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Divergence in replicated phylogenies: the evolution of partial post-mating prezygotic isolation in bean weevils

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natural selection;
parallel speciation;
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Abstract

By tradition, speciation research has been focused on processes leading to either premating or post-zygotic reproductive isolation. The processes which generate isolation after mating but before zygote formation are less well understood. Here, we study divergence in characters which contribute to post-mating prezygotic isolation, such as egg production and remating rate. We propose that 'replicated' laboratory phylogenies with known histories can be used to yield insights into the processes of divergence. We performed a series of cross-matings between populations within two strains of the bean weevil *Callosobruchus maculatus*. Each strain has a unique and independent origin and both have been kept in the same set of laboratories during the last few decades. Our results show that divergence has occurred between laboratory populations within strains with regards to the effects that mating has on female reproductive behaviour, showing that the evolution of partial post-mating prezygotic isolation can be rapid. More importantly, the pattern of divergence across populations was distinct in the two strains, suggesting that coevolutionary trajectories are not determined by environmental factors but are to some extent arbitrary. We discuss the limitations of the novel empirical strategy employed here, and conclude that our results lend support to the hypothesis that post-mating sexual selection is capable of rapidly generating post-mating prezygotic isolation.

Introduction

There has been a recent surge in interest into the process of speciation (Howard & Berlocher, 1998; Schluter, 2000, 2001; Barraclough & Nee, 2001; Orr & Turelli, 2001; Panhuis *et al.*, 2001), motivated by the fact that several aspects of the evolution of reproductive isolation are still incompletely understood. For example, new efforts have been made to link adaptation with speciation (Orr & Smith, 1998; Schluter, 2001), to characterize the evolutionary processes leading to premating isolation (Parker & Partridge, 1998; Panhuis *et al.*, 2001) and to better understand the evolution of post-zygotic isolation (Orr & Presgraves, 2000; Turelli & Orr, 2000). One line of research which has received increased attention is motivated by the realization that a number of events occurring after mating but prior to zygote formation can lead to significant reproductive isolation (Howard, 1999; Eady, 2001). Here, most attention has been given to sperm competition. Several independent studies have shown that conspecific sperm precedence can function as an effective barrier to hybrid formation (Nakano, 1985; Hewitt *et al.*, 1989; Robinson *et al.*, 1994; Wade *et al.*, 1994; Price, 1997; Howard *et al.*, 1998a; Fricke & Arnqvist, 2004). However, other reproductive behaviours can also contribute to such post-mating prezygotic isolation, such as differential female reproductive output favouring conspecific males and a lower female propensity to remate after mating with conspecifics (Andrés & Arnqvist, 2001). If we wish to understand post-copulatory prezygotic divergence, it is therefore important to study these reproductive parameters. Yet only a few studies to date have been performed to investigate parameters other than sperm competition in any detail [*Drosophila* (Jamart *et al.*, 1995; Knowles & Markow,

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2001; Price *et al.*, 2001), *Tribolium* (Nilsson *et al.*, 2002), *Musca* (Andrés & Arnqvist, 2001), *Callosobruchus* (Brown & Eady, 2001)].

The post-copulatory behaviour of many female insects, including sperm utilization, reproductive effort and remating, is known to be affected by 'signal' substances transferred by the male in the ejaculate (see Chen, 1984; Wolfner, 1997; Chapman, 2001; Gillot, 2003). Evolution of such seminal 'signals', and/or of the female response to these, can lead to differential fertilization and potentially speciation (Markow, 1997; Rice, 1998). Genetic variation in reproductive proteins across species is striking in many different groups (Swanson & Vacquier, 2002) and is known to sometimes cause assortative fertilization (Palumbi, 1999). Studies of divergence between populations are important to determine to what extent compatibility between male substances and female receptors, which influences different stages of the fertilization process, can actually lead to reproductive isolation (Markow, 1997; Andrés & Arnqvist, 2001; Eady, 2001; Swanson & Vacquier, 2002). It is also worth noting that any given species has a great variety of male seminal 'signals' (e.g. >80 in *Drosophila*; Chapman, 2001), and that male–female coevolution could thus potentially occur along a near infinite number of trajectories.

