Physiological Entomology

Physiological Entomology (2009) **34,** 370–378 DOI: 10.1111/j.1365-3032.2009.00700.x

Diet affects female mating behaviour in a seed-feeding beetle

CHARLES W. FOX[1] and JORDI MOYA-LARAÑO[2]

[1]Department of Entomology, University of Kentucky, Lexington, Kentucky, U.S.A. and [2]Departmento de Biología de Organismos y Sistemas, Cantabric Institute of Biodiversity (ICAB), Unviersidad de Oviedo, Oviedo, Spain

Abstract. In species where males provide nuptial gifts, females can improve their nutritional status and thus increase their fecundity by mating when in need of resources. However, mating can be costly, so females should only mate to acquire resources when the need for resources is large, such as when females are nutritionally-deprived. Two populations of the seed-feeding beetle *Callosobruchus maculatus*, a species that produces relatively large nuptial gifts, are used to test whether female nutritional status affects mating behaviour. Female access to water, sugar and yeast are manipulated and the fitness consequences of these manipulations are examined together with the effects of diet on the propensity of nonvirgin females to mate. Access to water has a small but significant effect on mass loss over time, lifespan and fecundity of females, relative to unfed controls. Access to sugar (dissolved in water) improves female fecundity and lifespan above that of hydrated females but access to yeast has no positive effects on female survival or reproduction. Diet has a large effect on both receptivity of nonvirgin females to a male and how quickly they accept that male. Unfed females are both more likely to mate, and accept a mate more quickly, than females provided access to water, which are more likely to mate and accept a mate more quickly than females provided with sugar. This rank order of behaviours matches the order predicted if females increase their mating rate when nutritionally deprived (i.e. it matches the effect of diet on female fitness). The results obtained also suggest that mate choice may be condition-dependent: females from one population (Burkina Faso) show a preference for large males when well-fed but not when unfed, although this result is not found in a second population (South India). It is concluded that nutritionally-deprived females are more receptive to mates than are well-fed females, consistent with the hypothesis that females 'forage' for nuptial gifts, or at least more willingly accept sperm in exchange for nuptial gifts, when they are nutritionally deprived.

Key words. *Callosobruchus*, mating behaviour, nuptial gift, polyandry, seed beetles, spermatophore.

Introduction

Males of many arthropod species produce nuptial gifts that are given to females either before or during mating (Vahed, 1998; Gwynne, 2008). Large nuptial gifts affect male fitness by increasing female fecundity (and thus the number of offspring sired) and by delaying female remating (i.e. reducing the risk of future sperm competition)(Vahed, 2006, 2007). Nuptial gifts also provide an obvious direct benefit to females: increased nutrients for reproduction and somatic maintenance. However, mating can be costly for females and it is unclear whether the benefits of obtaining nuptial gifts outweigh the cost of mating. For example, in the seed-feeding beetle *Callosobruchus maculatus* (F.), males do substantial physical damage to females during mating (Crudgington & Siva-Jothy, 2000; Edvardsson & Tregenza, 2005). Physical damage and other costs of mating may offset the nutritional benefits that

Correspondence: Charles Fox, Department of Entomology, S-225 Agricultural Science Center North, University of Kentucky, Lexington, Kentucky 40546-0091, U.S.A. Tel.: +1 859 257 7474; fax: +1 859 323 1120; e-mail: fox@uky.edu

females receive from nuptial gifts (Gwynne, 2008), leading to complex relationships between mating rate and female fitness (Arnqvist *et al*., 2005).

The extent to which females benefit from the nutrients in nuptial gifts will depend on their ability to acquire resources in other ways. For example, females that have ready access to nectar or other food sources as adults may gain relatively little from male nuptial gifts, and may thus be selected to reduce their mating rate (Boggs, 1990, 1995; Gwynne, 2008). By contrast, females that have less access to adult-acquired resources (e.g. those that do not feed as adults), or that are lacking particular required nutrients, may receive benefits from nuptial gifts that outweigh the mating costs, and thus be favoured to mate often. Comparative studies (i.e. among populations or among species) have shown that adult food intake rates correlate with mating rates (Boggs, 1990). Similarly, mating rate may be phenotypically plastic, with females that are unsuccessful at finding alternate resources increasing their mating rate (i.e. females may forage for nuptial gifts), and females that have adequate alternative sources of nutrients reducing their mating rate to avoid the costs of mating (Boggs, 1990).

