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Female *Callosobruchus maculatus* mate when they are thirsty: resource-rich ejaculates as mating effort in a beetle

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Because male uncertainty over parentage limits the value of paternal investment in offspring, mate attraction and facilitation of ejaculate transfer are thought to be important functions of nuptial gifts. However, these are unlikely functions for valuable resources in ejaculates delivered inside the female. Instead, ejaculates containing costly nuptial gifts may be maintained because females alter their mating behaviour in response to the trade-off between the costs and benefits of mating. The value of receiving an additional gift should decrease with improved female physiological condition. Providing a female with a substantial gift will therefore make it less profitable for her to remate and reduce the risk of future sperm competition. Females of the bruchid beetle *Callosobruchus maculatus* are harmed by the spiny male genitalia during copulation but also appear to derive material benefits from the large ejaculates. I kept female *C. maculatus* with access to water and other females without access to water. All females were given the opportunity to mate with a new male every day. Females without access to water mated more frequently than females with access to water. I suggest that female *C. maculatus* mate more frequently to obtain water when dehydrated and that this may select for ejaculates containing large amounts of water in males. By providing their mates with a large amount of water, males can delay female remating and reduce the risk of future sperm competition.

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Males of some insect species, in particular among the Lepidoptera, Orthoptera and Coleoptera, provide females with nuptial gifts of very large ejaculates (Thornhill 1976; Vahed 1998). These ejaculates sometimes make up a substantial part of the males' body weight and at least some appear to contain valuable material resources since a number of studies have found that receiving large amounts of ejaculate improves the fecundity of females suffering from a lack of nutrients or water (e.g. the bruchid beetle *Caryedon serratus*: Boucher & Huignard 1987; the grasshopper *Chortippus brunneus*: Butlin et al. 1987; the fly *Drosophila mojavensis*: Markow et al. 1990; the bruchid beetle *Callosobruchus maculatus*: Fox 1993; the cricket *Gryllus sigillatus*: Ivy et al. 1999). From a male's point of view, nuptial gifts

containing material resources may function either as paternal investment, increasing female offspring production, or as mating effort, increasing the proportion of eggs fathered by the gift-giving male (Gwynne 1997; Vahed 1998). These functions are not mutually exclusive and their relative importance has been the subject of much debate. Male uncertainty over parentage will diminish the payoff from paternal investment (e.g. Wickler 1985; Vahed 1998). Most female insects are promiscuous and last-male sperm precedence is often high (Simmons 2001). Much of a male's investment is therefore likely to be wasted on production of offspring fathered by other males. Hence, mating effort is currently viewed as the most prevalent function of nuptial gifts (Vahed 1998).

Two main hypotheses regarding the function of nuptial gifts are relevant to how material resources per se may function as mating effort (Gwynne 1997; Vahed 1998). First, gifts may entice females to mate. Second, gifts may prolong ejaculate transfer. The second hypothesis clearly

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applies to the spermatophylax, a large spermless gelatinous mass that is part of the spermatophore of some male bushcrickets (Orthoptera: Tettigoniidae; Wedell & Arak 1989; Wedell 1991). Males attach the spermatophore externally to females and sperm transfer is interrupted when females remove the spermatophore. Since females remove and consume the spermatophylax before removing the sperm-containing ampulla, a large spermatophylax will prolong sperm transfer. However, it is not obvious how either of these two hypotheses may apply to material resources in ejaculates delivered inside the female's reproductive tract. Large ejaculates cannot be used to entice females to mate since it should be difficult for females to assess ejaculate size prior to mating (Kaitala & Wiklund 1995). Nor can they be used to maximize ejaculate transfer unless females somehow control copulation duration and allow ejaculate transfer to continue as long as males maintain a high enough rate of resource transfer.