To fully understand speciation we need to establish which processes are involved in evolutionary divergence. Natural selection is commonly important, in that isolation can occur as a by-product of adaptation to environmental conditions (Dobzhansky, 1937; Mayr, 1963; Schluter, 2000). It has also become clear that sexual selection can lead to rapid divergence, as such selection is acting directly on traits which affect the reproductive success of individuals (Lande, 1980; West-Eberhard, 1983; Panhuis *et al.*, 2001; Kirkpatrick & Ravigné, 2002). For example, if divergence is driven by sexual selection, reproductive proteins should evolve faster than somatic proteins (Civetta & Singh, 1995; Rice, 1998). This prediction is widely supported by comparative data (Civetta & Singh, 1995, 1998; Swanson *et al.*, 2001; Galindo *et al.*, 2003; for a review see: Swanson & Vacquier, 2002). One important aspect of divergence by natural selection is that it should in some sense be repeatable, such that independent episodes of adaptation to a given ecological environment yield similar results. This property of adaptive radiation has been exploited in several recent studies of parallel speciation (Rundle *et al.*, 2000; Johannesson, 2001; Nosil *et al.*, 2002). In contrast, we expect coevolution of male signals and female responses driven by sexual selection to be more arbitrary (Lande, 1981; Arak & Enquist, 1993, 1995; Schluter & Price, 1993; Rice, 1998), such that the particular coevolutionary trajectory followed in a given environment is essentially unrepeatable. Thus, if we could replay (or replicate) any given allopatric divergence, we expect natural selection to lead to similar, but sexual selection to result in distinct, end points.

Although we can never hope to replay evolution, laboratory model systems offer a possibility to compare replicated episodes of allopatric divergence. In many cases, each of several founding populations (e.g. strains collected at a given location) has been split and kept in the same set of laboratories. As each laboratory offers a distinct 'ecological' environment (e.g. a unique rearing protocol), such cases are in a sense replicated episodes of allopatric divergence. Here, we expect sexual selection to generate dissimilar patterns of divergence across such episodes. In contrast, natural selection should result in (i) similar patterns of divergence, by replicated exposure to distinct laboratory environments, or in (ii) no significant divergence (if the environment is the same in different laboratories). We are, however, unaware of any studies that have attempted to systematically compare such episodes of divergence (see Boake *et al.*, 2003). Here, we compare the pattern of recent divergence in male and female traits which contribute to post-mating prezygotic isolation in two strains of the bean weevil *Callosobruchus maculatus* (Coleoptera, Bruchidae), each of which is represented with the same set of three allopatric laboratory populations (see Fig. 1). By comparing divergence in the effects that mating has on female post-mating reproductive behaviour, such as egg production rate and remating propensity, we can assess the rate and compare the pattern of divergence.

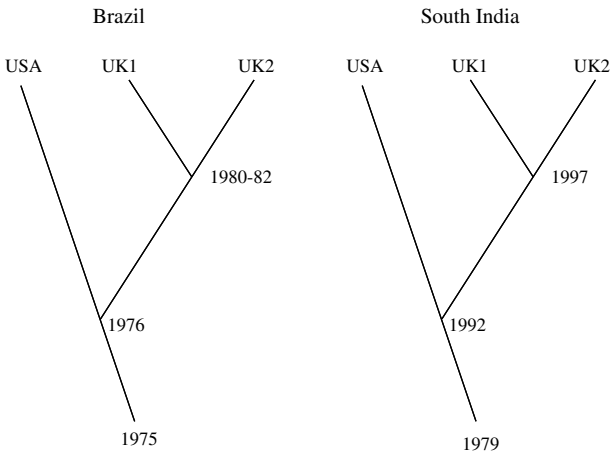

Fig. 1 Two strains of *Callosobruchus maculatus* (Brazil and South India) with independent origin have been kept in the same set of laboratories and thus share a similar evolutionary history, in essence representing 'replicated' phylogenies. Brazil was collected in 1975 in Campinas, Brazil (Credland & Wright, 1989), while South India was collected in 1979 in Tirunelveli (Mitchell, 1991). Brazil came in 1976 to the National Resource Institute in England and was further distributed in 1980 to the University of Leicester (UK 2) and in 1982 to the University of London (UK1). The University of London (UK 1) population of South India was derived in 1997 from the population at the University of Leicester (UK 2), which came from the original strain in 1992 from the USA to England. Note that branch lengths in the figure are arbitrary.

Materials and methods

We used two strains of *C. maculatus* for our experiments: Brazil (Br) and South India (SI), which have both been in culture for more than 20 years. The generation time of this beetle under laboratory conditions is approximately 3–4 weeks. The laboratory populations we used were collected from three different laboratories: University of London (UK 1, Peter Credland), University of Leicester (UK 2, Robert Smith) and Ohio State University (USA, George Keeney).

