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Mating stimulates female feeding: testing the implications for the evolution of nuptial gifts

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Abstract

Nutritional benefits from nuptial gifts have been difficult to detect in some species, raising the question: what maintains nuptial feeding when gifts do not benefit females? The sensory trap hypothesis proposes that nuptial feeding may be explained by pre-existing sensory responses that predispose females to ingest gifts. Recent studies have shown that male seminal proteins can induce a nonspecific increase in female feeding after mating, which may represent a sensory trap for nuptial feeding if it results in increased intake of post-mating gifts. I tested these ideas using female beetles that ingest a spermatophore after mating. I show that males stimulate strongly increased female feeding post-mating. However, there was little evidence for dose dependence in the feeding response that could allow males to stimulate feeding beyond the female optimum. Moreover, the post-mating feeding response could not explain nuptial feeding: despite feeding more in general, newly mated females were less likely than nonmated females to ingest spermatophore gifts.

Introduction

Nuptial gifts are well-known mating phenotypes in which males transfer edible material to females, typically as a form of mating effort (reviewed by Vahed, 2007a; Gwynne, 2008). In some insects, males provide females with a large and calorie-rich nuptial gift, and there is evidence that ingestion of these gifts increases female reproductive output (particularly in some orthopterans, e.g. Gwynne, 1984; Simmons, 1988; Brown, 1997; see also Engqvist, 2007a). Yet in other species, it is surprisingly difficult to detect the expected nutritional benefits of gifts. Examples with no detectable benefits include other orthopteran species (Wedell & Arak, 1989; Will & Sakaluk, 1994; Vahed & Gilbert, 1997; Vahed, 2003), as well as cockroaches (Mondet *et al.*, 2008) and coccinellid beetles (Perry & Rowe, 2008a). Furthermore, one frequently reported effect of gift ingestion is to increase female re-mating resistance (e.g. Simmons & Gwynne, 1991; Engqvist, 2007b; Perry & Rowe, 2008a) – a response that is clearly in the interests of males but may or may not benefit females, implying the possibility of sexual conflict over gift ingestion. In these cases, it is unclear what maintains nuptial feeding despite an apparent lack of nutritional benefit (see Arnqvist & Rowe, 2005; Arnqvist, 2006; Vahed, 2007a).

One hypothesis for the maintenance of nuptial feeding without benefit is that nuptial gifts exploit pre-existing female responses that have evolved by natural selection in another context, such as foraging (a type of sensory trap, Christy, 1995; Endler & Basolo, 1998; Sakaluk, 2000; reviewed by Vahed, 2007a). One potentially exploitable response is females' preference for foods with a particular chemical profile (e.g. Sakaluk, 2000; Sakaluk *et al.*, 2006; Vahed, 2007b; see also Bilde *et al.*, 2007). For example, free amino acids in the nuptial gifts of the cricket *Gryllodes sigillatus* apparently entice females by mimicking their preferred food but offer little useful nutrition (Warwick *et al.*, 2009; see also Wada-Katsumata *et al.*, 2009). A second potentially exploitable response (and the focus of this study) involves a more general feeding response that follows mating. Several recent studies of arthropods show that male seminal proteins transferred during mating can stimulate female feeding (Carvalho *et al.*, 2006; Kaufman, 2007; Barnes *et al.*, 2008). Such a general feeding response may increase the likelihood of gift ingestion after mating, and this could benefit males if, for example, gifts make females less likely to mate again (references above). This

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post-mating feeding response hypothesis has several relevant implications. First, it suggests that selection cannot easily separate a general feeding motivation from the motivation to eat nuptial gifts in particular, and in this way, nuptial feeding could be maintained even if gift ingestion provides no benefit for females. Second, because post-mating effects on foraging appear widespread (Browne, 1993; Kaufman, 2007), the hypothesis proposes a mechanism that could facilitate the evolution of nuptial gifts in many species. Still, no studies have tested whether a generalized post-mating feeding response can explain nuptial feeding in any species.

Here, I test the post-mating feeding hypothesis using the two-spot ladybird beetle *Adalia bipunctata* Linnaeus. Females of this species typically eject and eat a spermatophore after mating, and they do not appear to gain increased fecundity or longevity from doing so (Perry & Rowe, 2008a,b). Instead, spermatophore ingestion causes faster egg deposition and increases female resistance to re-mating (Perry & Rowe, 2008a), suggesting a benefit to males. Finally, a general increase in feeding after mating is plausible for this species (see Stewart *et al.*, 1991b), making it particularly suitable for investigating the influence of mating on spermatophore feeding.

The experiments here address four objectives. First, I test for a post-mating increase in feeding that is both specific to females and present in the period in which nuptial feeding occurs, in food-deprived and satiated individuals. Second, I consider several proximate factors that might cause increased feeding: (1) increased activity after mating (e.g. leading to more frequent encounters with food; see Isaac *et al.*, 2009); (2) recouping energy expended in mating; (3) an association with increased egg production after mating (see Barnes *et al.*, 2008); and (4) a response to ejaculate products transferred during mating (as observed in *Drosophila melanogaster*; Carvalho *et al.*, 2006). Third, I look for evidence of an assumption from verbal models of sexual conflict (e.g. Arnqvist & Nilsson, 2000; Johnstone & Keller, 2000; Wiklund *et al.*, 2001; Arnqvist & Rowe, 2005; Rowe *et al.*, 2005; Arnqvist, 2006; Engqvist, 2007b), that female reproductive responses to further male stimulus increase continuously (i.e. in a dose-dependent manner) rather than as a switch-like threshold (see Eberhard, 1996). A dose-dependent feeding response to mating predicts the potential for males to stimulate more feeding than is optimal for females, whereas a threshold response provides no scope for such over-stimulation. Fourth, I tested whether the general post-mating feeding response actually results in increased feeding on spermatophore nuptial gifts.