The material benefits that some females appear to obtain from ejaculates may be derived from ejaculate components involved directly in sperm competition, such as the spermatozoa themselves, or from seminal substances manipulating female oviposition or mating propensity to avoid sperm competition (e.g. Wickler 1985; Eberhard 1996; Vahed 1998; Arnqvist & Nilsson 2000). If so, large ejaculates may both provide females with nutrients and at the same time serve as male mating effort (Wiklund & Kaitala 1995). However, males of gift-giving species that are able to satisfy the nutritional requirements of their mates may thereby make them less likely to remate (Thornhill 1976). That the transfer of material resources per se functions as mating effort may be particularly likely in species where mating conveys not only benefits but also substantial costs to females. While the costs of mating are likely to be additive or even multiplicative (Arnqvist & Nilsson 2000; Johnstone & Keller 2000), any material benefits should show diminishing returns since there must be an upper limit to the amount of resources any particular female is able to use (Arnqvist & Nilsson 2000). Gaining additional resources from nuptial gifts may be most valuable to females that are in poor condition. Hence, if females can adjust their mating behaviour actively in response to their need for additional resources, they should mate only when the benefits outweigh the costs. This provides males with an opportunity to influence female mating behaviour. Males that donate large gifts will make it less beneficial for their mates to remate and will therefore reduce the risk of future sperm competition. In addition, the more they invest, the more of their investment will go into production of their own offspring.

The bruchid beetle *Callosobruchus maculatus* (F.) is a good candidate for a species in which a large ejaculate containing valuable material resources may be maintained because females trade-off the costs of mating against the benefits and therefore delay remating when they receive large gifts that reduce the payoff of remating. Males of this species transfer large ejaculates in spermatophores, which weigh up to 10% of the body weight of virgin males (Savalli & Fox 1998; personal observation). Evidence suggests that females derive material resources from the

ejaculates. Several studies have found positive associations between the amount of ejaculate received and female fecundity or life span (but see Eady 1995). Fox (1993) found that multiple mating increased female longevity but only when females were kept without access to yeast and sugar-water. Ofuya (1995) and Savalli & Fox (1999) both found that the number of previous matings by males had a negative effect on female fecundity. Ejaculate size decreases with the number of previous matings in *C. maculatus* and these results were therefore likely to be caused by reduced amounts of transferred ejaculate (Savalli & Fox 1999). Edvardsson & Canal (2006) found that manipulation of copulation duration significantly affected the size of the transferred ejaculate in *C. maculatus*. Furthermore, females that received relatively large ejaculates had a relatively high lifetime offspring production. There was no effect of copulation duration on rate of oviposition suggesting that this result was not caused by oviposition stimulants in the ejaculates.

Mating also appears to carry substantial costs to *C. maculatus* females. The male genitalia bear spines that unfurl during copulation and puncture the female's reproductive tract (Crudgington & Siva-Jothy 2000). Females use their hindlegs to kick their mates during the last part of the copulation and, if they are prevented from kicking, copulations last longer and the injuries they sustain are more severe (Crudgington & Siva-Jothy 2000). The injuries appear to be costly to females since they produce fewer offspring over their lifetime if they are prevented from kicking during copulation (Edvardsson & Tregenza 2005). The costs and benefits of mating seem to cause a complex relation between mating rate and female fitness (e.g. Arnqvist et al. 2004).

Two predictions can be made if the large ejaculate of *C. maculatus* is maintained because females delay remating when they receive large gifts that reduce the benefits of remating. First, ejaculate size should have a negative effect on female remating propensity. This has been found in a number of species (reviewed in Eberhard 1996; Vahed 1998) including *C. maculatus*. Two studies where ejaculate size of male *C. maculatus* was manipulated by controlling male mating history found a negative effect of ejaculate size on female remating propensity after 16 h (Eady 1995; Savalli & Fox 1999) and 24 h (Eady 1995). Similarly, Edvardsson & Canal (2006) found a trend for a negative effect on remating propensity after 48 h when ejaculate size was manipulated by controlling copulation duration. These results are consistent with females delaying their remating when they receive valuable gifts but they do not confirm a direct effect of material resources since large ejaculates may influence female mating behaviour in other ways. For instance, in many species seminal substances and/or stretching of the female's reproductive tract make females less receptive to males (reviewed in Eberhard 1996; Simmons 2001). The second prediction is that females should reduce their mating rate when they are able to obtain resources included in the ejaculates at a lower cost from an alternative source. Some evidence supports this in *C. maculatus*. Savalli & Fox (1999) found that allowing females to feed on yeast and a sucrose-water solution after their first mating reduced the

proportion of females remating after 28 h. However, this effect was seen only when females had mated with virgin males in their first mating.