Although the environment experienced by the beetles is 'controlled' in all laboratories, each of the laboratories has maintained the beetles in a unique environment (see Appendix for details). For both strains, the three populations are monophyletic. The topology of the phylogenies is identical, although the branch lengths differ in the two strains (Fig. 1). All beetles were maintained, and all experiments were performed, at 27 °C and 45% (±10%) RH under a 12 : 12 h light–dark cycle. All populations were kept in our laboratory for at least two generations prior to the experiments and were maintained on cowpeas (*Vigna unguiculata*), apart from SI UK 2 and SI USA, which were reared on mung beans (*Phaseolus aureus*). Virgin 12–26 h post-eclosion females and virgin 12–36 h old males were used in all mating experiments described below. For both strains, we used a full 3×3 mating design, such that each sex of each population was mated to the other sex of all populations within that strain. In all experiments and for each cross within each strain, 20 replicates were carried out.

Female egg and offspring production rate

Virgin females were mated once to a virgin male and then allowed to oviposit on their habitual host for their entire lifespan. Individual pairs (kept in a 30 mm diameter petri dish) were observed for the first 30 min following introduction, and we recorded whether mating occurred or not. The frequency of matings in these trials thus offers a measure of premating isolation (i.e. a no-choice design). Females that mated were transferred daily into vials with a superabundant supply of fresh beans for the first 7 days of their life, and were then left in the last vial until their death (egg production rate decreases markedly over life; e.g. Credland & Wright, 1989). After incubating the eggs for 7 days, the number of hatched and unhatched eggs was counted. The total number of adult offspring produced by each female was scored on day 32 after oviposition.

Female remating rate

Virgin males of all populations were introduced to virgin females of all populations, using the same mating protocol as described above. Each female was then given the opportunity to remate with a male from their own population, at 6, 12 and 24 h following the first copulation. Each of these remating opportunities lasted for 30 min, after which males and females were separated if no remating had occurred. If a successful remating was observed, females were deemed remated and were not given further remating opportunities.

Statistical rationale

We denote effects of the population origin of each sex as either 'male' or 'female' effects in the analyses presented below. In all models, a male effect thus suggests that males from different populations elicit different overall magnitudes of post-mating responses in females. A female effect indicates that females from different populations show different overall rates of post-mating behaviours. The male × female interaction term is of particular interest to us, as it indicates more complex and qualitative evolutionary modifications of the 'signal-receptor' system, which determines post-mating reproductive behaviour (see also Clark *et al.*, 1999; Pitnick & Brown, 2000; Andrés & Arnqvist, 2001). In our case, the pattern of the male × female interaction can be said to characterize the coevolutionary trajectories taken by the different populations within a given strain, and the three-way interaction strain × male × female thus provides a direct test of whether divergence has been replicable in the two strains.

For all continuous response variables, analyses were performed with conventional analyses of variance. For other variables, we used the appropriate generalized linear model analogues. For models of larval survival, egg hatching rate and female mating propensity we used binomial errors, whereas female remating propensity was modelled as a multicategory variable (remating at 6, 12, 24 h or not at all) with Poisson errors. Only females which produced eggs were included in the analyses. A few deviant cells (|studentized residual| > 2.5) were excluded from the data. All means are presented with standard errors. Analyses were performed with Systat® and GLIM®.

Results

We found little evidence for divergence in traits affecting premating interactions in our analyses of whether virgin females mated or not during the first 30 min. Among the South Indian populations, 95% of the females mated and the propensity to mate did not vary significantly across females or males (overall test of model: $\chi^2_8 = 9.9$; ns). Among the Brazil populations, 88% of the females mated. Here, females did show different overall tendencies to mate ($\chi^2_2 = 6.0$; $P = 0.050$) but there was no significant effect of males ($\chi^2_2 = 4.8$; $P = 0.091$) nor of the interaction between sexes ($\chi^2_4 = 4.9$; ns; overall test of model: $\chi^2_8 = 15.8$; $P < 0.05$).

Female lifespan and fitness

Females from different strains and populations had significantly different lifespans (see Table 1). However, it is also interesting to note that female lifespan depended on which male they mated with and that this effect varied across females (Table 1; male × female interaction). In five of six cases, females had shortest lifespans when mated to males from their own population (Fig. 2). The probability of this happening five times or more by chance is $P = 0.018$. Further, the pattern of the male × female interaction was not significantly different in the two strains (see three-way interaction in Table 1), and was primarily because of UK 2 females dying sooner when mated to their own males compared with when mated with males from other populations (Fig. 2).

