were reared on a diet of bran, yeast and glycerol (approximate ratio = 10:1:1). Adults and larvae were maintained at 28 °C, on a 14:10 h light:dark regime.

We obtained virgin individuals by initially removing, and discarding, all adults from large stock culturing pots early each morning and then removing all subsequently emerging adults every 2 h for 6 h. As almond moths do not mate immediately after emergence (K. B. McNamara, personal observation), and given that copulation continues for approximately 1.5 h, adults that were not already mating when removed were assumed to be virgins. Following removal, adults were weighed, sexed, and stored in 5 ml polypropylene vials without food or water for 12–48 h.

Prior to the experiment, males were randomly assigned to provide either only a dose of prespermatophoric seminal compounds (S) or a complete ejaculate (E; a dose of prespermatophoric seminal compounds, in addition to the subsequent spermatophore). We interrupted the copulation of S males after 30 min, prior to the transfer of the spermatophore (Norris 1932), and allowed E males to complete copulation.