Female modulation of reproductive rate and its role in postmating prezygotic isolation in *Callosobruchus maculatus*

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Summary

1. Mechanisms that come into play after mating but prior to fertilization can prevent hybrid formation and thus promote reproductive isolation. Recent research indicates that the evolution of such barriers to gene exchange between incipient forms appears to be common and is essential for speciation.

2. We aimed to test if female Bean Weevils (*Callosobruchus maculatus*) modulate their reproductive rate and/or remating propensity in response to mating with males with varying degrees of relatedness in a manner that limits the number of 'hybrids' produced. We also tested if remating with a male from a female's own population, following a first mating with a foreign male, would elevate egg production.

3. Females varied their egg-production rate depending on the relatedness of their mates, but this effect was not in the predicted direction. Heterospecific *C. analis* males actually elicited the strongest reproductive response in females, which resulted in up to 9% higher egg production. Male relatedness did not significantly affect female propensity to remate with a second male. Further, females did not generally show a compensatory increase in reproductive rate following rematings with males from their own population.

4. The mechanisms documented here do not act to limit gene flow and are costly to females, as they suffer reduced life span and egg production late in life following a high reproductive rate early in life. We suggest that sexually antagonistic coevolution within species may have caused the pattern observed.

Introduction

In order to understand speciation, we need to understand factors that limit gene flow between species or forms. By tradition, two sets of reproductive isolation mechanisms have been recognized: premating isolation and postzygotic isolation (Dobzhansky 1937; Mayr 1970; Coyne & Orr 1989, 1997). Premating isolation occurs when matings between forms are less likely, as when different cues for mate recognition are involved in the acceptance or rejection of mates. Postzygotic isolation is the result of genetic incompatibilities in hybrids, resulting in hybrid infertility or inviability. More recently, attention has been given to a third form of reproductive isolation: postmating prezygotic, or cryptic, isolation (for reviews see Howard 1999; Eady 2001). This form of isolation reduces gene

flow after a mating has taken place but prior to zygote formation, and occurs whenever conspecific males have some form of fertilization advantage over heterospecific males. Although Dobzhansky (1937) was already aware of what he termed 'gametic isolation', we have only recently gained a more detailed understanding of this more subtle set of barriers to interbreeding (Albuquerque, Tauber & Tauber 1996; Markow 1997; Howard 1999; Alipaz, Wu & Karr 2001; Eady 2001).

For a potential isolation mechanism to be efficient, it should limit, or even prevent, gene flow between diverging populations or incipient species. The most widely cited example of a cryptic isolation mechanism is conspecific sperm precedence (Howard 1999). Although this form of isolation has been observed in several taxa (Nakano 1985; Hewitt, Mason & Nichols 1989; Robinson, Johnson & Wade 1994; Wade *et al*. 1994; Carney, Hodges & Arnold 1996; Price 1997; Howard *et al*. 1998a), our understanding of the

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mechanisms that generate conspecific sperm precedence is limited. Better understood in this respect are cases where direct interactions between the egg and sperm determine fertilization success. For example, incompatibilities between molecules involved in gamete recognition/fusion cause differential fertilization success in many plants (Carney *et al*. 1996; Kachroo *et al*. 2001); in marine free-spawning invertebrates (Palumbi 1999; Swanson & Vacquier 2002a); and in *Drosophila* (Alipaz *et al*. 2001). However, a large number of steps lead to the fusion of a given sperm and egg (Eberhard 1996; Markow 1997; Bloch Qazi, Heifetz & Wolfner 2003), and all such steps can potentially contribute to cryptic isolation. Thus there is a suite of potential postmating mechanisms that can reduce interbreeding and gene flow (Albuquerque *et al*. 1996; Markow 1997; Price *et al*. 2001). Proteins that are known to be involved in postmating male–female interactions have been shown to evolve rapidly under positive selection and to be polymorphic (Vacquier 1998; Palumbi 1999; Swanson & Vacquier 2002a, 2002b) and often species-specific (Chen 1984; Vacquier 1998), demonstrating the importance of cryptic isolation. However, the general role of cryptic isolation for the speciation process is not well understood. For example, postmating prezygotic traits limiting hybrid formation can appear late in the speciation process when two species are already partly isolated (Howard *et al*. 1998b), or they might occur early in the divergence process of two populations prior to other forms of isolation mechanism (Fricke & Arnqvist 2004a), and thus possibly fuel the early stages of speciation by limiting gene flow.