Methods

Experimental animals

Adalia bipunctata is an aphid predator with Holarctic distribution. Females can lay 10–30 eggs daily in abundant food conditions (Stewart *et al.*, 1991a). Both sexes mate multiply. During mating (mean duration: 140 min ± 65 SD, Perry & Rowe, 2008a), males transfer sperm via a spermatophore capsule composed of seminal fluids that solidify within the female reproductive tract. Shortly after mating (typically 2–4 min), females usually eject (90% of matings) and ingest the spermatophore capsule (>90% ingest at least a portion, whereas two-thirds ingest the entire capsule; Perry & Rowe, 2008a). The beetles used in this study were of the third generation from a laboratory stock initially obtained from a supply company (Natural Insect Control, Stevensville, ON, Canada). Adult beetles were fed excess UV-sterilized flour moth eggs (*Ephestia kuehniella* Zeller, a standard diet, de Clerq *et al.*, 2005). *Adalia bipunctata* larvae consume these eggs at a rate that is indistinguishable from the rate for aphid prey (Jalali *et al.*, 2009).

General experimental procedures

I conducted six experiments; five involved both a mating or nonmating (control) treatment and a feeding trial as described in the following sections.

Mating and control treatments

Each experiment involved randomly assigning initially virgin females to a mating or nonmating treatment. I made every effort to handle nonmating females identically as for mated females. In most of the experiments, pairs of mating and nonmating females moved through the phases of the experiment as a unit to control for variation in copulation duration (e.g. when a mating female completed copulation and spermatophore ejection, this female and the paired nonmating female were both placed in feeding arenas). To begin each experiment, females were placed in new Petri dishes and a male was introduced to the dish of the 'mating' female. I removed the male when copulation ended and removed the spermatophore capsule upon ejection. The actions of introducing and removing a male and removing the spermatophore were simulated for the nonmating female.

Measuring feeding

I measured food ingestion by providing individuals with a known mass of flour moth eggs (hereafter 'food') and re-weighing the remaining food after a period of feeding. For each group of mating and nonmating individuals, the feeding trial began immediately following spermatophore ejection by the mated female. Each individual was introduced to a feeding arena consisting of an aluminium weighing boat (containing the preweighed food) covered with a Petri dish lid lined with filter paper. Along with the feeding arenas assigned to the experimental individuals, a control arena with food only was set up to account for changes in food mass in the absence of feeding.

I simulated the introduction and removal of a beetle from this control arena. I calculated food ingestion by subtracting the final mass of food from the initial mass and subtracting from this the mean mass loss of food in control dishes. To examine the relationship between feeding and egg-laying, I recorded the number of eggs laid in each feeding arena. Feeding trials took place at 21 ± 1°C and 50% humidity. The duration of the trials varied, as described below.

Experiment 1: post-mating behaviour in males and females

This experiment was designed to establish whether females display a sex-specific post-mating increase in feeding, which might lead to feeding on spermatophores as a sensory trap, and to test whether such a post-mating change in feeding represents a specific foraging response or is (1) part of a general post-mating increase in activity or (2) indirectly stimulated by a post-mating increase in reproduction. I measured food ingestion at two points: 250 min after copulation and spermatophore ejection, and from that point until 24 h after copulation. The first feeding arenas initially contained 5.00 mg ± 10% of food, and the second arenas contained 10.0 mg ± 10%.

I conducted the experiment by setting up groups of two male–female pairs and assigning each pair to the mating or nonmating treatment by a coin toss; 25 groups were tested. During the first period, I assessed beetle activity beginning 10 min into the feeding trial and subsequently every 30 min, using the following index: 0 (no movement); 1 (slow forward or side-to-side movement); 2 (slow and steady forward movement); and 3 (rapid forward movement). I scored activity blind to the assigned treatments.

Experiment 2: the schedule of the post-mating feeding response in food-deprived and satiated females

Here the goal was (1) to establish the schedule of the post-mating feeding response and in particular to test whether it occurs immediately after mating when females normally would encounter spermatophores and (2) to test whether the effect of mating on feeding occurs in hungry females as well as the well-fed females used in Experiment 1. I manipulated female state (hungry or satiated) and mating status in three time periods after mating and spermatophore ejection: within 30 min, from 30 to 120 min, or 120 to 240 min. The feeding arenas in each period initially contained 1.0 mg ± 10%, 2.0 mg ± 10% and 2.5 mg ± 10% of food, respectively. To generate hungry and satiated females, I placed initially virgin females on a 'no food' (n = 42) or *ad libitum* diet (n = 43) 48 h before the experiment.

Experiment 3: is the feeding response an attempt to recoup energy expended in copulation?

I tested whether the post-mating increase in female feeding is an effort to recoup energy expended in carrying males during copulation, by randomly assigning females to one of the three treatments: mating, weight-bearing, or a control (n = 40 per treatment). Groups of three females (each assigned to a different treatment) moved through the phases of the experiment as a unit to control for copulation duration. To begin, all females in a group were lightly anesthetized with CO_2. For the weight-bearing treatment, I affixed a lead weight, equivalent in mass to a large male (13.1 mg ± 10%, the third quartile of mass for a sample of laboratory males), to the female's elytra in a similar position to that of a mating male, using a small piece of double-sided tape. I made every effort to handle the control (to which I applied double-sided tape only) and mating females exactly as for the weight-bearing treatment. When copulation ended for the mating female within each group, I removed the male and removed the weight and tape from the other treatments within the group (simulating this for the mated female). Each female was transferred to an initial feeding arena containing 5.00 mg ± 10% of food for 250 min and then to a second arena containing 10.0 mg ± 10% of food until 24 h total.