Substantial effort has been dedicated to modelling (Kondoh, 2001) and testing hypotheses (Katvala *et al*., 2008) regarding the evolution of mating rate, and the evolution of nuptial gift sizes in response to mating rate. However, little attention has been dedicated to the question of whether individual females increase their mating rate when deprived of nutritional resources, which is the key prediction of the hypothesis that females forage for nuptial gifts to offset nutritional restrictions. Diet manipulation studies have shown that food-deprived females increase mating rates in katydids (Gwynne, 1990; Simmons & Gwynne, 1991), sagebruch crickets (Johnson *et al*., 1999), seed beetles (Takakura, 2004) and nursery-web spiders (Bilde *et al*., 2007). Interestingly, in species without nuptial gifts, food-deprived females either tend to mate at a lower rate relative to well-fed females (waterstriders, Ortigosa & Rowe, 2002) or no differences are found (spiders, Wilder & Rypstra, 2008), consistent with the hypothesis that an increase in the mating rate in food-deprived females of species with nuptial gifts is a result of the nutritional value of the gift.

In the seed-feeding beetle *C. maculatus*, virgin males produce ejaculates that typically weigh 6–10% of the male's body mass (Fox, 1993a; Savalli & Fox, 1998a), although the proportion of their mass transferred declines substantially in subsequent matings (Fox *et al*., 1995; Savalli & Fox, 1999b). For all seed beetle species in which nuptial gifts have been studied, substances in male ejaculates are incorporated into female eggs and somatic tissues (Das *et al*., 1980; Huignard, 1983; Boucher & Huignard, 1987). These male nuptial gifts appear to have positive effects on female fitness but the fitness advantages of multiple mating are dependent on female diet (Fox, 1993a, 1993b). For example, multiply-mating females, which receive multiple nuptial gifts, generally have higher fecundity (Fox, 1993b; Savalli & Fox, 1999a; Wilson *et al*., 1999; but see also Arnqvist *et al*., 2005), an increased adult lifespan (Fox, 1993b; but see also Savalli & Fox, 1999a) and their egg size declines more slowly with age (Wasserman & Asami, 1985; Fox, 1993a), and all are consistent with females using materials in male nuptial gifts for egg production and somatic maintenance. However, males also do substantial physical damage to females during mating (Crudgington & Siva-Jothy, 2000; Edvardsson & Tregenza, 2005), which appears to offset much of the advantage that females gain from multiple mating (Eady *et al*., 2007), such that an intermediate mating rate maximizes females fitness (Arnqvist *et al*., 2005).

Female *C. maculatus* that mate with nonvirgin males (which produce smaller ejaculates than virgin males), or those that produce small nuptial gifts, are more likely to remate than are females that mate with virgin males (Savalli & Fox, 1999a; Edvardsson & Canal, 2006), and these effects are not likely caused by sperm limitation (Fox *et al*., 2007). These results suggest that water, nutrients or other components of the male ejaculate affect both female fitness and female propensity to remate. However, none of these results can disentangle the nutritional effects of ejaculates from the effects of other substances found in the ejaculate (e.g. chemical manipulation of female receptivity). Because *C. maculatus* inhabits environments that are arid for much of the year, and because the beetle mainly occurs in storage environment (where they have access only to metabolic water), it is suggested that the main advantage to females of multiple mating is the acquisition of water rather than metabolizable nutrients, and this hypothesis is consistent with the experimental results obtained for *C. maculatus* (Edvardsson, 2007; Ursprung *et al*., 2009).

In the present study, the hypothesis that females of the seed-feeding beetle *C. maculatus* forage for matings when in need of nutrients for reproduction is tested in two populations previously demonstrated to differ in female receptivity to mating. First, in a series of three experiments, female nutritional status is manipulated by differentially allowing females access to food and water in six diet treatments, and the fitness effects of these six female diets is then quantified. Second, whether access to water and/or sugar, relative to unfed controls, affects subsequent female willingness to remate in the pattern predicted by the effects of diet of female survival and reproduction is tested.

Material and methods

Study populations

The life cycle of *C. maculatus* revolves around seeds of its host plant. Females cement their eggs to the surface of host seeds, particularly beans of the genus *Vigna*. First-instar larvae burrow through the seed coat and into the seed. Larval development and pupation are completed entirely within a single seed; they do not emerge until adulthood. Emerging adults mate and females begin to lay eggs within hours of emerging from the seed. Adults are facultatively aphagous (i.e. they require neither food nor water in the adult stage). Adults reproduce using primarily metabolic water and the resources acquired during larval development (i.e. they are capital breeders) (Messina & Slade, 1999). In some bruchids, the adults commonly feed on pollen, floral or extra-floral nectaries or leaf fungi, and these nutrients are used for maturation of eggs (Clement, 1992). However, in *Callosobruchus*, access to adult resources has only a small, but detectable, positive effect on female fecundity and improves adult lifespan (Shinoda & Yoshida, 1987; Fox, 1993a; Tatar & Carey, 1995).