One distinct form of cryptic isolation occurs when females modulate their reproductive rate depending on the specific status of their mate, although this phenomenon has been poorly documented (Eberhard 1996; Nilsson, Fricke & Arnqvist 2002; Arnqvist & Rowe 2005). Reyer, Frei & Som (1999) showed that waterfrog females of the *Rana lessonae/esculenta* complex reduce their clutch size when amplexed by non-compatible males. Further, females can modulate their reproductive investment according to their mate preferences (Sheldon 2000). Females can, for example, invest differently in offspring production depending on male attractiveness (Cunningham & Russell 2000; Kolm 2002; Gil *et al*. 2004). It is also known that, at mating, males of many taxa transfer to females gonadotropic ejaculatory substances that affect female egg production (Herndon & Wolfner 1995; Eberhard 1996; Gillot 1996; Wolfner 1997; Heifetz *et al*. 2000). Cryptic isolation would result if females exhibited depressed egg-production rates when receiving heterospecific ejaculatory substances (Nilsson *et al*. 2002).

Here we test if modulation of female reproductive rate is an incipient postmating prezygotic isolation mechanism in the Bean Weevil, *Callosobruchus maculatus*. Previous research has shown that female reproductive output early in life is affected by the population origin of her mate (Fricke & Arnqvist 2004b). Here we ask whether the reproductive rate of females after mating is a decreasing function of decreased phylogenetic relatedness with their mates (Fricke & Arnqvist 2004a). One underlying assumption is that forms build co-adapted gene complexes over time that cause lower hybrid fitness. This can also be true for different conspecific populations, especially if they are spatially/geographically subdivided such that gene flow and recombination are suppressed (Tregenza & Wedell 2000). If this is true, females may have evolved to adaptively suppress reproduction when mated to less-compatible males. We mated focal females from several populations to males with increasing phylogenetic distance, and recorded female egg production. Our *a priori* prediction is that females should tend to lay fewer eggs when mated to males to which they are more distantly related. By doing so, females would avoid the cost of producing 'hybrid' offspring following matings with less-compatible males. Female Bean Weevils are polyandrous, and females could thus allocate relatively more reproductive resources to future egg production following subsequent matings with more compatible males. This would then, in effect, limit the formation of hybrid offspring and hence constitute a cryptic isolation mechanism.

Materials and methods

The basic design used was one where females were mated twice with males of different population origin. By recording egg production after their first and second mating, we were able to relate egg production to male type. We used four different strains of the Bean Weevil, *Callosobruchus maculatus* – Brazil (Campinas), Mali, Yemen and Oman – and one strain of the closely related species *Callosobruchus analis*. Figure 1 shows the phylogenetic relationship between strains and species. All colonies were kept at $30 \pm 0.5\,°C$ and 45% ($\pm 10\%$) RH under a 12 : 12 h light : dark cycle, and all beetles used in the experiments were virgins. Virgin males and females were isolated after emerging from the host beans and were kept individually prior to matings. The virgin beetles were mated on day 1 after hatching at room temperature (20–21 °C) by introducing one male and one female into a small Petri dish (3 cm diameter).