Experiment 4: the feeding response and transfer of sperm and seminal proteins

In this experiment, I aimed to test whether sperm or seminal protein transfer, if either, causes increased female feeding. Copulation in *A. bipunctata* is characterized by early transfer of seminal fluids (including those that form the spermatophore; Ransford, 1997; Haddrill, 2001); seminal proteins can be detected in the female's reproductive tract after only a minute of copulation (J. Perry, unpublished). In contrast, sperm transfer begins at least 30 min after mating is initiated (Ransford, 1997; Haddrill, 2001). I randomly assigned females to one of the seven treatments: a nonmating treatment, a complete mating, or interrupted mating occurring at 1, 15, 30, 60 or 120 min (n = 41–44 per treatment). I monitored mated females for spermatophore ejection for up to 1 h after mating, and after 1 h transferred the females to a feeding arena containing 12.0 mg ± 10% of food for 24 h. As a check on sperm transfer, I retained eggs from each female and monitored them for hatching success.

Experiment 5: multiple mating and the dose dependence of the feeding response

To test the hypothesis that female feeding is further elevated after a second mating, which could permit males to stimulate female feeding to a level above the female optimum, I assigned females to an initial mating or

nonmating treatment and then to a second mating or nonmating treatment 9 days later ($n = 23$ per group), such that females experienced 0, 1 or 2 matings. This interval between matings is at least as long as natural mating intervals in this species (Haddrill *et al.*, 2008). I measured female feeding in the 24 h following the second mating (or control) treatment, in feeding arenas containing 13.0 mg ± 10% of food.

Experiment 6: does increased feeding after mating translate into increased nuptial feeding?

Having detected a post-mating elevation in female feeding, I conducted an experiment to directly test the hypothesis that this feeding response explains nuptial feeding on spermatophores. If the hypothesis is correct, then newly mated females will be more likely than nonmated females to ingest spermatophores they encounter. I presented recently mated and nonmated females ($n = 23$ per group) with a spermatophore and monitored their feeding. As above, mated females were not permitted to eat their own ejected spermatophores after mating. To obtain a supply of spermatophores, I collected ejected spermatophores from additional mating pairs before and during the experiment and stored them in small humidity chambers to prevent drying (on a dry surface within a covered Petri dish containing a saturated salt solution; Winston & Bates, 1960). Spermatophores were used in the experiment within 1 h of collection.

To conduct the experiment, at 3 min after spermatophore ejection by a mated female, I transferred paired mated and nonmated females to individual 1.5-mL microtubes with a small hole in the cap and a fresh spermatophore inside. I monitored females continuously for 1 h and recorded whether the spermatophore was wholly or partially eaten.

Statistical analyses

I used one-way or two-way mixed-model ANOVAS to analyse the effect of mating, along with any other experimental treatments and the interaction term, on food ingestion and the number of eggs laid (by females that laid ≥1 egg). Most models included the random effect 'group'. I tested whether continuous responses met the assumptions of parametric statistics and where necessary applied a transformation or used a nonparametric test. I report least square means ± SE, back-transformed where appropriate. I analysed categorical responses by chi-square tests.

In Experiment 1, I tested for a relationship between egg-laying and feeding using a mixed-model ANCOVA, including mating, the number of eggs and group. To examine the effect of mating and sex on activity, I conducted a two-way ANOVA using the summed activity scores (0–27) as the response variable. I then tested for a correlation between the activity score and food ingestion.

Results

Experiment 1: effects of mating on feeding, activity and reproduction

I measured food ingestion by mated and nonmated males and females over two time periods. Because results were similar within each time period, I present results for total food ingestion.

Both sex and mating had substantial influence on feeding (Fig. 1a; sex: $F_{1,92} = 31.8$, $P < 0.0001$; mating: $F_{1,92} = 6.3$, $P = 0.01$; square-root transformed data), and there was a marginally significant sex × mating interaction effect ($F_{1,92} = 3.9$, $P = 0.05$). Mated females ate 46% more than nonmated females, whereas there was minimal difference in feeding by mated and nonmated males (Fig. 1a). These results were qualitatively similar when feeding rate was analysed by an ANCOVA including premating mass as a covariate (results not shown).

There was no indication that increased feeding was attributable to increased activity after mating: activity rates were similar regardless of sex or mating (Fig. 1b; sex: $F_{1,96} = 0.2$, $P = 0.68$; mating: $F_{1,96} = 0.9$, $P = 0.34$; sex × mating: $F_{1,96} = 0.7$, $P = 0.39$; square-root transformed data), and there was no significant relationship between activity rate and feeding ($R^2 = 0.03$, $P = 0.09$).

In contrast, feeding was strongly and positively correlated with egg-laying (Fig. 1c; ANCOVA: $F_{1,44} = 32.9$, $P < 0.0001$). Further, egg-laying was stimulated by mating: mated females were more likely to lay eggs (23/25 vs. 11/25; $\chi^2_1 = 12.8$, $P = 0.0004$) and tended to lay more eggs, although this difference was not statistically significant (22.4 ± 2.8 vs. 14.9 ± 4.1, $F_{1,32} = 2.9$, $P = 0.11$). The variation in egg-laying could entirely account for the effect of mating on feeding: when the number of eggs was controlled for in an ANCOVA, mating itself no longer had explanatory power ($F_{1,44} = 2.8$, $P = 0.10$; no significant interaction, $F_{1,44} = 2.3$, $P = 0.14$).

Experiment 2: female nutritional state and the schedule of the post-mating feeding response

Here, I tested the feeding rates of satiated or food-deprived females at three points soon after mating. I found weak evidence of increased feeding by mated females within 30 min of mating, and strong evidence of increased feeding by 2 and 4 h after mating (Fig. 2, Table 1). Hungry females ate more than satiated females, but the effect of mating on feeding was similar in both groups (Fig. 2, Table 1).