Diet effects on female survivorship, reproduction and mating behaviour were examined in two populations of *C. maculatus* that were collected from and maintained on different legume hosts. The South India (SI) population was collected in 1979 from infested pods of mung bean *Vigna radiata* (L.) Wilczek and the closely-related black gram *Vigna mungo* (L.) Hepper in Tirunelveli, India (Mitchell, 1991). The Burkina Faso (BF) population was collected in 1989 from infested pods of cowpea *V. unguiculata* (L.) Walp. in Ouagadougou, Burkina Faso (Messina, 1993). These two populations differ in body size, lifetime fecundity, patterns of egg dispersion, oviposition preference and adult longevity. They also differ in female receptivity to males (in within-population pairings, SI females mate more readily than do BF females)(Messina *et al*., 2007). Both populations were maintained in laboratory growth chambers on seeds of *V. radiata* (SI) or *V. unguiculata* (BF) at >1000 adults per generation for >100 generations (BF) or >200 generations (SI) prior to this experiment.

All experiments were performed under an LD 14:10 h photoperiod at 26°C.

Mass loss

Experiment 1. This first experiment was performed to confirm that diet manipulation affects the nutritional status of females. Virgin females ($n = 40$ per diet per population) were confined individually in 60-mm sterile Petri dishes and assigned to one of six diet treatments: 'Control' (no access to food or water); 'Yeast' (5 mg of dry baker's yeast, *Saccharomyces cerevisiae*, presented in a small parafilm cup); 'Water' (a 38-mm braided dental cotton roll soaked in distilled water); 'Sugar' (a cotton roll soaked in a 10% sucrose solution); or 'Water + Yeast' and 'Sugar + Yeast'. Beetles were weighed on an electronic microbalance (Mettler-Toledo AT261; Mettler-Toledo Ltd, U.K.) to within 0.01 mg precision within 24 h of their emergence from their rearing seed and then again each 24 h until day 15 (or until death). Water and sugar water were replenished daily either by spraying cotton wicks (if they were clean) or replacing cotton wicks (when mold began to grow on them). Periodic observations (at 12-h intervals for a subsample of the beetles) demonstrated that beetles came into regular physical contact with all diet sources. The experiment was also performed on males, although the results obtained were qualitatively identical to those for females, and thus are not reported here.

Diet effects on lifespan and female reproduction

In two sequential experiments, how diet manipulation (the same six treatments as above) affects the lifespan of virgin beetles (*Experiment 2*) and the lifespan and fecundity of mated beetles (*Experiment 3*) was examined.

Experiment 2. This experiment was identical to Experiment 1, except that beetles were weighed only on day 1 and not handled afterwards. The date of death was recorded for all beetles ($n = 30$ females per diet per population).

Experiment 3. This experiment was identical to Experiment 2, except that females were mated once (within 24 h of emergence) to a single virgin male that was 24–48 h post-emergence (male age was controlled because male age affects ejaculate size ; Fox *et al*., 1995). Females were then confined in a 100-mm Petri dish with approximately 150 seeds of mung (*V. radiata*) (estimated by volume) on which they could lay eggs. The date of death and lifetime fecundity were recorded for all females up until day 25, after which offspring produced by females started emerging. Dishes were thus frozen in a freezer at −20°C at 25 days, and all females still alive at 25 days were treated as censored data in subsequent lifespan analyses. Note that females remained alive at 25 days in only two treatments (greater than half remained alive in the 'Sugar' and 'Sugar + Yeast' treatments), so means for lifespan of mated females in these treatments should be treated as significant underestimates. Females lay the overwhelming majority of their eggs within 8 days of mating (Fox, 1993b) and rarely lay any eggs at ages this advanced; it is thus unlikely that truncating the experiment at 25 days had any effect on fecundity estimates.

After mating, males were transferred to a clean 35-mm Petri dish with the same treatment as their mate, and maintained as in Experiment 1 until death. However, the results obtained were qualitatively identical to those for females and thus are not reported here.

Diet effects on female mating

Experiment 4. To test the hypothesis that nutritional deprivation affects mating behaviour of females, the prediction that female diet (which clearly affected female nutritional status) affects female remating probability was tested. As in Experiment 3, newly-emerged virgin females were mated with a 24–48-h-old adult virgin male. Immediately after mating, females were confined in a 60-mm Petri dish with approximately 50 mung seeds (estimated by volume) and one of three diet treatments, 'Control', 'Water' or 'Sugar', as described above ($n = 32–39$ females per diet per population). Yeast was not used because the previous experiments demonstrated that access to yeast had no significant positive effect on lifespan or fecundity of females.