Each focal female was first mated with a male of a certain type and then with another male of either the same type as the first mate, or with a male of her own type. We used females from the Brazil, Mali and Yemen strains as focal females, giving a total of 15 combinations (3×5) for first matings (Fig. 2). In first matings, pairs were observed for a 30-min period and the start and end of each mating was recorded. Pairs were separated after a successful copulation and females were transferred into Petri dishes (9 cm diameter) with 50 black-eyed beans (*Vigna unguiculata*) as
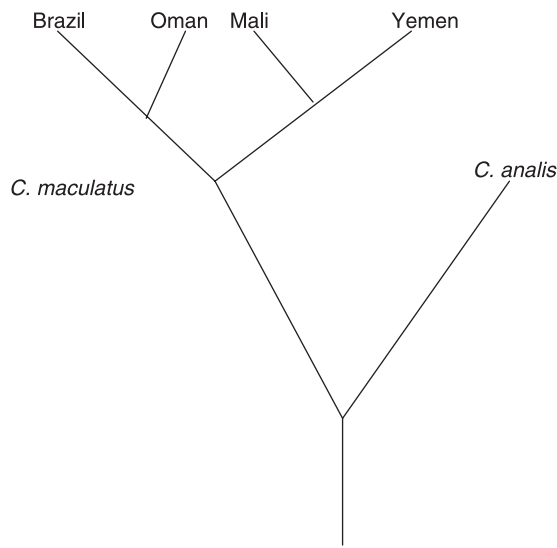
Fig. 1. The phylogenetic relationship assumed for the populations and species used in this experiment (arbitrary branch lengths). *Callosobruchus analis* and *Callosobruchus maculatus* are closely related species within the genus. The topology for the populations of *C. maculatus* is based on data from eight polymorphic enzyme loci (Abdel-Gabe 1992).
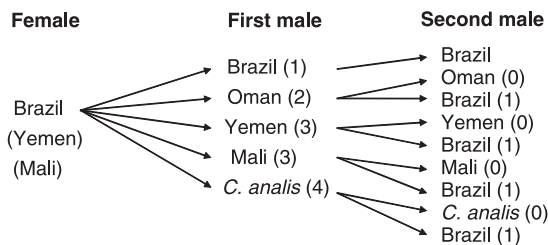
Fig. 2. Experimental design of the double-mating protocol, illustrated by showing all possible mating combinations for the Brazil females, split into two groups after the first mating. Numbers in brackets behind the first males represent the coding used in the statistical evaluation of results. Numbers in brackets behind the second males indicate the coding used in analyses of the rescue effect. This design for Brazil females only; the same basic design logic applied to females from the Yemen and Mali populations.

Table 1. Results of a generalized linear model of female propensity to remate, using binomial errors and a logit-link function

Source	LLR	df	P
Female	185·48	2	<0·001
First male	5·40	3	0·14
Second male	9·36	3	0·02
Female × first male	5·82	6	0·44
Female × second male	7·86	6	0·25

First and second males were coded according to their relative phylogenetic rank relative to the focal female. The contribution of each term was tested by sequentially adding them to a null model. LLR, log-likelihood ratio.

oviposition vial until their death, which we recorded through daily spot checks. After 7 days' incubation the number of eggs laid by each female was counted for each oviposition vial.

STATISTICAL ANALYSIS

Statistical analyses were performed using either ʀ (www.r-project.org) or sʏsᴛᴀᴛ (SPSS Inc., version 10). In all analyses, males were defined statistically according to the relative relatedness to their mates, such that variance in our response variables due to males here reflects their relatedness to females rather than their strain identity *per se*. This was achieved by coding males according to their phylogenetic distance rank relative to the focal female (Fig. 2).

Females first mated to foreign males may adaptively upregulate egg production following a subsequent mating to males of their own type, a phenomenon referred to here as a 'rescue effect'. To test for a rescue effect among females first mated to foreign males, we focused on egg production during the 24 h following the second mating (day 2 of the experiment). Here we compared females remating with males of their own type (coded 1) with those mating a second time with the same type of foreign male as in their first mating (coded 0) (Fig. 2). Females that mated twice to males of their own strain were excluded from this analysis.

Because residuals of all models were non-normally distributed, and several response variables were non-continuous, we used generalized linear models with appropriate error and link functions (McCullagh & Nelder 1989). Statistical inferences were based on analyses of deviance. Means are presented with their associated standard error throughout this paper.