Experiment 3: post-mating feeding in relation to energy expended in copulation

I tested whether increased female feeding could be explained by energy expended in carrying males during copulation by subjecting females to a mating,

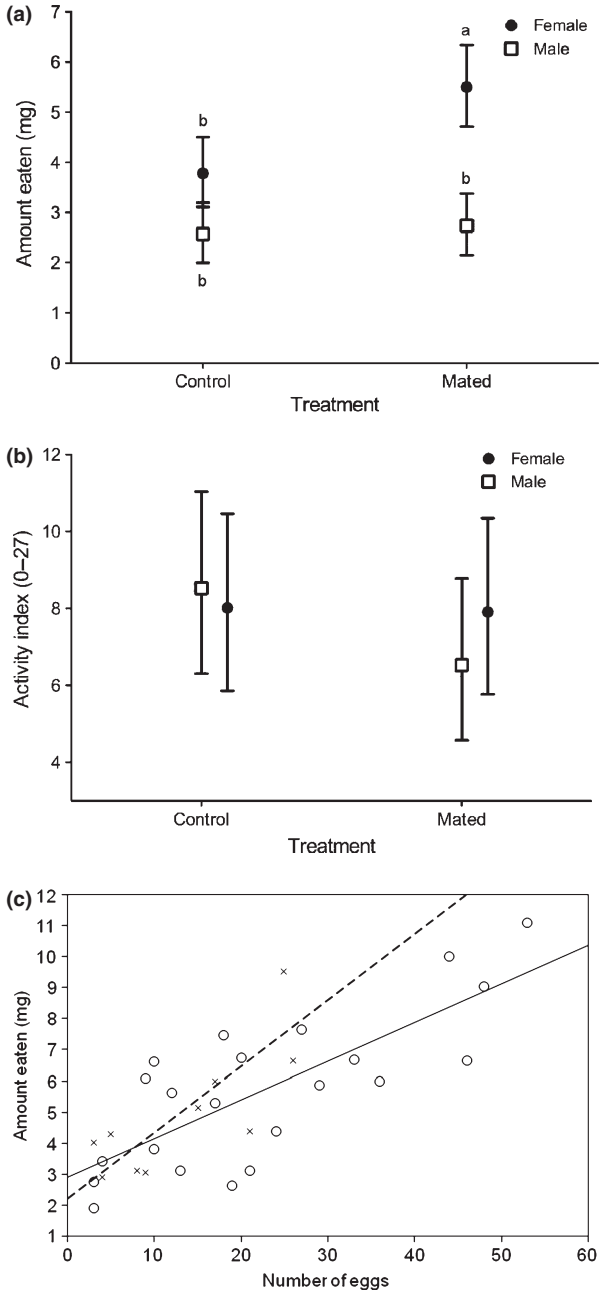

Fig. 1 Male and female beetles were assigned to a mating or control (nonmating) treatment to test the effects of sex and mating on (a) food ingestion (flour moth eggs, ±95% CI) in the 24 h following mating and (b) activity level scored in the 4 h after mating. (c) Feeding was positively correlated with egg-laying during the feeding trial, in both mated (circles, solid line; $R^2 = 0.59$, $\beta = 0.12 \pm 0.02$, $F_{1,20} = 28.7$, $P < 0.0001$) and control females (×, dashed line; $R^2 = 0.68$, $\beta = 0.21 \pm 0.05$, $F_{1,9} = 19.0$, $P = 0.002$). Letters indicate significant differences among groups by a Tukey HSD test.

weight-bearing or control treatment and measuring food ingestion over two time periods. Both periods yielded similar results and so total feeding is presented here.

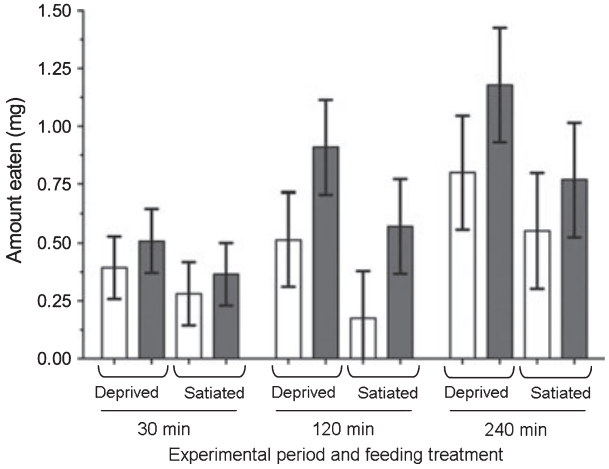

Fig. 2 Food ingestion (flour moth eggs, ±95% CI) by nonmated (open bars) and mated (solid bars) females in each of the three time periods following mating (see text). Females were deprived of food or satiated before the experiment as indicated.

Contrary to the prediction of this hypothesis, there was no evidence that artificially weighted females behaved like mated females (Fig. 3). Mated females ate more than both weighted and control females, which fed at similar rates ($F_{1,117} = 6.6$, $P = 0.002$).

Experiment 4: the feeding response and transfer of sperm and seminal proteins

Here I examined whether seminal fluid or sperm transfer could account for increased female feeding by subjecting females to matings of different durations.

Females that mated for any length of time ate more than control females that did not mate, although only matings of 60 min or longer resulted in significantly increased feeding (Fig. 4; $F_{6,281} = 2.7$, $P = 0.01$; Box–Cox transformation). As a test of whether the feeding response is induced after only 1 min of mating, I compared the 1-min, nonmating and complete mating treatments together in a separate *post hoc* ANOVA. Female feeding after 1 min of mating was indistinguishable from feeding after a complete mating (5.7 ± 1.0 vs. 6.1 ± 1.0 mg, respectively), and both were elevated compared with nonmating females (4.5 ± 1.0 mg; $F_{2,281} = 5.9$, $P = 0.004$).