Twenty four hours after their first mating, females were presented with a new virgin male, who was 24–48 h post-emergence, in a clean 60-mm Petri dish. Beetles were observed for 10 min and scored for whether they mated within this period and when they mated (in 1-min intervals). If a male did not attempt to mate with a female within 1 min of their initial contact, the male was replaced with a new virgin male; however, this was necessary in only approximately 1% of pairings and did not differ between diet treatments. All mating trials were performed at mid-day, approximately 5 h after lights on in growth chambers.

In the SI population, the majority of females remated after 24 h. However, remating rate after 24 h was much lower in the BF population, and the results were unclear (trends were observed but none were statistically significant; see Results). This experiment was thus repeated using only the BF population and examining female remating probability after 48 h (instead of after 24 h) ($n = 43–45$ females per diet).

The effects of diet, female size, male size (first and second male) and female egg production (i.e. fecundity between the first and second mating) on the probability of females remating was examined with logistic regression. Females that failed to lay eggs in the 24 h (or 48 h) after their first mating (eight BF females and no SI females) were not included in the analysis.

Results

Diet affects mass loss of virgin females

There was no significant difference in the mass of newly-emerged females that were assigned to the six food treatments ($F_{5,473} = 0.22$, $P = 0.95$). However, there was a large effect of diet on the amount of mass lost by virgin females over their lifetime [repeated measures analysis of variance (ANOVA), diet × age interaction, $F > 11.4$, $P < 0.001$ for both beetle populations; Fig. 1]; the change in mass with age differed between all pairs of treatments (all linear contrasts, $P < 0.1$), except for between the 'Sugar' and 'Sugar + Yeast' treatments ($P = 0.66$). Rank ordered by treatment, from most to least mass loss, Control > Yeast > Water > Water + Yeast > Sugar = Sugar + Yeast. Although females from the SI population are larger than females from the BF population (by approximately 10%), there was no significant population × treatment interaction and no significant population × age interaction.

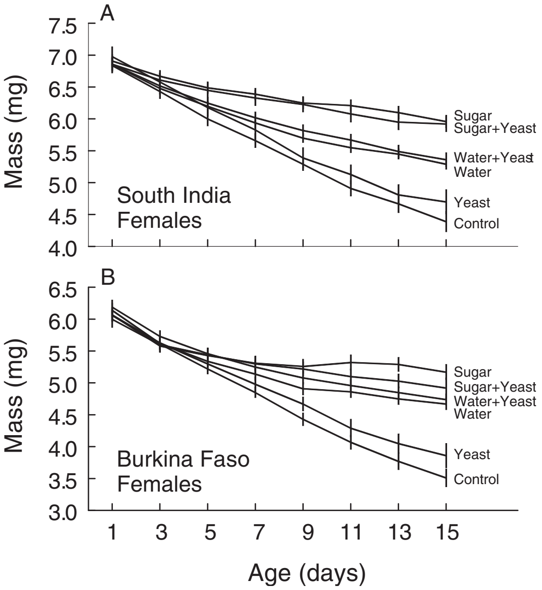

Fig. 1. Mean ± SE loss of mass (mg) over a 15-day period in virgin *Callosobruchus maculatus* females fed six different diets. (A) South India population, (B) Burkina Faso population.

This first experiment demonstrates that the diet treatments affect beetle nutritional status, quantified by effects on mass loss with age.

Diet affects lifespan and reproduction

Diet had a large effect on the lifespan of virgin females (Cox proportional hazards, $\chi^2 = 120.6$, d.f. = 5, $P < 0.001$) and mated females ($\chi^2 = 120.0$, d.f. = 5, $P < 0.001$; Fig. 2A,B). However, only one particular diet supplement (i.e. 'Sugar') was responsible for the majority of the diet effect. Linear contrasts indicated that lifespan of beetles in both sugar treatments ('Sugar' and 'Sugar + Yeast') differed significantly from all other treatments ($P < 0.001$ for all contrasts), although the two sugar treatments did not differ from each other ($P > 0.13$ for each analysis). Because sugar was provided in solution, sugar is always confounded with the presence of water. However, providing water alone had little or no effect on lifespan (linear contrasts, 'Water' versus 'Control', $\chi^2 = 0.12$, d.f. = 1, $P = 0.73$ for virgin females, and $\chi^2 = 1.21$, d.f. = 1, $P = 0.27$ for mated females), whereas providing sugar dissolved in water (the 'Sugar' treatment) significantly improved survival over water alone ($P < 0.001$ for both virgin and mated females).