To test whether variation in female lifespan was associated with variation in fitness (lifetime offspring production) within a population, while controlling the potentially confounding effect of differences in female condition across populations, we proceeded in the following way. For each strain and female population ($n = 6$), we calculated three separate average lifespans and lifetime offspring productions for each of the three male populations they were mated to. We then correlated average lifespan with average offspring production within each female population. We next tested whether the mean Pearson product–moment correlation thus derived ($n = 6$) was different from zero across females from all strains and populations. This analysis showed that shortened female lifespan was strongly associated with depressed female fitness (mean $r = 0.63$, $t = 4.17$; $P < 0.05$).

Female early fecundity

We defined early fecundity as the number of eggs laid during the first 24 h following mating. This corresponds to about 35% of the lifetime fecundity in our experiment. Again, females from different strains and populations differed significantly in their early fecundity. Early fecundity also depended on the male (Brazil), and male and female population interacted in their effect on early fecundity in both strains (see Table 2). Most importantly, a full model revealed that the pattern of this interaction was different in the two strains (strain × female × male interaction; $F_{4,329} = 4.02$, $P < 0.01$). A visual inspection (Fig. 3) reveals a very different pattern in the female × male interaction in the two strains. In both strains, the interaction is primarily because of male population having a different effect on female response among UK

Table 1 The results of a full analysis of variance of female lifespan for populations from the Brazil and the South India strain.

Source	*SS*	d.f.	*F*	*P*-value
Strain	221.9	1	35.12	<0.001
Female	212.7	2	16.83	<0.001
Male	131.9	2	10.44	<0.001
Female × male	133.3	4	5.28	<0.001
Strain × female × male	17.6	4	0.70	0.594
Error	2059.6	326		

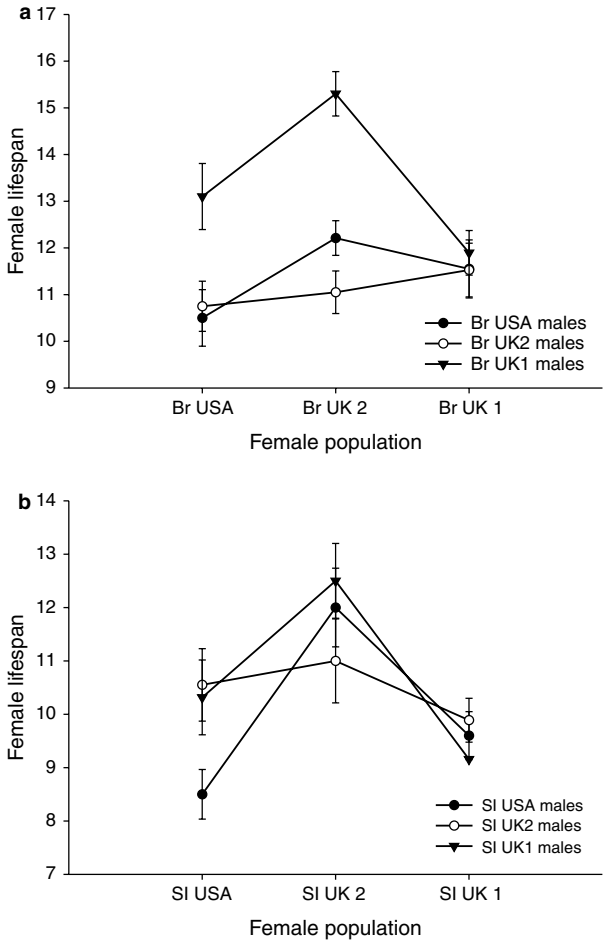

Fig. 2 Female lifespan in days (±SE) in the various population crosses for the Brazil (a) and the South Indian (b) strains.

Table 2 The results of analyses of variance of female early fecundity in the two strains used. Residuals from these models did not differ significantly from normality (Kolmogorov–Smirnov One Sample Test; $P = 0.328$ for SI and $P = 0.185$ for Br).

