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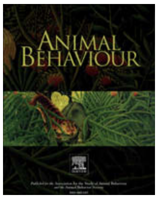

Condition-dependent female remating resistance generates sexual selection on male size in a ladybird beetle

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Behavioural resistance to remating by females is common, but the causes and consequences of resistance are rarely explained. Prominent hypotheses include resistance as a means of avoiding costly and superfluous mating, or as a means of biasing mating towards high-quality males. In species in which males produce nutritious nuptial gifts, females may further modulate resistance according to their need for nutrition. We investigated these hypotheses in the ladybeetle *Adalia bipunctata*, in which females frequently display vigorous resistance before copulation and ingest a spermatophore after copulation. In two experiments, we manipulated female nutritional state, depriving or satiating females for a short (16 h) or long (96 h) interval before a remating trial. We found that food-deprived females resisted mating more frequently and for longer periods than satiated females and consequently remated less frequently. This condition dependence of resistance supports the hypothesis that resistance functions to reduce superfluous and costly mating. Our finding that food-deprived females were more resistant suggests that mating imposes energetic costs, and that nuptial feeding does not offset these costs. In a third experiment, we investigated whether the extent of resistance depended on male size or whether resistance itself biased mating towards large males. The extent of female resistance was independent of male size, but resistance itself resulted in a mating bias towards large males. In summary, our results support the hypotheses that females resist mating simply because it is costly and superfluous, and that a side effect of resistance is sexual selection for large male size.

Behavioural resistance to mating by females is a common feature of mating systems. It may function to reduce female mating frequency, and in some cases, it biases mating success of males towards phenotypes that can overcome resistance (Arnqvist & Rowe 2005). Forms of resistance range from vigorous struggles with males (e.g. Rowe et al. 1994; Day & Gilburn 1997; Jormalainen 1998; Blanckenhorn et al. 2002) to avoidance of males through habitat switching (e.g. Krupa et al. 1990; Stone 1995; Rowe et al. 1996). In several of these examples, experiments have shown that resistance is costly to females. These costs include physical harm or elevated mortality (e.g. Mesnick & Le Boeuf 1991; Rowe 1994; Mühlhäuser & Blanckenhorn 2002) and missed opportunities such as foraging (Rowe 1992; Stone 1995). Evidence that females may pay a cost for resistance implies that some direct or indirect benefit offsets these costs.

There are several nonexclusive hypotheses that may account for female resistance to mating, yet there have been few attempts to experimentally distinguish among them. First, females may resist simply because additional mating is superfluous for fertilization and is costly. Although there is substantial support for the existence of costs to superfluous matings (reviews in Thornhill & Alcock 1983; Gwynne 1989; Choe & Crespi 1997; Arnqvist & Nilsson 2000), experimental support for the hypothesis that these costs account for female resistance is minimal. Direct support comes from economic studies where the costs (or benefits) of mating to females are manipulated, and the extent of resistance is then monitored (e.g. Lauer 1996; Blanckenhorn et al. 2002; Hosken et al. 2003; Teuschl & Blanckenhorn 2007). For example, in water striders, hungry females tend to increase resistance to mating, as expected because mating conflicts with female foraging (Rowe 1992), and females with stored sperm are more resistant than those depleted of sperm (Ortigosa & Rowe 2003).

The economics of female resistance to mating in species with nuptial gifts may be a particularly interesting case. In these species,

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it has been argued that some mating occurs as a means of acquiring resources from males (Gwynne 1984). If so, then one would expect resistance to decline when females are hungry, the opposite of the pattern observed in water striders. The evidence here is mixed. In some species with nuptial gifts, nutritionally deprived females do tend to be less resistant (e.g. Thornhill 1984; Gwynne 1990; Simmons & Bailey 1990; Bilde et al. 2007). However, in other species, nutritional state appears to have no effect on willingness to mate (Engqvist 2007b), suggesting that either nuptial gifts are not valuable to females as food items (see Vahed 1998), or some other factor is determining resistance.

A second hypothesis for female resistance is the male screening hypothesis, which relies on indirect rather than direct selection on resistance (West-Eberhard 1983; Wiley & Poston 1996; Eberhard 2002; Kokko et al. 2003). In short, females may resist males selectively so that mating is biased towards males of high genetic quality; females pay a direct cost of resisting males to obtain the indirect benefit of improved offspring quality. In some species, resistance does favour certain male phenotypes; however, there is little evidence that females modulate their level of resistance based on male phenotype (Arnqvist & Rowe 2005). If female resistance does not depend upon male phenotype, but male phenotype does affect the success of males in overcoming resistance, then biases may simply be a by-product of a general resistance by females to costly mating as per the first hypothesis above. In two well-studied systems (seaweed flies and water striders) the by-product hypothesis is supported: resistance depends on ecological circumstance rather than the phenotype of the male (Crean & Gilburn 1998; Shuker & Day 2001; Ortigosa & Rowe 2002). The by-product hypothesis can account for resistance and biases in male mating success (i.e. direct selection on male phenotypes that help males overcome resisting females), but does not speak to any positive (or negative) indirect selection that may result from these biases (i.e. a good genes process is not necessarily predicted).