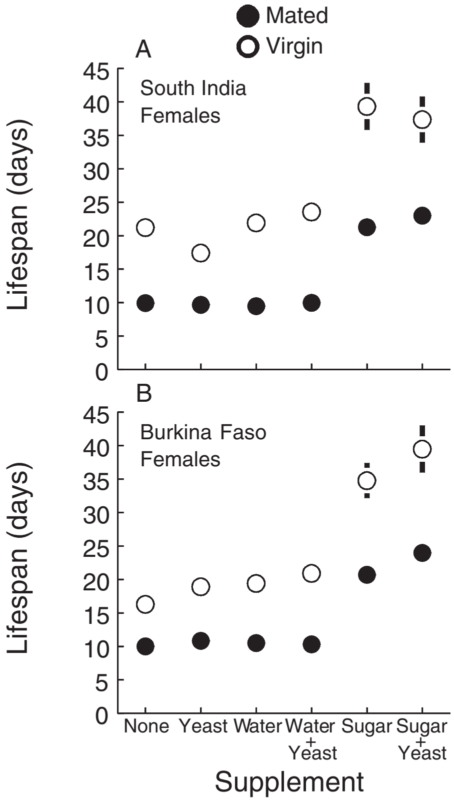

Fig. 2. Mean ± SE lifespan (days) of mated and virgin *Callosobruchus maculatus* females fed six different diets. (A) South India population, (B) Burkina Faso population. Error bars are present but smaller than most of the points.

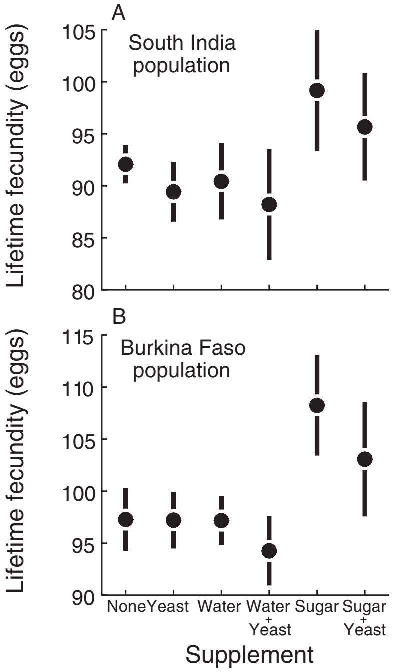

Fig. 3. Mean ± SE fecundity (eggs) of *Callosobruchus maculatus* females fed six different diets. (A) South India population, (B) Burkina Faso population.

Although there was a significant population × treatment interaction for lifespan of virgin females ($\chi^2 = 18.0$, d.f. $= 5$, $P = 0.003$), this indicated a small difference in the magnitude of diet effects between populations rather than differences in the pattern of diet effects. There was no significant population × treatment interaction for lifespan of mated females ($\chi^2 = 2.86$, d.f. $= 5$, $P = 0.72$).

The pattern of results for female lifetime fecundity was qualitatively the same as for lifespan. Diet had a large effect on the lifetime fecundity of females that had been mated only one time ($F_{5,329} = 2.7$, $P = 0.02$; Fig. 3), but only one particular diet supplement (i.e. sugar) affected fecundity. Fecundity of females in both sugar treatments differed significantly from all other treatments ($P < 0.05$ for each linear contrast) but the 'Sugar' treatment did not differ significantly from the 'Sugar + Yeast' treatment ($P = 0.26$). Neither the 'Water' nor the 'Yeast' treatments differed significantly from the 'Control' treatment (linear contrast, $P > 0.73$ for both). There was no treatment × population interaction, indicating that diet had similar effects on females from both populations ($F_{1,329} = 0.04$, $P = 0.99$). Diet treatment had no effect on the probability that an egg developed or the probability that a developed egg hatched ($F_{1,329} < 0.49$, $P > 0.78$).

These adult lifespan and reproduction data demonstrate that, although all five diet treatments reduce the amount of mass lost by females (and thus presumably beetle nutritional status) relative to unfed controls, only one diet component affects female fitness; female access to sugar has a very large effect on lifespan, and a moderate but ecologically significant effect on fecundity.