Source	*SS*	d.f.	*F*	*P*-value
Brazil				
Female	7088.4	2	87.75	<0.001
Male	3127.2	2	38.72	<0.001
Female × male	1096.2	4	6.79	<0.001
Error	6461.9	160		
South India				
Female	6456.9	2	46.69	<0.001
Male	255.2	2	1.85	0.162
Female × male	1296.6	4	4.69	0.001
Error	10303.5	149		

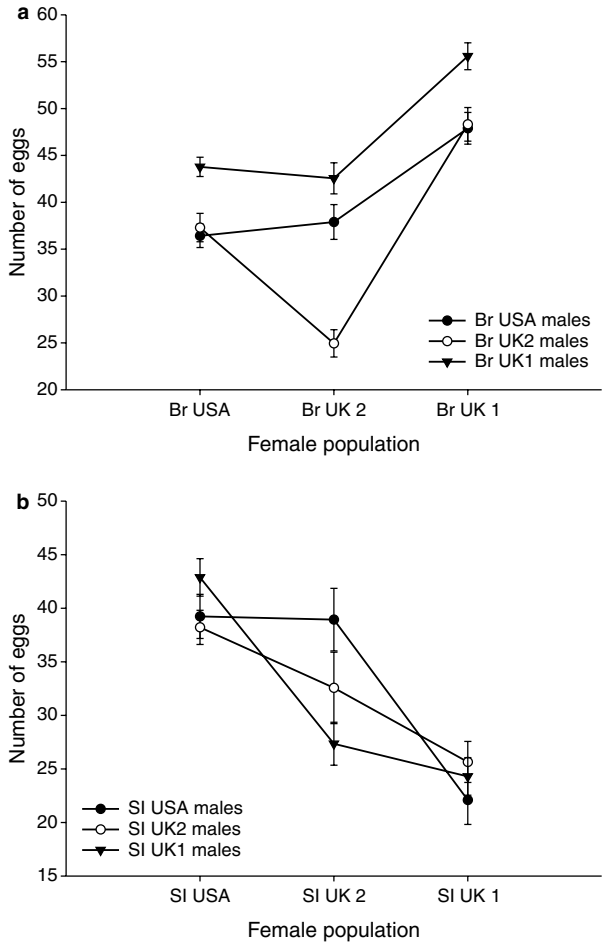

Fig. 3 Female early fecundity, measured as the number of eggs laid during the first day following mating (±SE), in the various population crosses for the Brazil (a) and the South Indian (b) strains.

2 females compared with females from the other two populations. However, the three-way interaction suggests that UK 2 females cause the interaction in distinct ways in the two strains.

High reproductive effort early in life may increase age-specific mortality rates in *C. maculatus* (see Tatar *et al.*, 1993), and we thus tested for a trade-off between early fecundity and female lifespan using the same inferential strategy as in the test of a covariation between female lifespan and fitness (see above). However, we found no evidence for a cost of elevated early fecundity in terms of reduced lifespan: average early fecundity did not significantly relate to average lifespan (mean $r = 0.13$, $t = 0.45$; ns).

Female lifetime fecundity

Overall, the effects on female lifetime egg production were fairly similar to those on early female fecundity. Lifetime fecundity differed across both strains and female populations. Further, the fecundity depends on which male females are paired with, and females and males interact significantly in their effect on fecundity in one of the two strains (Table 3). A full model showed that the interaction was significantly different in the two strains (strain × female × male interaction; $F_{4,329} = 3.72$, $P < 0.01$). A visual inspection (Fig. 4) reveals a quite different pattern in the two strains. Again, the interaction is primarily caused by the differential response of the Brazil UK 2 females.

Table 3 The results of analyses of variance of lifetime fecundity in the two strains used. Residuals from these models did not differ significantly from normality (Kolmogorov–Smirnov One Sample Test; $P = 0.068$ for SI and $P = 0.715$ for Br).

Source	*SS*	d.f.	*F*	*P*-value
Brazil				
Female	8963.4	2	20.12	<0.001
Male	4382.6	2	9.84	<0.001
Female × male	2278.6	4	2.56	0.041
Error	35421.8	159		
South India				
Female	117071.0	2	50.53	<0.001
Male	8151.4	2	3.52	0.032
Female × male	4414.1	4	0.95	0.436
Error	171443.0	148		

Hatching rate

In the Brazil strain, hatching rate of eggs was not significantly affected by females and/or males (overall test of model; $\chi^2_8 = 12.66$; ns). This contrasts with the South Indian strain (overall test of model; $\chi^2_8 = 20.61$; $P < 0.01$). Here, the hatching rate is influenced by both females ($\chi^2_2 = 8.41$; $P < 0.05$) and, more importantly, by the interaction between females and males ($\chi^2_4 = 9.66$; $P < 0.05$). The main effect of males, however, was not significant ($\chi^2_2 = 2.47$; ns).