Results

Females of the three *C. maculatus* strains used in this experiment were significantly different in their propensity to remate after 24 h (Table 1). Mali females remated most frequently, with 85·20% of all remating opportunities leading to mating; Brazil females remated at an intermittent rate (39·83%); and Yemen

oviposition medium. Each of the 15 female–male combinations was subsequently divided into two equally sized groups for the second mating. In the second matings, one group of females (20 replicates) were remated with a male of the same origin as their first mate, while the second group (20 replicates) was remated to a male of the same origin as the female (Fig. 2). Second matings were conducted 24 h after the first, and we allowed for rematings to occur for an observation period of 45 min. We recorded whether or not a remating occurred, and females who did not remate were discarded. For females who remated, we recorded the time at which copulation started and ended. Females were subsequently transferred to a new Petri dish with fresh black-eyed beans. Females were then transferred on days 3, 4, 6 and 8 to a new oviposition vial with black-eyed beans *ad libitum*. They were kept in the last

Table 2. Results of a generalized linear model of the number of eggs laid during the 24 h following the first mating

Source	LLR	df	*P*
Female	45·20	2	0·10
First male	297·10	3	<0·001
Female × first male	68·00	6	0·30

Because data were overdispersed (dispersion parameter = 9·75) we used a quasi-Poisson error distribution and a log-link function. First males were coded according to their phylogenetic rank relative to females. The contribution of each term was tested by adding them sequentially to a null model. LLR, log-likelihood ratio.
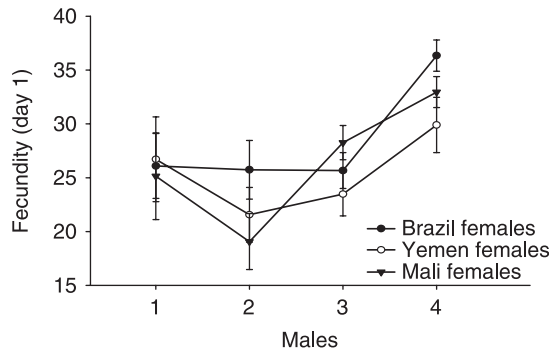
Fig. 3. Fecundity early in a female's life, measured here as the number of eggs laid during 24 h after first mating. Males are coded according to their phylogenetic distance relative to females: 1 = males from their own strain to 4 = heterospecific *Callosobruchus analis* males.

females showed the lowest remating rate (35·02%). The females' first mate had no significant influence on a female's propensity to subsequently remate, while the second male did have such an effect. Female remating rates were 43·30% when the second male was of her own type; 52·42% when the second male was from the females' 'sister strain' (male 2); 51·87% when the second male was from the most distantly related conspecific strain; and 43·75% when the second male was heterospecific (male 4).

It is clear that the first male to mate with a given female elicited significantly different rates of early reproductive output in their mates depending on relatedness, measured as egg production during the 24 h following the first mating (Table 2), while the three female strains on average did not differ significantly in this regard (Brazil, 28·19 ± 1·10 eggs; Mali, 26·99 ± 1·10 eggs; Yemen, 24·68 ± 1·29 eggs). As shown in Fig. 3, females tended to have a lower early fecundity when mated to their conspecific sister strain (males 2) compared with their own males, while the heterospecific *C. analis* males caused females of all three strains to lay the greatest number of eggs during the first 24 h after the initial mating. To assess whether the significant effect was driven solely by heterospecific matings, we repeated the model given in Table 2 but excluding *C. analis* males. In this model, the effect of

Table 3. Generalized linear model of the number of eggs laid on day 2, using quasi-Poisson errors and a log-link function (dispersion parameter = 3·942)

Source	*F*	df	*P*
Female	10·02	2	<0·001
Early egg production	170·52	1	<0·001
First male	0·10	2	0·90
Rescue effect	2·02	1	0·16
Female × first male	4·05	4	0·003
Female × rescue effect	3·94	2	0·02
First male × rescue effect	3·13	2	0·04
Female × first male × rescue effect	1·22	4	0·30