Diet affects female mating

When presented with a new male 24 h after their first mating, BF females were much less likely to remate than were SI females (45 versus 86% for BF and SI, respectively, averaged across treatments; highly significant population effect in logistic regression, $\chi^2 = 30.07$, d.f. $= 1$, $P < 0.001$). Of those females that mated, BF females also mated less quickly than did SI females (within 3.8 min for BF females, versus 2.6 min for SI females, averaged across treatments; population effect in ANOVA, $F_{1,145} = 49.7$, $P < 0.001$). The effect of diet treatment on the female remating probability after 24 h also differed between populations (there was a significant population × treatment interaction, $\chi^2 = 10.6$, d.f. $= 2$, $P = 0.005$). The two populations were thus analysed separately.

South India population. Most SI females remated when presented with a new male 24 h after first mating (86%, averaged across treatments). However, the proportion remating varied among treatments ($\chi^2 = 6.9$, d.f. $= 2$, $P = 0.03$). Only one of the females denied access to water or sugar failed to remate (Fig. 4A). By contrast, only 74% of the females provided sugar remated, with the frequency of remating intermediate (88%) for females provided access to only water. All linear contrasts between the treatments were significant ($\chi^2 > 4.3$, d.f. $= 1$, $P < 0.04$) indicating that females provided sugar were less likely to remate than females provided only water, and females provided only water were less likely to remate than females with no diet supplement. The difference in remating probability among treatments was not explainable by effects on female fecundity; the number of eggs laid by females in the 24 h between their first mating and exposure to a second male did not differ among treatments ($F_{2,114} = 2.6$, $P = 0.08$) and, when fecundity was included as a covariate in the logistic regression, there was no effect of the number of eggs laid on the probability of remating ($\chi^2 = 0.02$, d.f. $= 1$, $P = 0.88$).

Of SI females that remated when presented with a second male, diet affected how quickly they remated ($F_{2,98} = 7.1$, $P = 0.001$; Fig. 4B); 'Control' females remated sooner (in 1.9 ± 0.2 min) than did females provided access to either water or sugar (2.9 ± 0.3 and 3.1 ± 0.5, respectively; linear contrasts, $F > 8.4$, $P < 0.005$ for both). However, there was no difference in how quickly females remated when provided water versus sugar ($P = 0.76$).

On average, larger females were slightly more likely to remate than were smaller females (female size as an independent variable in the logistic regression; $\chi^2 = 6.3$, d.f. $= 1$, $P = 0.01$), and larger females remated more quickly than did smaller females (female size as covariate in an ANOVA, $t = -2.53$, $P = 0.01$). Neither first male nor second male size had any effect on whether females remated ($P > 0.14$ for both), although females remated more quickly when paired with a larger second male ($t = -2.59$, $P = 0.011$). None of the effects of female and male size on female remating and time to remate differed between treatments (i.e. there were no significant interactions), and inclusion of body size did not affect the significance of the treatment effects or any linear contrast.

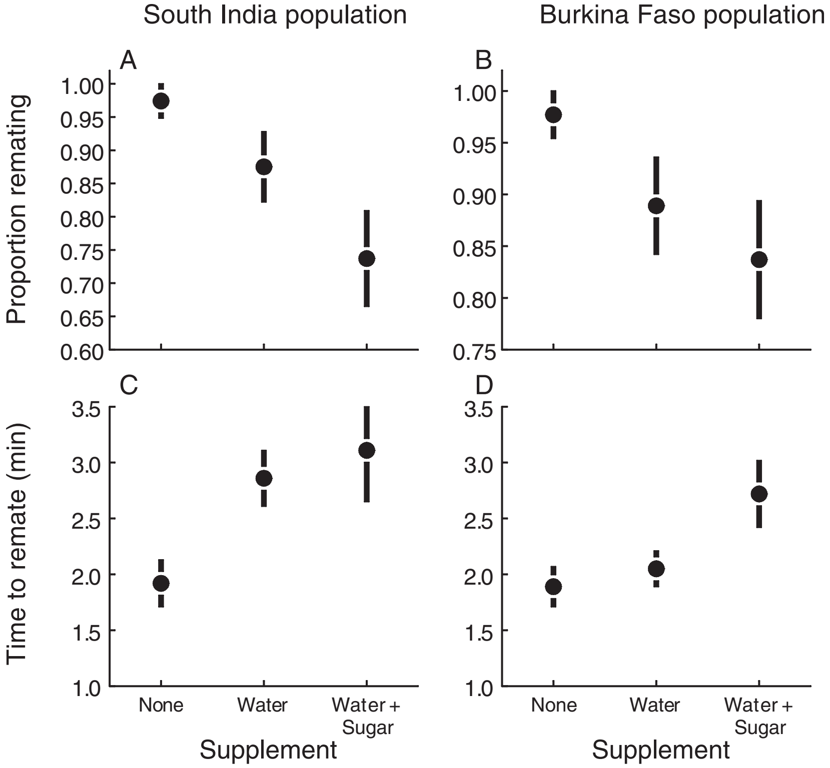

Fig. 4. Frequency of mating (A, B) and time to mate (C, D) of nonvirgin *Callosobruchus maculatus* females presented a virgin male 24 h (South India population) or 48 h (Burkina Faso population) after their first mating.