Larval survival

In direct contrast to the hatching rate, larval survival was not significantly affected by females and/or males in the South Indian strain (overall test of model; $\chi^2_8 = 10.92$; ns) but was so in the Brazil strain (overall test of model; $\chi^2_8 = 21.27$; $P < 0.01$). In the latter strain, both females ($\chi^2_2 = 6.93$; $P < 0.05$) and the interaction between females and males had significant effects ($\chi^2_4 = 14.02$; $P < 0.01$). This was not true for the main effect of males ($\chi^2_2 = 0.42$; ns).

It might seem as if our analyses of hatching and larval survival rates suggest that there are emerging post-zygotic incompatibilities among the populations used, but that these incompatibilities affect different ontogenetic stages in the two strains. However, these interactions are caused by within-population crosses being

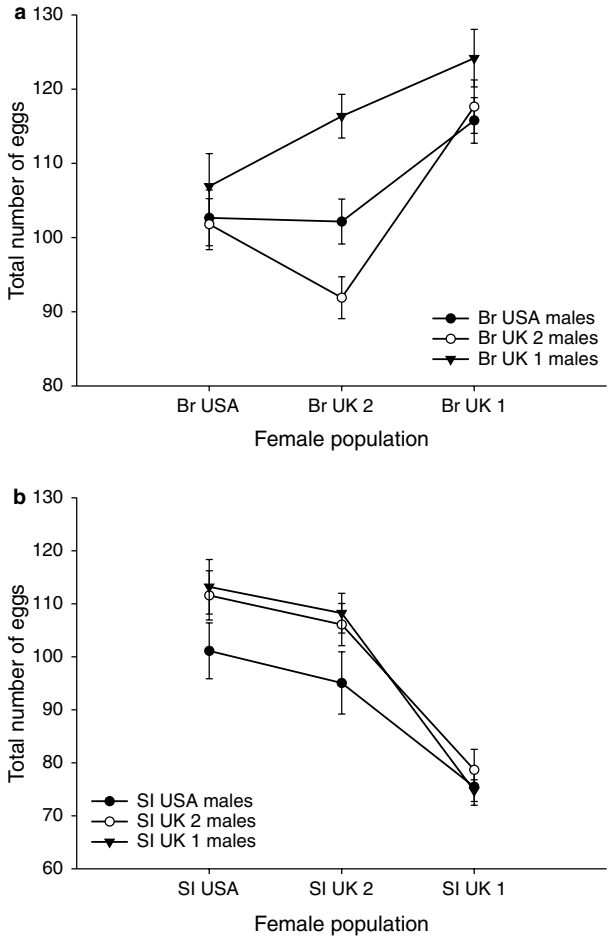

Fig. 4 Female lifetime egg production (±SE) in the various population crosses for the Brazil (a) and the South Indian (b) strains.

lowest in rates (Tables 4 and 5) in five of six cases ($P = 0.018$). This instead suggests that some populations suffer from mild inbreeding. The overall hatching and survival rates are, nevertheless, very high (Tables 4 and 5). Whatever the causes are of this incompatibility, one might hypothesize that females adaptively adjust their reproductive effort according to male compatibility (see Tregenza & Wedell, 2000, 2002). We tested for this by performing a rank correlation between early reproductive effort and measures of compatibility, in the following way. For each strain and female population ($n = 6$), we

Table 4 Average hatching rate of eggs (±SE) in crosses between populations of the South Indian strain.

	♂		
♀	SI USA	SI UK 2	SI UK 1
SI USA	0.97 (±0.01)	0.97 (±0.01)	0.94 (±0.04)
SI UK 2	0.97 (±0.01)	0.93 (±0.03)	0.97 (±0.004)
SI UK 1	0.93 (±0.02)	0.97 (±0.01)	0.88 (±0.03)

Table 5 Average rates (±SE) of larval survival in crosses between populations of the Brazil strain.

	♂		
♀	Br USA	Br UK 2	Br UK 1
Br USA	0.87 (±0.04)	0.94 (±0.01)	0.92 (±0.01)
Br UK 2	0.90 (±0.03)	0.80 (±0.03)	0.91 (±0.01)
Br UK 1	0.91 (±0.01)	0.92 (±0.01)	0.87 (±0.02)

ranked (i) fecundity early in life, (ii) egg hatching rate and (iii) larval survival rate separately for the three male populations each were mated to. We then performed rank correlations using these ranks. Fecundity early in life was, however, not significantly correlated with either egg-hatching rate in the South India strain ($r_s = -0.167$, d.f. = 7, ns) or with larval survival in the Brazil strain ($r_s = 0.333$, d.f. = 7, ns). We note that the observed male × female interaction on female fecundity is a pure mating effect, and that the interaction is not caused by females laying more eggs with males which would father more outbred offspring.