The material resource that female *C. maculatus* obtain from male ejaculates could be either nutrients or water. Although most studies of nuptial gifts have focused on nutrients, Ivy et al. (1999) found that females of the cricket *Gryllobates sigillatus* benefit from the water they obtain when consuming the gelatinous spermatophylax. *Callosobruchus maculatus* inhabits areas that are arid for much of the year (Labeyrie 1981). It is a pest of stored legumes and is likely to encounter a very dry environment in the stores it infests. Furthermore, *C. maculatus* is generally reared without access to water in the laboratory. Dehydration may therefore often limit female reproductive output and life span.

If female *C. maculatus* derive water from the large ejaculates and are able to change their mating behaviour in response to the trade-off between the need for additional water and the costs of mating, males would benefit from transferring large amounts of water by decreasing the females' remating propensity. Females would then be expected to mate less frequently when they have access to an alternative source of water. In three experiments, I evaluated the effects of having access to water on female longevity, fecundity and mating behaviour in *C. maculatus*.

METHODS

All individuals used in this study came from cultures maintained on black-eyed beans, *Vigna unguiculata*, and kept at 28°C. The females were all aged 1–2 days after eclosion at the time of their first mating. Eppendorf tubes filled with deionized water and sealed with cotton wool were used to provide females with water. No females had access to any other source of food or fluids. All mating trials were conducted at 21°C under ambient room lighting. Females and males taking part in the experiments were kept at 28°C and with a 12:12 h light:dark cycle in between mating trials.

Control of Female Drinking

The first experiment was done to verify that females drink from the Eppendorf tubes. I weighed virgin females ($\pm 1 \mu\text{g}$) on a Sartorius microbalance and then kept them isolated individually at 28°C either without access to water ($N = 35$) or with access to three Eppendorf tubes filled with water ($N = 32$). The females were weighed again after 24 h.

Benefits of Having Access to Water

The aim of the second experiment was to evaluate whether females benefit from having access to water in terms of longevity and lifetime offspring production. Virgin females were mated to virgin males and then placed in 60-mm petri dishes containing approximately 60 black-eyed beans and remained there until they died.

Females were kept with or without access to water and with or without two males. Thus, I had four experimental groups: group 1: no water, no males ($N = 18$); group 2: water, no males ($N = 15$); group 3: two males, no water ($N = 15$); group 4: two males, water ($N = 14$). Water was provided in three Eppendorf tubes as described above and males were virgin when they were placed with the once-mated females. The Eppendorf tubes were checked daily and refilled when necessary. I recorded the day of death for all females and the number of eggs laid.

Mating Behaviour and Access to Water

I conducted the third experiment to assess the effects of having access to water on female mating rate. Virgin females were mated to virgin males and then kept individually in petri dishes with approximately 60 beans. Half of the females had access to water provided in three Eppendorf tubes ($N = 40$) and half of the females did not have access to water ($N = 40$). Every 24 h, females were offered a virgin male for 10 min and I recorded whether they mated. This continued until all females were dead. I also recorded female life span and lifetime offspring production.

Statistical Analyses

All statistical analyses were carried out using SYSTAT 10 (Systat Software Inc., San Jose, U.S.A.). Data are presented as mean \pm SEM unless otherwise stated. Statistical tests are two tailed.

RESULTS

Control of Female Drinking

Females that did not have access to water lost significantly more weight than females that had access to water (without water: $0.132 \pm 0.008 \text{ mg}$; with water: $0.042 \pm 0.019 \text{ mg}$; Student's t test: $t_{65} = -4.50$, $P < 0.001$). Some females with access to water actually gained weight. Females were also observed to sit on the wet cotton wool and engage in what appeared to be drinking. Hence, females did drink from the Eppendorf tubes.