Mating duration influenced egg fertilization in a manner consistent with previous reports that sperm transfer typically does not begin until 30 min into copulation (see Methods; Supporting Information, Table S1).

Experiment 5: dose dependence in the feeding response

I tested the hypotheses that the female feeding response increases further with additional matings (which may allow males to over-stimulate female feeding) or that

Table 1 Tests of the effect of mating and hunger on food ingestion in three intervals after mating. Significant *P*-values are given in bold.

	Interval after mating (min)		
	0–30	30–120	120–240
*Food ingestion (mg)**			
Mating	$F_{1,166} = 3.6$, $P = 0.06$	$F_{1,116} = 10.7$, **$P = 0.001$**	$F_{1,166} = 5.7$, **$P = 0.02$**
Hunger	$F_{1,166} = 1.2$, $P = 0.27$	$F_{1,116} = 14.6$, **$P = 0.0002$**	$F_{1,166} = 6.9$, **$P = 0.01$**
Mating × hunger	$F_{1,166} = 0.0$, $P = 0.94$	$F_{1,116} = 0.0$, $P = 0.99$	$F_{1,166} = 0.4$, $P = 0.53$

*Box–Cox transformations were applied.

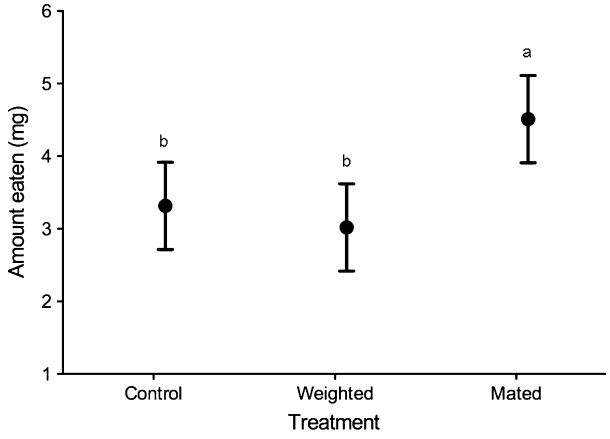

Fig. 3 Food ingestion (flour moth eggs, ±95% CI) over 24 h by females subjected to one of the three treatments: mating, artificially weighted to simulate carrying a male during copulation or a control treatment. Letters indicate significant differences among groups by a Tukey HSD test.

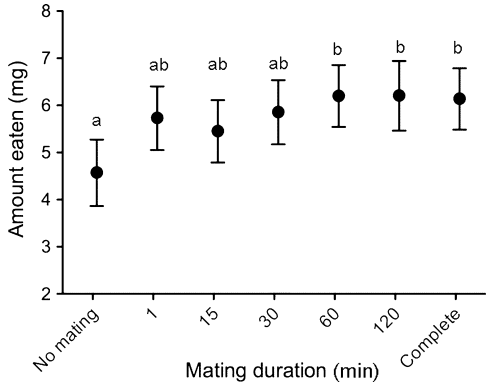

Fig. 4 Food intake (flour moth eggs, ±95% CI) in 24 h by females assigned to matings of varying duration or control (nonmated) females, with letters indicating significant differences among groups.

feeding is switched on after a single mating and remains on, by subjecting initially virgin or previously mated females to a second mating or no mating. The results support at most only weak dose dependence in feeding. Previously mated females had a slight and nonsignificant

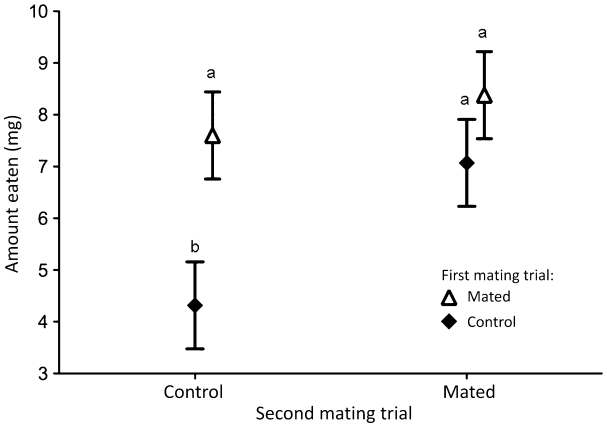

Fig. 5 Food intake (flour moth eggs, ±95% CI) by initially virgin females assigned to a mating or nonmating treatment in each of the first and second mating trials. Letters indicate significant differences among groups by a Tukey HSD test.

increase in feeding after a second mating, compared with previously mated females that did not mate a second time (Fig. 5; effect size: 0.8 ± 0.6 mg; 95% CI: −0.8, 2.4; $P = 0.58$). This contrasts with the large and significant increase that initially virgin females experienced after mating compared with nonmating females (Fig. 5; effect size: 3.3 ± 0.6 mg; 95% CI: 1.7, 4.9; $P < 0.0001$). These different responses to the second mating trial between initially virgin and previously mated females are reflected in a significant interaction effect on feeding ($F_{1,88} = 5.8$, $P = 0.02$; main effects: initial mating trial: $F_{1,88} = 26.3$, $P < 0.0001$; second mating trial: $F_{1,88} = 15.6$, $P = 0.0003$).

Experiment 6: the post-mating feeding response and nuptial feeding

I tested whether elevated feeding in recently mated females did in fact increase the likelihood of ingesting spermatophore nuptial gifts. This prediction was not supported; in fact, the opposite occurred: nonmated females were more likely than recently mated females to eat part or all of the spermatophore (part: 21/23 [91%] vs. 14/23 [61%], $\chi^2_1 = 6.2$, $P = 0.01$; all: 16/23 [70%] vs. 9/23 [39%], $\chi^2_1 = 4.4$, $P = 0.04$). To assess whether this pattern

was explained by decreased female activity soon after mating, I examined the activity scores from Experiment 1 at 10 min after mating; however, mated and nonmated females displayed similar activity levels (respectively, medians: 1 (range 0–2) vs. 1 (range 0–3); means: 0.8 ± 0.2 vs. 0.9 ± 0.2; Wilcoxon test: $\chi^2_1 = 0.0$, $P = 0.83$).