Female propensity to remate

Female propensity to remate with a male from their own population was not affected by the population origin of their first mate or by the interaction between female and male population (Table 6). Within the South Indian strain, females from different populations did exhibit different propensities to remate: whereas 77% (±0.035) of the USA females mated a second time, only 18% (±0.039) of the UK 2 females and 8.3% (±0.033) of the UK 1 females did.

Discussion

We have documented divergence in the effects that mating has on female post-mating reproductive behaviour across allopatric populations, which were separated very recently. Our results have three general implications. First, post-mating prezygotic barriers apparently evolved prior to any premating barriers across our beetle populations. This mimics the results of several other insect systems (Nakano, 1985; Howard *et al.*, 1998a, b; for a review see Howard, 1999). Secondly, incipient post-mating prezygotic isolation evolved very rapidly among our populations. Assuming a generation time of 1 month, the longest within-strain branch length in our data is about 312 and the shortest about 60 generations (Fig. 1). We note that divergence has occurred during this short time period despite the lack of intentional differences in selection regimes across populations in the different laboratories. Thirdly, our results show that much of the evolutionary divergence across populations has not been replicable. The pattern of divergence was distinct in the two strains, suggesting that the coevolutionary trajectories

Table 6 The results of general linearized models (analyses of deviance) of female propensity to remate with males from their own population following a mating with a focal male. Propensity to remate was treated as a multi-category response variable with poisson errors.

	South India			Brazil		
Source	χ^2	d.f.	*P*-value	χ^2	d.f.	*P*-value
Female	21.48	2	<0.001	2.50	2	0.287
Male	0.022	2	0.990	0.55	2	0.760
Female × male	0.322	4	0.988	1.07	4	0.899
Full model	21.83	8	0.005	3.67	8	0.886

taken are dissimilar. In the light of the great number of male traits which potentially affect female reproductive rate (e.g. seminal substances, behaviours, morphology), this is perhaps not surprising. Nevertheless, our results do provide some insights into the processes which might cause post-mating prezygotic divergence.

Natural vs. sexual selection

As pointed out in the Introduction, divergence by natural selection should be relatively deterministic and repeatable. In contrast, divergence by sexual selection should be more arbitrary. By comparing the pattern of divergence in our two replicated phylogenies, we have exploited this fact to assess which of these processes has been most important. As the coevolutionary trajectories taken are dissimilar in the two strains, at least with regards to traits affecting female egg production rates, our results suggest that sexual selection has been the key. This suggestion is also supported by the sheer rapidity of the coevolution we observed (Panhuis *et al.*, 2001; Turelli *et al.*, 2001; Kirkpatrick & Ravigné, 2002).

The occurrence of significant male × female interactions is strongly indicative of several 'signals' and 'receptors' being involved (Pitnick & Brown, 2000; Andrés & Arnqvist, 2001). Male Bruchid beetles are known to transfer seminal peptides/proteins which stimulate egg production in females (e.g. Huignard *et al.*, 1977; Das *et al.*, 1980; Huignard, 1983). We suggest that populations may have diverged in such seminal gonadotropic 'signals' and female responsiveness to them. As there is typically a large number of such seminal signals in insects (Chen, 1984; Chapman, 2001; Gillot, 2003), assuming there is post-mating sexual selection on such traits (Eberhard, 1996; Andrés & Arnqvist, 2001), coevolution could proceed along any of a very great number of potential trajectories (Arak & Enquist, 1993, 1995; Schluter & Price, 1993). Although we currently do not know which traits in the two sexes are responsible, this scenario would explain our results.

The experimental strategy we employ here, i.e. to compare the pattern of divergence in male × female interactions over replicated laboratory phylogenies, is to our knowledge novel. Although we do believe that it promises to provide valuable insights, it also suffers at least three important limitations. First, phylogenies might not be perfectly replicated. One of the assumptions of this empirical strategy is that the environmental differences between strains within laboratories are negligible compared with differences between laboratories. This may not always be true. Similarly, branch lengths in the phylogenies may differ to some extent. We should therefore generally be cautious when concluding that differences in natural selection have not contributed to differences in the pattern of divergence between replicated phylogenies, but these complications are unlikely to have contributed to the results of the current study. Differences in the pattern of divergence seen were in no case caused by populations from the laboratory where conditions to some extent differed between strains (i.e. UK 1 – see Appendix). Further, differences in branch lengths in our phylogenies were not predictive of differences in response. For example, the UK 1–UK 2 split is deeper in the Brazil strain (Fig. 1) but UK 1 and UK 2 females did not respond more differently to USA males in the Brazil strain compared with the South Indian strain (Figs 2–4). Another potential problem is the possibility that genetic variability may have been different among founders of different strains. Although it is difficult to assess the gravity of this problem, such differences would primarily result in differences in rates of divergence (Bieri & Kawecki, 2003). It is more difficult to see how such dissimilarities could generally result in distinct patterns of divergence.