First males were coded according to their phylogenetic rank relative to the focal female. The rescue effect was coded in the following way: remating with a female's own male = 1; remating with foreign males = 0. The contribution of each term was tested with *F* tests using a sequential model approach, in which terms were added in the order given above.
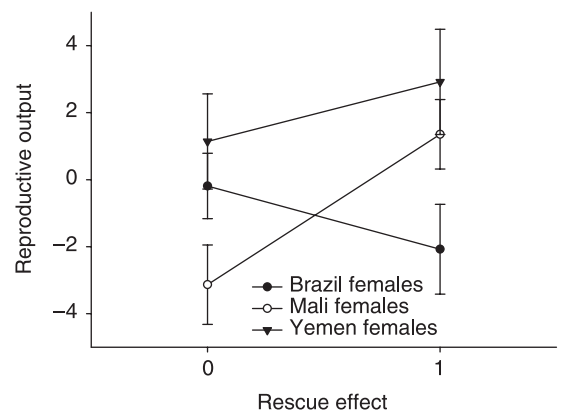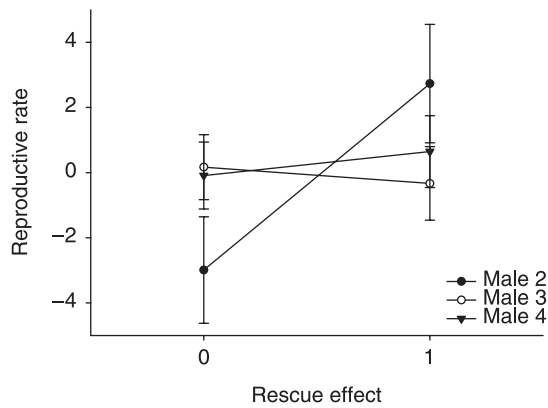

Fig. 4. Reproductive rescue effect across female strains (0 = remating with yet another foreign male; 1 = remating with a male from the female's own strain). Reproductive rate represents residual reproductive rate from a regression of the number of eggs laid 24 h after the second mating on the number of eggs produced 24 h after the first mating.

the first male was no longer significant (log-likelihood ratio, LLR = 44·2, df = 2, *P* = 0·10), showing that the observed effect was generated primarily by higher egg production following matings with heterospecific *C. analis* males.

Female strains did differ in the number of eggs they produced during day 2, following their second mating (Table 3) (Brazil, 26·01 ± 0·94 eggs; Mali, 26·78 ± 0·86 eggs; Yemen, 30·80 ± 1·22 eggs). We failed, however, to find a general reproductive rescue effect: remating with a male from a female's own strain did not generally elevate reproductive rate during the 24 h following the second mating compared with remating with yet another foreign male. However, as revealed by the significant female × rescue effect interaction term (Table 3), female strains differed in this respect. While Mali females did show a rescue effect, as they laid ≈15% more eggs after remating with their own males, the females of the other strains showed no apparent rescue effect (Fig. 4). We also note that a rescue effect

Fig. 5. Effect of relative relatedness of the first mate on subsequent reproductive rescue (0 = remating with yet another foreign male; 1 = remating with a male from the female's own strain). First males were coded according to their phylogenetic distance relative to the focal female (2 = closely related males, sister strain; 3 = more distantly related conspecific males; 4 = heterospecific *Callosobruchus analis* males). Reproductive rate represents residual reproductive rate from a regression of the number of eggs laid 24 h after the second mating on the number of eggs produced 24 h after the first mating.

occurred when the first male was foreign but of close phylogenetic distance rank, but not when the first male was more distantly related (Fig. 5). Heterospecific *C. analis* males, which triggered a high reproductive rate in females during day 1 (see above), did not elicit a similar strong response during day 2. In summary, while females did not generally show the expected adaptive increase in reproductive rate following matings with their own males, such effects did occur, but were contingent on female strain and female relatedness to the first male.