Benefits of Having Access to Water

An analysis of variance of female lifetime offspring production revealed a significant positive effect of water and a significant negative effect of presence of males (group 1, no water, no males: 86.6 ± 4.9 eggs; group 2, water, no males: 107.9 ± 4.5 eggs; group 3, males, no water: 66.2 ± 6.6 eggs; group 4, males, water: 99.9 ± 4.3 eggs; Table 1). Female longevity followed a similar pattern: an analysis of variance of female longevity (number of days until death) showed a significant positive effect of water and a significant negative effect of presence of males (group 1: 6.0 ± 0.4 ; group 2: 7.9 ± 0.4 ; group 3: 4.5 ± 0.3 ; group 4: 6.1 ± 0.2 ; Table 2). There was no significant interaction between access to water and presence of males

Table 1. Analysis of variance of female lifetime egg production in experiment 2

Source	Type III sums of squares	df	F	P
Access to water	11637	1	27.74	<0.001
Presence of males	3075	1	7.33	0.009
Water*males	592	1	1.41	0.24
Error	24331	58		

Multiple $R^2 = 0.379$.

for either lifetime offspring production or longevity (Tables 1, 2).

Mating Behaviour and Access to Water

In experiment 3, females that had access to water lived for significantly longer than females that did not have access to water (with water: 9.6 ± 0.3 days; without water: 8.1 ± 0.3 days; Mann–Whitney U test: $U = 354$, $N_1 = N_2 = 40$, $P < 0.001$). Water also had a significant positive effect on female lifetime offspring production with females that had access to water laying more eggs than females that did not have access to water (with water: 112.2 ± 3.9 ; without water: 84.9 ± 3.0 ; Student's t test: $t_{78} = -5.55$, $P < 0.001$). Females with access to water were significantly less likely to remate at the first remating opportunity after 24 h than females that did not have access to water (13/40 with water and 23/40 without water remated; Fisher's exact test: $P = 0.042$). They also had a lower mating rate over their entire life span resulting in a significantly lower total number of matings (with water: 1.95 ± 0.12 ; without water: 2.73 ± 0.15 ; Mann–Whitney U test: $U = 1161$, $N_1 = N_2 = 40$, $P < 0.001$).

DISCUSSION

My results show that female *C. maculatus* benefit from having access to water in terms of both longevity and lifetime offspring production. Presence of males, on the other hand, had a negative net effect on female longevity and offspring production regardless of whether females had access to water. Fox (1993) found that although female *C. maculatus* may benefit from receiving multiple ejaculates when housed without males, females confined continuously with three males did not differ in their lifetime offspring production from females mated singly. In

Table 2. Analysis of variance of female longevity in experiment 2

Source	Type III sums of squares	df	F	P
Access to water	50.05	1	31.42	<0.001
Presence of males	42.44	1	26.65	<0.01
Water*males	0.25	1	0.16	0.69
Error	92.38	58		

Multiple $R^2 = 0.495$.

that study and in my second experiment, females confined with males should have benefited from receiving additional ejaculates. However, when kept in petri dishes with only a single layer of beans, escaping the harassment of courting males is likely to be difficult for females and this may have resulted in significant costs of male harassment that outweighed the benefits derived from the ejaculates. Other insect studies have found similar costs of exposure to males (e.g. Partridge & Fowler 1990; Friberg & Arnqvist 2003).

My results also show that in spite of living longer and producing more offspring, females that have access to water mate fewer times than females that do not have access to water. This result could be an artefact of dehydrated females not having the strength to fend off harassing males during the mating trials. However, this seems unlikely for two reasons. First, there was already a difference in mating propensity at the first remating opportunity when all females should have been in good condition. Second, most females appeared to be very weak during the last day of their lives but this did not lead to an increase in mating propensity of females kept with access to water. In spite of undiminished male persistence, none of these females mated during the last day of their lives. Refusing to mate during a mating trial does not seem to be difficult even for females in poor condition. Hence, access to water appears to have a direct effect on female mating behaviour.