Discussion

I found strong evidence that female ladybirds experience a rapid and substantial post-mating increase in feeding that is initiated by mating interactions with males. The data suggest that this general feeding response is part of a cascade of reproductive responses (including oviposition) that are 'switched on' by mating. However, recently mated females were in fact less likely to ingest spermatophore nuptial gifts compared with nonmated females. Hence, this result clearly contradicts the hypothesis that the post-mating feeding response is a sensory trap that accounts for nuptial feeding.

Mating turns on feeding

Increased female feeding after mating was consistent throughout this study (Figs 1–5; Table 1). The feeding response was specific to females: mating did not influence male feeding within 24 h of mating, which is surprising given that both mating and ejaculate production are energetically costly for male *A. bipunctata* (J. Perry and C. Tse, unpublished; Perry & Rowe, 2010). Increased female feeding began within 2 h of mating and possibly within 30 min; thus, feeding appears to be elevated in the period in which females normally encounter gifts (within minutes to an hour of mating).

Mating stimulated feeding independent of female nutritional state, suggesting that the response is not simply an artefact of using satiated or food-deprived laboratory females. Further, the results indicate that females' response is specific to foraging. The increase in feeding could not be attributed to energy expended in carrying males during copulation – although it is possible that mating generates energetic costs beyond bearing a male's weight – nor could it be attributed to increased female activity after mating. The feeding response was tightly linked to increased egg-laying after mating; in fact, the increase in egg-laying could completely account for the general post-mating feeding response (Experiment 1). Hence, a reasonable hypothesis is that mating stimulates egg production in *A. bipunctata*, which in turn stimulates feeding. This also appears to be the case in *D. melanogaster* (Barnes *et al.*, 2008).

The results above establish that interactions with males stimulate female feeding (potentially indirectly, as noted previously), and in fact, only 1 min of mating was sufficient to increase feeding (Experiment 4). This is consistent with the specific male stimulus being either physical contact with males (including mechanical stimulation from the aedeagus) or seminal proteins, which are detectable in the female reproductive tract after 1 min of mating (J. Perry, unpublished). Studies from other arthropods suggest that seminal proteins are a likely candidate (Carvalho *et al.*, 2006; Kaufman, 2007). In contrast, receiving sperm cannot explain increased feeding because sperm is not transferred until 30 min into copulation (current study; Ransford, 1997). It is also unlikely that female stretch receptors in the reproductive tract produce the response because a minimum of 20 min of mating is required for the transfer of the entire spermatophore (Ransford, 1997).

Potential for conflict over feeding?

Reproductive responses that exhibit dose dependence in response to a male stimulus give rise to the potential for sexual conflict (Arnqvist & Nilsson, 2000; Johnstone & Keller, 2000; Wiklund *et al.*, 2001; Arnqvist & Rowe, 2005; Rowe *et al.*, 2005; Arnqvist, 2006). By supplying additional stimulus, males can potentially increase female responses beyond the female optimum (which should benefit males in polyandrous species; see Arnqvist, 2006). In turn, females should evolve resistance to such over-stimulation (reducing the magnitude of dose dependence). Still, given that many female reproductive responses (including feeding) will be favoured by natural selection, perfect female resistance is probably rare and so some dose dependence is expected to remain. I found evidence of only a weakly dose-dependent feeding response, if any: feeding levels were statistically indistinguishable in females mated once or twice. Furthermore, the interval between matings in the experiment was similar to or longer than natural mating intervals in this species (Haddrill *et al.*, 2008); thus, the weak or absent dose dependence cannot be explained by matings being unnaturally crowded together in time. This result is consistent with either (1) weak dose dependence resulting from past sexual conflict over feeding combined with strong female resistance or (2) a switch-like response (no dose dependence) indicating little potential for sexual conflict over feeding. The latter could result from female feeding being coordinated with other post-mating responses that together respond to a cue that mating has occurred (see Eberhard, 1996; Ram & Wolfner, 2007); indeed, this view is consistent with the observed correlation between feeding and egg-laying after mating. Future work should examine dose dependence in the response more closely, for example by manipulating ejaculate receipt within a single mating (e.g. with males of different size or recently mated males).

Does a generalized feeding response explain nuptial feeding?

Ingesting spermatophores gives no detectable nutritional benefit to female *A. bipunctata* (Perry & Rowe,

2008a,b; Perry *et al.*, 2009). The sensory trap hypothesis attempts to reconcile this observation with the maintenance of nuptial feeding, suggesting that nuptial feeding is a by-product of female responses that originated in another context (Sakaluk, 2000). However, the results of this study do not support the hypothesis that the feeding response accounts for gift ingestion in this species. Surprisingly, mated females were less likely than virgin females to eat spermatophores presented to them immediately after mating, despite their generally elevated foraging during this period compared with virgins.

Three explanations for this result are possible. First, perhaps females discriminated against spermatophores that they themselves have not ejected, implying an experimental artefact. This appears unlikely. For such 'nonself' discrimination to have influenced the result, females must be able to distinguish self-ejected from nonself spermatophores, they must have a distaste for nonself spermatophores that is initiated only after mating, and this distaste must be strong enough to not only reduce feeding on the nonself spermatophore but counteract the generally elevated post-mating feeding rate. Although possible, it is hard to imagine how females are able to distinguish self from nonself spermatophores and why females would have evolved a strong distaste for nonself spermatophores given that they must rarely encounter them in a natural environment. Furthermore, to my knowledge, self vs. nonself discrimination of gifts is not known from any nuptial feeding species that have been studied with similar experimental procedures (e.g. Gwynne, 1984; Engqvist, 2007a; Perry & Rowe, 2008b; Wedell *et al.*, 2008).