The three different female strains differed significantly in the number of eggs they laid during their lifetime (Table 4). Mali females laid the greatest number, with an average of 100·60 (±1·90) eggs. Brazil and Yemen females laid 93·44 (±1·72) and 94·28 (±1·98) eggs, respectively, during their life. This difference is, to some extent, a result of differences in life span, as the relative order among strains from most to least fecund is the same as for longest to shortest life span. Female life span differed significantly between the three strains used in this experiment (Table 5). On average, the adult life span of Mali females was $12·24 \pm 0·19$ days, while Brazil and Yemen females lived $10·70 \pm 0·17$ and $10·53 \pm 0·20$ days, respectively. Within strains, however, female life span tended to correlate negatively with lifetime fecundity (Brazil, $r = -0·004$, $n = 189$, $P = 0·956$; Yemen, $r = -0·278$, $n = 186$, $P < 0·001$; Mali, $r = -0·124$, $n = 190$, $P = 0·089$).

There was a significant negative phenotypic correlation between female egg production during the first 24 h after a first mating and total life span, across all females ($r = -0·200$, $n = 561$, $P < 0·001$) as well as within all three strains when analysed separately (Brazil, $r = -0·188$, $n = 189$, $P = 0·009$; Yemen, $r =$

Table 4. Results of a generalized linear model of female lifetime fecundity, using quasi-Poisson errors and a log-link function

Source	F	df	P
Female	4·28	2	0·01
First male	1·48	3	0·22
Second male	1·53	3	0·21
Female × first male	0·78	6	0·59
Female × second male	1·18	6	0·31

First and second male were coded according to their phylogenetic distance relative to females. The contribution of each term was tested by *F*-tests where each term was sequentially added to a null model.

Table 5. Results of a generalized linear model of female life span, using Poisson errors and a log-link function

Source	F	df	P
Female	14·79	2	<0·001
First male	1·65	3	0·18
Second male	0·52	3	0·67
Female × first male	0·93	6	0·47
Female × second male	1·91	6	0·07

First and second male were coded according to relative phylogenetic distance for each female type. The contribution of each term was tested by sequentially adding them to a null model.

$-0·223$, $n = 183$, $P = 0·002$; Mali, $r = -0·237$, $n = 189$, $P = 0·001$). However, both life span and egg production may correlate phenotypically with body size (Messina & Slade 1999; Messina & Fry 2003). To remove the potential impact of confounding variables such as body size from this analysis, we first calculated average female life span and egg production for each focal female/first male mating-treatment combination. For each focal female strain we then correlated the five paired averages, and finally tested whether the mean Pearson product-moment correlations across the three female strains was different from zero (Fricke & Arnqvist 2004b). This analysis showed a negative, but marginally non-significant, impact of a female's early reproductive output on her life span (mean $r = -0·635$, $n = 3$, $t = -3·262$, $P = 0·083$). However, the power of this test is very limited due to the low sample size. A correlation of the early reproductive output (egg during the first 24 h) and late fecundity (number of eggs after 24 h until natural death of females) revealed that there is a weak but significant negative relationship between early and late fecundity across all females (Pearson correlation: $r = -0·221$, $n = 566$, $P \leq 0·001$). An assessment of the mean Pearson product-moment correlations across strains, as described above, again revealed a negative but non-significant relationship between these parameters (mean $r = -0·370$, $n = 3$, $t = -2·590$, $P = 0·122$). To conclude, our data strongly suggest that females that reproduce at a high rate early

in life generally suffer a reduced life span and reduced egg production later in life (Fricke & Arnqvist 2004b).

Discussion

Our study revealed that females' reproductive rate early in life was indeed affected by the identity of their first mate. However, the pattern observed was clearly not in accordance with our *a priori* predictions. Females did not lay fewer eggs after mating with more distantly related males. On the contrary, for all three strains used here, females had a consistently higher reproductive rate after mating with heterospecific males. Furthermore, the origin of the second male to mate with a female did not change a female's reproductive output significantly, either in the short term (24 h after second mating) or in the long term (female lifetime fecundity). It also seems clear that a high reproductive rate early in life has negative consequences for components of female late life fitness, in terms of both a reduced life span and lower future egg production. We first ask what processes could generate the pattern observed here, then discuss some other implications of our findings.