Studies have shown that female *C. maculatus* benefit from receiving large ejaculates (Fox 1993; Ofuya 1995; Savalli & Fox 1999; Edvardsson & Canal 2006). There is also evidence that females may benefit from receiving multiple ejaculates but only when they are kept without access to yeast and sugar–water (Fox 1993). That females mated more frequently in the current study when they did not have access to water suggests that the benefits are, at least partly, hydration benefits. Although water has been put forward as a potentially valuable component of nuptial gifts (e.g. Thornhill 1976), the only other study to demonstrate hydration benefits to females of receiving nuptial gifts is Ivy et al.'s (1999) study of the cricket *G. sigillatus*. Previous studies of *C. maculatus* have also demonstrated costs of mating caused by the spines on male genitalia (Crudginton & Siva-Jothy 2000; Edvardsson & Tregenza 2005) and have shown that females reduce their remating propensity when they receive relatively large ejaculates (Eady 1995; Savalli & Fox 1999). In combination, the earlier work and the present study suggest that females face a trade-off between the costs and benefits of mating (Arnqvist et al. 2004) and respond actively to this trade-off by changing their mating rate in accordance with their need for additional material resources. Males that provide females with a large amount of water, and possibly also nutrients, will make it less beneficial for females to remate, and benefit from delayed female remating. This logic has parallels to Johnstone & Keller's (2000) suggestion that males may benefit from harming their mates when there are escalating costs of cumulative damage: males make it more costly for females to remate the more harm they inflict; here, males make it less beneficial for females to remate the more resources they provide.

There must be a reason why males evolved ejaculates that are large enough for females to derive material resources from in the first place. This may have occurred because males benefit from paternal investment but also because of selection on males to transfer large numbers of sperm or manipulative seminal substances to meet sperm competition (Wickler 1985; Simmons & Parker 1989; Cordero 1996; Arnqvist & Nilsson 2000). Females may then have evolved the ability to metabolize sperm, spermatophores and seminal substances or to derive water from ejaculates.

The reasoning outlined here is based on the idea that there are substantial costs of mating to females. Although usually less obvious than in species such as *C. maculatus* (Crudgington & Siva-Jothy 2000) and the dung fly *Sepsis cynipsea* (Blanckenhorn et al. 2002), where males harm females physically during mating, some costs of mating appear to be ubiquitous (e.g. Daly 1978; Arnqvist & Nilsson 2000). For example, the seminal fluids of male insects can be toxic to females (Chapman et al. 1995) and there can be energetic costs of mating (Watson et al. 1998). Mating can also entail costs of increased risk of predation (e.g. Arnqvist 1989) and parasite transfer (e.g. Hurst et al. 1995). Females also need to be able to change their mating propensity in response to their need for material resources independently of the manipulative substances that males transfer with the ejaculates (Eberhard 1996; Simmons 2001). It appears that *C. maculatus* is not the only species in which this occurs since females of two katydid species have been found to shorten their refractory periods when nutritionally stressed (Gwynne 1990; Simmons & Gwynne 1991). In addition, female sagebush crickets, *Cyphoderris strepitans*, which feed on the fleshy hindwings of the male during mating, increase their mating propensity when maintained on a nutrient-poor diet and remate sooner when they have received a relatively small wing meal (Johnson et al. 1999). The mating behaviour of females of the water strider *Gerris buenoi*, a species without nuptial gifts, also seems to depend on female condition. In this species, starvation makes females more reluctant to mate, presumably because mating conflicts with foraging (Ortigosa & Rowe 2002). In all, this makes an active female trade-off between costs and material benefits a potentially important mechanism behind the maintenance of large ejaculates containing valuable material resources. The possible functions of nuptial gifts are not mutually exclusive, however (Gwynne 1997; Vahed 1998). If females metabolize sperm for example, sperm could serve both as paternal investment by providing females with nutrients and as mating effort by making it less valuable for females to remate and by competing to fertilize the eggs. Future research into the composition of ejaculates and the effects of seminal substances on females will shed more light on the relative importance of material resources, manipulative substances and sperm in the evolution of insect ejaculates.

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