Burkina Faso population. When BF females were presented to a virgin male 24 h after their first mating, diet treatment had no effect on either the probability of female remating ($\chi^2 = 3.3$, d.f. = 2, $P = 0.19$) or on the time for females to remate ($F_{2,41} = 0.05$, $P = 0.95$). The linear contrasts were consistent with the overall analyses in that none of the pairwise contrasts were significant; treatment did not affect female behaviour.

However, when females were presented with a virgin male 48 h after their first mating, females confined with sugar were significantly less likely to remate than were females confined with only water ($\chi^2 = 4.0$, d.f. = 1, $P = 0.045$), and females confined with only water were less likely to remate than were 'Control' females ($\chi^2 = 3.6$, d.f. = 1, $P = 0.057$). Only one of the females denied access to water or sugar failed to remate (Fig. 4). By contrast, only 83% of females provided access to sugar remated. The probability of remating was intermediate for females provided access to water (87%). The difference in remating probability among treatments was not explainable by effects on female fecundity; the number of eggs laid by females in the 48 h between their first mating and exposure to a second male did not differ among diet treatments ($F_{2,130} = 0.6$, $P = 0.55$).

Of BF females that remated, diet affected how quickly they remated ($F_{2,116} = 3.1$, $P = 0.049$). Control females remated more quickly (average of within 1.9 ± 0.2 min) than did females provided access to sugar (2.7 ± 0.3 min, $P = 0.01$), although the time to remate for females provided access to water was not significantly different from either other treatment ($F < 2.1$, $P > 0.15$ for both linear contrasts).

There was no detectable effect of female body size, first male body size or second male body size on the probability that a female remated ($P > 0.08$ for all size effects and all interactions). Similarly, there was no effect of female size or first male body size on the time to remate, nor was there a significant interaction between these two variables and treatment. Inclusion of male and female body size did not affect the significance of the linear contrasts. However, there was a significant second male body size × treatment interaction ($F_{2,107} = 6.0$, $P = 0.003$), which was the result of a negative relationship between second male body mass and the time to remate in females provided sugar ($F_{1,32} = 8.7$, $P = 0.006$), and no significant relationships in the other two treatments (both $P > 0.18$).

Discussion

If nuptial gifts provide females with nonspecialized nutrients, including water and a source of calories, and if females forage for nuptial gifts when in need of these nutrients, the receptivity of females to courting male would be expected to correlate inversely with female dietary status and/or access to other sources of adult nutrition (Boggs, 1990; Gwynne, 2008). The present study demonstrates that the receptivity of female *C. maculatus* to courting males changes as predicted in response to their dietary status; females denied access to food and water are more likely to remate, and remate sooner, than females provided either water or sugar. This result agrees in part with previous studies of *C. maculatus* (Edvardsson, 2007; Ursprung *et al*., 2009) reporting that female water status affects remating probability. However, in contrast to the suggestion of Edvardsson (2007), and the conclusions of Ursprung *et al*. (2009), that females remate mainly to acquire the water in male ejaculates, the present study shows that water deprivation is not the only dietary variable affecting female remating. In the present study, females supplemented with water have an intermediate rate of mass loss relative to the nutrient-deprived and sugar treatments (note that the 'Sugar' treatment is sugar dissolved in water). Also, in the present study, females supplemented with water have neither higher fecundity nor longer lifespan than nutrient-deprived females, in contrast to the results of Edvardsson (2007) and Ursprung *et al*. (2009), whereas sugar-supplemented females have both higher fecundity and longer lifespan than nutrient-deprived females. These effects of diet correlate inversely with effects of diet on female mating behaviour; females with no access to sugar or water are more willing to remate, and remate more quickly, than do females supplemented with water or sugar water, but females supplemented with water remate more readily, and remate sooner, than do females provided access to sugar.