A second potential explanation is that mated females may have a brief quiescent period after mating, which limits their feeding. However, this is not supported by the observation that movement levels are similar between mated and nonmated females 10 min after mating and that within 30 min of mating, mated females tend to eat more than nonmated females. Third, given that mated females feed more in general but less on spermatophores in particular, an intriguing possibility is that mated females have some degree of aversion to eating spermatophores to avoid the effects of spermatophore ingestion, such as increased resistance to re-mating (Perry & Rowe, 2008a). This hypothesis is at odds with the observation that most females (~90%) do in fact ingest at least part of the spermatophore after mating, with 67% of females eating the entire spermatophore (Perry & Rowe, 2008a). Yet, this latter observation also indicates that females do not eat the entire spermatophore in one-third of opportunities. Experiments testing the effect on female fitness of the increased re-mating resistance induced by spermatophore feeding (Perry & Rowe, 2008a; see Gwynne, 2008) may clarify whether selection favours or disfavours nuptial feeding in this species.

Conclusions

This study demonstrates that female ladybirds display a post-mating increase in feeding that is stimulated by males, but this response does not account for nuptial feeding in *A. bipunctata*. There is growing interest in determining which female post-mating responses are independently stimulated by mating and (or) interacting with males and which are part of a cascade of reproductive responses in the mated female (e.g. Barnes *et al.*, 2008; Isaac *et al.*, 2009; Ja *et al.*, 2009). A generalized feeding response may potentially link some of the most striking post-mating responses in insects, such as increased reproduction and decreased longevity. Current research in this area is overwhelmingly based on *D. melanogaster*, and it is timely to expand these studies to other taxa.

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References

Arnqvist, G. 2006. Sensory exploitation and sexual conflict. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* **361**: 375–386.

Arnqvist, G. & Nilsson, T. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Anim. Behav.* **60**: 145–164.

Arnqvist, G. & Rowe, L. 2005. Sexual conflict. *Monographs in Behavior and Ecology*. Princeton University Press, Princeton, NJ.

Barnes, A.I., Wigby, S., Boone, J.M., Partridge, L. & Chapman, T. 2008. Feeding, fecundity and lifespan in female *Drosophila melanogaster*. *Proc. R. Soc. Lond. B* **275**: 1675–1683.

Bilde, T., Tuni, C., Elsayed, R., Pekar, S. & Toft, S. 2007. Nuptial gifts of male spiders: sensory exploitation of the female's maternal care instinct or foraging motivation? *Anim. Behav.* **73**: 267–273.

Brown, W.D. 1997. Courtship feeding in tree crickets increases insemination and female reproductive life-span. *Anim. Behav.* **54**: 1369–1382.

Browne, L.B. 1993. Physiologically induced changes in resource-oriented behavior. *Annu. Rev. Entomol.* **38**: 1–25.

Carvalho, G.B., Kapahi, P., Anderson, D.J. & Benzer, S. 2006. Allocrine modulation of feeding behavior by the sex peptide of *Drosophila*. *Curr. Biol.* **16**: 692–696.

Christy, J.H. 1995. Mimicry, mate choice, and the sensory trap hypothesis. *Am. Nat.* **146**: 171–181.

de Clerq, P., Bonte, M., Van Speybroeck, K., Bolckmans, K. & Deforce, K. 2005. Development and reproduction of *Adalia*

bipunctata (Coleoptera: Coccinellidae) on eggs of *Ephestia kuehniella* (Lepidoptera: Phycitidae) and pollen. *Pest Manag. Sci.* **61**: 1129–1132.

Eberhard, W.G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton University Press, Princeton, NJ.

Endler, J.A. & Basolo, A.L. 1998. Sensory ecology, receiver biases and sexual selection. *Trends Ecol. Evol.* **13**: 415–420.

Engqvist, L. 2007a. Nuptial food gifts influence female egg production in the scorpionfly *Panorpa cognata*. *Ecol. Entomol.* **32**: 327–332.

Engqvist, L. 2007b. Nuptial gift consumption influences female remating in a scorpionfly: male or female control of mating rate? *Evol. Ecol.* **21**: 49–61.

Gwynne, D.T. 1984. Courtship feeding increases female reproductive success in bush crickets. *Nature* **307**: 361–363.

Gwynne, D.T. 2008. Sexual conflict over nuptial gifts in insects. *Annu. Rev. Entomol.* **53**: 5.1–5.19.

Haddrill, P.R. 2001. *The Development and Use of Molecular Genetic Markers to Study Sexual Selection and Population Genetics in the 2-Spot Ladybird, Adalia bipunctata (L.)*, PhD Thesis. University of Cambridge, Cambridge.

Haddrill, P.R., Shuker, D.M., Amos, W., Majerus, M.E.N. & Mayes, S. 2008. Female multiple mating in wild and laboratory populations of the two-spot ladybird, *Adalia bipunctata*. *Mol. Ecol.* **17**: 3189–3197.

Isaac, R.E., Li, C., Leedale, A.E. & Shirras, A.D. 2009. *Drosophila* male sex peptide inhibits siesta sleep and promotes locomotor activity in the post-mated female. *Proc. R. Soc. Lond. B* **277**: 65–70.

Ja, W.W., Carvalho, G.B., Zid, B.M., Mak, E.M., Brummel, T. & Benzer, S. 2009. Water- and nutrient-dependent effects of dietary restriction on *Drosophila* lifespan. *Proc. Natl Acad. Sci. USA* **106**: 18633–18637.