Secondly, the proposed approach can shed light on whether sexual selection *per se* is capable of generating divergent evolution in traits that contribute to reproductive isolation. However, because environmental differences across laboratories are typically small compared with such differences across natural populations, results of this kind might only poorly reflect the relative importance of natural and sexual selection in the wild. Thirdly, because the null hypothesis is that the pattern of divergence across replicated phylogenies is the same, and as we can never hope to prove that a null hypothesis is true, we cannot use this inferential logic to firmly conclude that natural selection has been of key importance. A failure to reject the null hypothesis of the same pattern of divergence, which is predicted under natural selection, could simply be due to a lack of statistical power.

Distinguishing between various sexual selection scenarios

There has recently been much discussion about the possibility of using population crosses to infer the

processes of population divergence (Chapman *et al.*, 2003; Rowe *et al.*, 2003; Arnqvist, 2004). Some people have argued that, during the initial phases of differentiation, sexually antagonistic coevolution should result in a pattern where females exhibit relatively weak reproductive responses to their own males whereas other sexual selection scenarios should lead to the opposite pattern (Parker & Partridge, 1998; Clark *et al.*, 1999; Andrés & Arnqvist, 2001; Knowles & Markow, 2001; Hosken *et al.*, 2002; Nilsson *et al.*, 2002, 2003). This is because females will have been unable to evolve resistance to males with which they do not share a coevolutionary history. This suggestion is, however, contentious (see Brown & Eady, 2001; Chapman *et al.*, 2003; Rowe *et al.*, 2003; Arnqvist, 2004, for discussions).

In our data on female reproductive rates and remating propensity, no obvious pattern emerged with regard to female response to their own males. We failed to demonstrate that the overall female response to conpopulation males was different from that to heteropopulation males. Although this contrasts with the findings of Brown & Eady (2001) for the same species, who found that females tended to respond strongest to their own males, it does not allow us to conclude much with regard to the sexual selection scenario that has caused divergence across our populations (see Rowe *et al.*, 2003).

We did find that females consistently lived shortest when mated with conpopulation males. We note that this is a pure mating effect and thus cannot be due to any inbreeding that might occur. Because there is evidence suggesting that *C. maculatus* males harm their females directly (Crudgington & Siva-Jothy, 2000; Morrow *et al.*, 2003) and as most theory implies that females should be better able to resist whatever direct costs of mating males impose when mating to their own males (e.g. Parker & Partridge, 1998), this result is perhaps somewhat surprising. It suggests that females are instead less resistant to their own males, thus illustrating the difficulties involved when using patterns from population crosses to infer coevolutionary process (Rowe *et al.*, 2003; Arnqvist, 2004). We also failed to document a trade-off between reproductive rate and lifespan (cf. Tatar *et al.*, 1993). It therefore seems that the differences in lifespan we detected were not the result of different reproductive rates triggered by males, but of different direct costs of mating. This is also supported by the fact that reductions in female lifespan were indeed associated with reduced lifetime offspring production (i.e. female fitness).

In conclusion, we used a novel empirical strategy to (i) establish whether very closely related populations (60–300 generations) have diverged in terms of the effects that mating has on female post-mating reproductive behaviour and to (ii) assess whether this divergence has been replicable over 'replicated' phylogenies. We found considerable divergence, which provides evidence for rapid evolution of partial post-mating prezygotic isolation. Furthermore, the pattern of divergence was distinct in different phylogenies, suggesting that post-mating sexual selection has caused the divergence seen.

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Appendix

	USA	UK 1	UK 2
Temperature	25 °C	27 °C	30 °C
Rel. humidity	50–70%	60–65%	70%
Light regime	14L : 10D	13L : 11D	12L : 12D
Food host	Br: cowpeas; Sl: mung beans	Br: cowpeas; Sl: cowpeas	Br: cowpeas; Sl: mung beans
Population density	100 beetles on 250 g of food	250 beetles on 430 g of food	350 beetles on 150 g of food
Development time	~30 days	26–27 days	21 days
Rearing protocol	Overlapping generations	Nonoverlapping generations; generation time variable (range 25–35 days)	Nonoverlapping generations; generation time 28 days