Modulation of reproductive rate is a form of postmating sexual selection among males (Eberhard 1996; Andrés & Arnqvist 2001; Nilsson *et al.* 2002) that offers females an opportunity to discriminate against non-compatible males. Contrary to our predictions, we found that increased reproductive rate was associated with mating with more distantly related males such that heterospecific matings caused the strongest response among females from all three *C. maculatus* populations used. In general, such reproductive responses could be interpreted in terms of adaptive inbreeding avoidance by females (Bateson 1983; Tregenza & Wedell 2000). However, two facts suggest that this is an unlikely explanation for our results. First, an earlier study showed no evidence for inbreeding among *C. maculatus* populations cultured under the same conditions as used here (Fricke & Arnqvist 2004b). Second, although the theoretical framework linking genetic distance to optimal outbreeding is poorly developed, optimal outbreeding would surely occur with one of the related conspecific populations rather than with heterospecific males (Pusey & Wolf 1996; Tregenza & Wedell 2000). Interestingly, a similar study of *Drosophila melanogaster* revealed female postmating discrimination against close kin, but not against more distantly related conspecific males (Mack, Hammock & Promislow 2002). Thus adaptive inbreeding avoidance is unlikely to explain the pattern observed here. Parker & Partridge (1998) suggested that postmating sexual selection generated by sexually antagonistic coevolution can lead to patterns such as those observed here (Andrés & Arnqvist 2001; Arnqvist & Rowe 2005). In short, this process can predict a high reproductive response when allopatric populations meet secondarily, because females may be poorly defended (have low resistance) against males with which they do not share a history of coevolution (females in a given population generally should be unable to evolve resistance to male traits specific to other populations). Because earlier research on Bean Weevils has shown that matings are potentially harmful for females (Crudgington & Siva-Jothy 2000) and that matings can carry substantial costs to females (Tatar, Carey & Vaupel 1993; Rönn, Katvala & Arnqvist 2006), this appears to be a more plausible explanation, although Brown & Eady (2001) found only limited support for this pattern in *C. maculatus*. While previous experimental support for the prediction of Parker & Partridge (1998) comes from cases where females show a stronger reproductive response to males from different allopatric populations of the same species than to males from their own population (Andrés & Arnqvist 2001; Knowles & Markow 2001; Hosken, Blanckenhorn & Garner 2002; Nilsson *et al.* 2002, 2003), our results demonstrate that this pattern can also extend to different species. This is somewhat surprising, as we expect different species to have accumulated genetic and/or molecular incompatibilities (Orr 1995; Orr & Presgraves 2000) even under sexually antagonistic coevolution (Fricke & Arnqvist 2004a; Arnqvist & Rowe 2005). This is especially true considering that reproductive traits under postcopulatory sexual selection are generally believed to evolve rapidly (Civetta & Singh 1995; Arnqvist 1998).

Our results raise the question as to whether *C. maculatus* and *C. analis* should be considered different species. There is little, if any, premating isolation and no evidence for postzygotic isolation, as F_1 hybrids are fully viable and fertile (personal observation). Although both species are now global pests on stored leguminous seeds, *C. maculatus* originated in west Africa while *C. analis* is thought to have originated in south-east Asia (Decelle 1981). It thus seems probable that these two taxa have largely evolved in allopatry with no or little opportunity for interbreeding. The two taxa have previously been classified as distinct species based on morphological characters (Southgate, Howe & Brett 1957); more recent phylogenetic work, based on molecular data, has confirmed their specific status and has further established that the two are not even strict sister species (M. Tuda, J. Rönn, S. Buranapanichipan, N. Wasano and G. Arnqvist, unpublished data). Thus, although these species have been separated geographically for a long time, they have not evolved complete reproductive isolation as an incidental by-product of adaptation to their environments (Mayr 1970). However, because of our explicit focus on female reproductive rate and remating propensity, our conclusions regarding postmating isolation are somewhat limited. An inclusive measure of net postmating isolation in this system would also need to incorporate measures of conspecific sperm precedence, which has been found to be important in other systems (Howard 1999; Eady 2001).