Jalali, M.A., Tirry, L. & de Clercq, P. 2009. Food consumption and immature growth of *Adalia bipunctata* (Coleoptera: Coccinellidae) on a natural prey and a factitious food. *Eur. J. Entomol.* **106**: 193–198.

Johnstone, R.A. & Keller, L. 2000. How males can gain by harming their mates: sexual conflict, seminal toxins, and the cost of mating. *Am. Nat.* **156**: 368–377.

Kaufman, W.R. 2007. Gluttony and sex in female ixodid ticks: how do they compare to other blood-sucking arthropods? *J. Insect Physiol.* **53**: 264–273.

Mondet, C., Abed-Vieillard, D., Gautier, P. & Farine, J.-P. 2008. Could male tergal secretions be considered as a nuptial gift in the Madeira cockroach? *Anim. Behav.* **75**: 451–460.

Perry, J.C. & Rowe, L. 2008a. Ingested spermatophores accelerate reproduction and increase mating resistance but are not a source of sexual conflict. *Anim. Behav.* **76**: 993–1000.

Perry, J.C. & Rowe, L. 2008b. Neither mating rate nor spermatophore feeding influences longevity in a ladybird beetle. *Ethology* **114**: 504–511.

Perry, J.C. & Rowe, L. 2010. Condition-dependent ejaculate size and composition in a ladybird beetle. *Proc. R. Soc. Lond. B* **277**: 3639–3647.

Perry, J.C., Sharpe, D.M.T. & Rowe, L. 2009. Condition-dependent female remating resistance generates sexual selection on male size in a ladybird beetle. *Anim. Behav.* **77**: 743–748.

Ram, K.R. & Wolfner, M.F. 2007. Seminal influences: *Drosophila acps* and the molecular interplay between males and females during reproduction. *Int. Comp. Biol.* **47**: 427–445.

Ransford, M.O. 1997. *Sperm Competition in the 2-Spot Ladybird, Adalia bipunctata*, PhD Thesis. University of Cambridge, Cambridge.

Rowe, L., Cameron, E. & Day, T. 2005. Escalation, retreat, and female indifference as alternative outcomes of sexually antagonistic coevolution. *Am. Nat.* **165**: S5–S18.

Sakaluk, S.K. 2000. Sensory exploitation as an evolutionary origin to nuptial food gifts in insects. *Proc. R. Soc. Lond. B* **267**: 339–343.

Sakaluk, S.K., Avery, R.L. & Weddle, C.B. 2006. Cryptic sexual conflict in gift-giving insects: chasing the chase-away. *Am. Nat.* **167**: 94–104.

Simmons, L.W. 1988. The contribution of multiple mating and spermatophore consumption to the lifetime reproductive success of female field crickets (*Gryllus bimaculatus*). *Ecol. Entomol.* **13**: 57–69.

Simmons, L.W. & Gwynne, D. 1991. The refractory period of female katydids (Orthoptera: Tettigoniidae): sexual conflict over the remating interval? *Behav. Ecol.* **2**: 276–282.

Stewart, L.A., Dixon, A.F.G., Ruzicka, Z. & Iperti, G. 1991a. Clutch and egg size in ladybird beetles. *Entomophaga* **36**: 329–333.

Stewart, L.A., Hemptinne, J.-L. & Dixon, A.F.G. 1991b. Reproductive tactics of ladybird beetles: relationships between egg size, ovariole number and developmental time. *Funct. Ecol.* **5**: 380–385.

Vahed, K. 2003. Increases in egg production in multiply mated female bushcrickets *Leptophyes punctatissima* are not due to substances in the nuptial gift. *Ecol. Entomol.* **28**: 124–128.

Vahed, K. 2007a. All that glisters is not gold: sensory bias, sexual conflict and nuptial feeding in insects and spiders. *Ethology* **113**: 105–127.

Vahed, K. 2007b. Comparative evidence for a cost to males of manipulating females in bushcrickets. *Behav. Ecol.* **18**: 499–506.

Vahed, K. & Gilbert, F.S. 1997. No effect of nuptial gift consumption on female reproductive output in the bushcricket *Leptophyes laticauda* Friv. *Ecol. Entomol.* **22**: 479–482.

Wada-Katsumata, A., Ozaki, M., Yokohari, F., Nishikawa, M. & Nishida, R. 2009. Behavioral and electrophysiological studies on the sexually biased synergism between oligosaccharides and phospholipids in gustatory perception of nuptial secretion by the German cockroach. *J. Insect Physiol.* **55**: 742–750.

Warwick, S., Vahed, K., Raubenheimer, D. & Simpson, S.J. 2009. Free amino acids as phagostimulants in cricket nuptial gifts: support for the 'Candymaker' hypothesis. *Biol. Lett.* **5**: 194–196.

Wedell, N. & Arak, A. 1989. The wartbiter spermatophore and its effect on female reproductive output (Orthoptera: Tettigoniidae, *Decticus verrucivorus*). *Behav. Ecol. Sociobiol.* **24**: 117–125.

Wedell, N., Tregenza, T. & Simmons, L.W. 2008. Nuptial gifts fail to resolve a sexual conflict in an insect. *BMC Evol. Biol.* **8**: 204.

Wiklund, C., Karlsson, B. & Leimar, O. 2001. Sexual conflict and cooperation in butterfly reproduction: a comparative study of polyandry and female fitness. *Proc. R. Soc. Lond. B* **268**: 1661–1667.

Will, M.W. & Sakaluk, S.K. 1994. Courtship feeding in decorated crickets: is the spermatophylax a sham? *Anim. Behav.* **48**: 1309–1315.

Winston, P.W. & Bates, D.H. 1960. Saturated solutions for the control of humidity in biological research. *Ecology* **41**: 232–237.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Oviposition behaviour during a feeding trial and egg fertilization after the trial for females subjected to matings of different durations, or control females that did not mate.

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