The result that nutritionally-deprived females increase their frequency of mating relative to fed females is evidence that nutrient-deprived females use males as a source of nutrition. Alternatively, the increased mating frequency of nutrient-deprived females may be as result of these females being smaller and/or weaker, and thus less able to resist mating attempts (Edvardsson, 2007). Additional experiments are necessary to distinguish these alternatives. However, a previous study on a different population of *C. maculatus* reports no evidence that female size affects the probability that she will remate (Savalli & Fox, 1999b). Similarly, old females do not increase their mating rate despite continued attempts by males (old females readily refuse to mate; Edvardsson, 2007). In the present study, nutrient-deprived females are not only more likely to remate, but also they remate sooner, most immediately after encountering the male, with little attempt at resistance. These observations, together with the fact that females remain active for many days after experimentation, searching for seeds and producing eggs, suggest that it is unlikely that control females are too small or have too little energy to flee males and resist matings. Instead, the lack of resistance to male courting suggests that females are more receptive to males when nutritionally-deprived (Ursprung *et al*., 2009). Even so, this may be a strategy by which nutrient-deprived females conserve resources (i.e. it is not that they are too exhausted to flee/resist but, instead, choose not to waste resources fleeing/resisting). However, considering the substantial costs to mating in this species, which would offset the small energetic costs of fleeing from a male (Crudgington & Siva-Jothy, 2000; Edvardsson & Tregenza, 2005; Eady *et al*., 2007), females should minimize their frequency of mating, except when the benefits are substantial (Arnqvist *et al*., 2005).

Both water and other nutrients are components of seed beetle ejaculates that could be important for females (Takakura, 2004; Arnqvist *et al*., 2005). The results obtained in the present study suggest that access to water does not affect female lifespan or fecundity in these seed feeding beetles, whereas access to sugar (calories) has a large effect on both the lifespan and fecundity of females. This is consistent with recent work on bushcrickets showing that females quickly metabolize carbohydrates in male nuptial gifts, and these gifts are an important energy resource for females (Voigt *et al*., 2008). However, this conclusion contrasts with those of Edvardsson (2007) and Ursprung *et al*. (2009) who show that access to water increases female fecundity. The population of origin is not mentioned in Edvardsson (2007), although it is likely to be different from the populations used in the present study, whereas Ursprung *et al*. (2009) report using the BF population as is used in the current study. Possibly the effect of water varies among populations and with the conditions under which the beetles are maintained. Regardless, the results obtained in the present study clearly indicate that water deprivation alone is unlikely to be the only source of selection favouring female multiple mating. Access to sugars may be important limiting factors for seed beetles, especially in grain stores where females have little access to other sugar sources (e.g. they have no access to floral or extra-floral nectar, which many bruchids use as a food source during the adult stage; Clement, 1992).

In some species, sexual selection depends on a female's nutritional status, possibly mediated through effects on mate choice (condition-dependent mate choice; Bonduriansky, 2001). Mate choice is expected to be costly and so females should be less choosy when the costs of choice are greater; for example, the ratio of benefit to costs of choice may be lower for females in poorer condition (Hunt *et al*., 2005). Alternatively, females may be less choosy when in need of the resources present in nuptial gifts. There are studies demonstrating that sexual selection varies with female diet (e.g. Ortigosa & Rowe, 2002), although few studies specifically demonstrate that female choosiness or preference are condition-dependent (Hunt *et al*., 2005; Fisher & Rosenthal, 2006; Hebets *et al*., 2008). Although previous studies in some seed beetles show that female remating is dependent on male body size (Savalli & Fox, 1998b), the results obtained in the present study are difficult to interpret. There is some evidence that females remate sooner when presented with a larger second male (in the SI but not BF population) and some evidence that diet affects this mate choice (a significant treatment × male size interaction for BF but not SI). Disentangling the nature of the treatment × male size interaction reveals that sugar-fed females mate sooner with larger second males, and this pattern is not found in other feeding treatments. This result might suggest that well-fed females can be more selective in mate choice, possible preferring larger, presumably higher-quality, mates for their second mating. However, these results are at best suggestive, and are not consistent between populations. Thus, further studies are needed before any robust conclusions can be reached regarding effects of female diet on female mate choice.

In conclusion, nutritionally-deprived female *C. maculatus* are more likely to mate with a second male than are well-fed females. The results are consistent with the hypothesis that nutrient-deprived females remate to acquire limiting resources, such as sugar and water, both of which are found to enhance some fitness components, with sugar (calories) being a more important limiting resource than water. The results also suggest that female nutritional status may affect mate choice, although the evidence is inconclusive.

Acknowledgements

We thank Frank Messina for providing the SI and BF colonies used in this experiment. We thank T. Tardy and W. Wallin for their assistance with these experiments. This work was funded in part by the University of Kentucky Agricultural Experiment Station.

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Accepted 13 July 2009