We suggest that our results are due to differences in the amount or type of seminal substances that males transfer to females during copulation. Female reproductive responses are often mediated by male accessory gland proteins (ACPs) in insects (Wolfner 1997, 2002; Chapman 2001; Gillot 2003). Such proteins are known to be polymorphic within species (Begun *et al.* 2000; Swanson & Vacquier 2002b) and to evolve rapidly (Civetta & Singh 1995). In *Drosophila* fruitflies, ACPs are known to modulate egg production (Chapman *et al.* 2001); this is also true for another bruchid beetle, *Acanthoscelides obtectus* (Das *et al.* 1980). Moreover, the fact that female *C. maculatus* emerge with only about eight mature eggs (Wilson & Hill 1989) suggests that the results seen in our experiments on egg production during the 24 h following the first mating (some 25 eggs) may involve differential effects of male ACPs on female egg maturation. In fruitflies, ACPs and the act of mating itself affect a multitude of behavioural and physiological traits in females, including ovulation rate and egg maturation rate (Wolfner 1997, 2002; Chapman 2001), as well as the pattern of female gene expression (McGraw *et al.* 2004). It is, perhaps, less clear whether different fruitfly populations are generally distinct with regard to ACPs. Although some allopatric populations show divergence of ACPs apparently caused by directional selection (Aguadé 1998, 1999), others show little genetic differentiation across ACP loci (Panhuis, Swanson & Nunney 2003).

In some taxa, such as free-spawning marine organisms (Palumbi 1999; Swanson & Vacquier 2002b), reproductive proteins are known to be polymorphic within and between species and to contribute to assortative mating. Genes coding for ACPs are thus obvious candidates for genes contributing to reproductive isolation and ultimately speciation. Further, work on fruitflies has revealed evidence for coevolution of male and female postmating reproductive traits within species (Pitnick *et al.* 2003), and there is some support for the idea that this can result in at least partial reproductive isolation (Alipaz *et al.* 2001). Despite the fact that there is clearly variation in postmating prezygotic traits across conspecific populations of *C. maculatus* (Brown & Eady 2001; Fricke & Arnqvist 2004b), we failed to find evidence for a role of such divergence in terms of generating partial reproductive isolation in this system.

Among females first mated to males from populations other than their own, we did not observe a general increase in female reproductive rate after a subsequent mating with their own males (i.e. no reproductive rescue effect). However, we note that females from different populations appeared to react somewhat differently in this regard.

We did not measure female body size, as the focus of our study was to assess effects of males on fecundity within female populations. Female body size is generally weakly but positively phenotypically correlated with fecundity within populations of these beetles (Messina & Slade 1999; Messina & Fry 2003; Arnqvist *et al.* 2004). While the inclusion of female body size in our models might have increased the statistical power by reducing residual within-treatment variance, body-size effects cannot have caused the treatment effects seen, as females were randomly assigned to mating treatments.

This study raises several questions. First, the fact that we found effectively the opposite of partial postmating reproductive isolation between *C. maculatus* and *C. analis* in terms of differential egg production suggests that isolating mechanisms other than those investigated so far may be in place. Unravelling such mechanisms may help to explain our results. Second, we do not know how far the pattern documented here is replicable across multiple species. The genus *Callosobruchus* contains several fairly closely related species, and it would be interesting to elucidate whether the pattern found is general within the genus. Third, theory relating the degree of population divergence to reproductive compatibility is rather poorly developed (Tregenza & Wedell 2000; Rowe, Cameron & Day 2003). This is particularly true for reproductive traits that coevolve in the two sexes, where empirical patterns across extant populations might help unravel past coevolutionary processes (Arnqvist & Rowe 2005).

Acknowledgements

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