In this study we examine each of these hypotheses for female resistance in a species with so-called nuptial gifts, the two-spot ladybird beetle *Adalia bipunctata*. Following copulation, females eject a spermatophore and consume it (Perry & Rowe 2008a). Nevertheless, females often vigorously resist remating by kicking at or running from males or by bending the abdomen to prevent genital contact. Ladybirds are known to face food-limited conditions in nature (Sloggett & Majerus 2000), and we reasoned that mating would conflict with female foraging. Mating involves females carrying males for several hours, and it is likely that foraging efficiency would decline and energy consumption would be elevated during this period. If females resist because mating interferes with foraging and is energetically costly, then hungry females should resist mating more than satiated females. Alternatively, if energy acquired through consuming spermatophores offsets these costs, then we would expect the opposite effect of hunger on resistance. In two experiments of the current study, we manipulated short-term and long-term female nutritional state to test these predictions.

In a third experiment, we determine whether resistance is dependent on the phenotype of males, and whether resistance tends to bias mating success of males. There are mixed reports of a mating advantage to large males in *A. bipunctata*, but the origin of these size biases have not been investigated (e.g. Tomlinson et al. 1995; Yasuda & Dixon 2002). One possibility is that females resist less with larger males as a means of biasing mating success towards them (i.e. the male screening hypothesis, Eberhard 2002). Another is that larger males are simply better able to overcome female resistance. To distinguish these hypotheses, we determined whether females altered their level of resistance based on male size, and whether resistance per se favoured larger males.

METHODS

Experimental Animals

Adalia bipunctata is an aphid predator widespread in temperate habitats (Omkar & Pervez 2005). Both males and females mate multiply. Females are typically larger than males and there is substantial variation in mass (range: female, 5.22–17.83 mg; male, 5.34–13.95 mg; this study). The beetles used in this study were from the F1 generation reared in our laboratory, from stock obtained from Natural Insect Control (Stevensville, Ontario, Canada). During maintenance periods, animals were provided daily with moistened cotton as a water source and fed pea aphids (*Acyrthosiphon pisum* reared on broad bean, *Vicia faba*) and UV-sterilized flour moth eggs (*Ephestia kuehniella*).

Assessing Female Remating Resistance

We investigated the effect of food level on female remating behaviour in two experiments. Each experiment consisted of an initial mating, followed by a feeding treatment and then a remating trial in which we monitored mating resistance. Females were housed individually in petri dishes (50×12 mm) throughout. For the initial mating, virgin females of similar age were mated once to a male from the laboratory stock. We did not interfere with spermatophore consumption after mating, which meant that most females probably ingested some or all of the spermatophore (>90%; Perry & Rowe 2008a). Following this mating and before the feeding treatment, females were fed excess flour moth eggs for several days. During this period, we monitored oviposition and discarded females that did not oviposit because it may have indicated a failure of sperm transfer.

For the remating trial, females were paired with a test male from the laboratory stock. All males had mated at least once previously. Males were maintained on excess flour moth eggs and kept isolated from females for at least 2 days before the trial. Males that did not attempt to mount the female within 10 min were replaced. We recorded whether the female resisted a male's mating attempt, and when resistance occurred, we measured the duration of resistance behaviour until mating began or until the male was dislodged. Males often remount females immediately after being dislodged. If the male did not remount the female within 1 min, we ended the trial. When males remounted within 1 min and females again resisted, we timed the duration of resistance and added it to the initial resistance time. We repeated this measurement of resistance until mating occurred or the female successfully eluded the male for at least 1 min. This design accounts for the likelihood that, in nature, a male may be able to immediately remount a female if dislodged but, we conjecture, would be less likely to remount if the female puts some distance between them. We separately analysed the remating responses of females considering only the first bout of resistance, but as the results were similar we do not report them here.

Short-term Hunger

To test the hypothesis that short-term hunger influences resistance, we began the food treatment 16 h before the mating trial. Females were transferred to new petri dishes, provided with moistened cotton as a water source, and either deprived of food ($N = 16$) or fed an excess of flour moth eggs ($N = 18$). Sixteen hours should have been sufficient time for gut clearance, which occurs in 2–12 h (McMillan et al. 2007). After 16 h, females were transferred to a new dish and paired with a male for the remating trial.